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Animal production in Europe: The way forward in a changing world

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Preface

By the president of the ISAH

Mr President, Ladies and Gentlemen,

As president of the ISAH I have a great honor to take part in this in-between Congress here in Saint-Malo. With no doubts this congress is a very important event in our scientific field.

ISAH is a society with traditions. Our teachers and older colleagues, who are unfortunately no longer active in this world, created this society in the early seventies.

The very first ISAH congress took place in 1973 in Budapest. At the moment we are preparing to the XII ISAH Congress, which will be held in Warsaw in Poland in September 2005. The scientific goals of our society were very clearly described from the beginning. One of the fundamental tasks of the society is to assist in the formation of a more uniform understanding of animal hygiene as one of the branches of veterinary and animal sciences as well as to help to realize new scientific achievements for the benefit of animal production and veterinary public health ie food safety and environment preservation.

Scientists - hygienists performed their task of caring about animal health and needs very well. They proved that there is a special place for animal hygiene, as a scientific branch, in animal production sector. In the recent years the scale and range of hygienist activities became wider and it has changed a lot. The world is still changing in every field and area, of part, because of the common globalization. European as well as worldwide contacts are getting closer. We had to accept and learn many new definitions like: prevention, biosecurity, sustainable animal production, organic production, welfare, public health and environment. In my opinion we now understand all these definitions and we are able to use them in practice. This is how in the recent years we have discovered the meaning of the word "environment" again. We consider it much wider now. It is not only microclimate but we also investigate the influence of many environmental factors on the welfare of animals. We are also trying to establish the welfare of animals by changing or limiting the influence of some environmental factors. That is a very up-to-date subject worth discussing.

Accession of many European countries to EU means that national regulations have to be adjusted to European ones and it will result in great changes in animal and plant production and in the food chain as a whole. The direction agriculture is taking to will draw the new EU member States to Europe but probably will also generate new concerns. Especially intensification of plant and animal production, growing plants in monocultures, increasing the size of farms and concentration of animal production will undoubtedly create new problems to those countries in the field of public health, feed and feeding hygiene, animal production technologies and influence of animal production on environment. We have a lot of work ahead of us.

I would like to wish you success and effective deliberations.

Prof Andrzej Krynski

Editorial foreword

On behalf of both the organising committee and the scientific committee, I am pleased to welcome you in this wonderful place of St Malo, to take part in the "in-between congress" of the International Society for Animal Hygiene.

The ISAH was founded in 1970 by a group of European scientists at veterinary research institutes and universities. It was the result of a demand for new information mainly regarding farm animal keeping in relation to animal/human health concerns as well as environmental protection. The vocable "Animal Hygiene" was given to gather all those aspects of animal health and welfare maintenance and of veterinary public health. Hence the word "hygiene" was here used in its etymological acceptation, much broader than the current usual interpretation.

The ISAH has a main congress every third year and in the mean time there is usually a so-called "in-between congress" with the same scientific standard as the main congress. The current St Malo meeting is focused on the European situation of animal production sector in connexion to animal hygiene. Needless to mention here the crises we had to face in Europe during the recent years. Among these, BSE was certainly the most detrimental but avian flu also had locally a severe impact. In addition to these temporarily acute problems, several other issues are daily concerns to the livestock sector: animal welfare, environmental pollution, foodborne infections, drug resistance…Those crises and endemic problems point out the increasing need to take care of the food chain in a holistic-integrated way. The preharvest stage looks essential in this respect. An important part of these items relates to veterinary public health and fall completely within the scope of the ISAH.

Within the scientific committee we had a deep reflection on those critical points and we decided to build up scientific sessions accordingly. The objective was to try to bring in the updated knowledge which was available in the scientific community, trying to overcome the difficulties encountered in the food chain and taking advantage of the lessons learnt from the past.

On the first day entitled "animal production and society", we will have a group of lectures about key-issues among which EU regulations and their impact. Then parallel sessions will focus on certain aspects of welfare in farm animals and on the integration of production systems in the environment. The second day has animal health as main topic. The presentations will give us key-points for disease prevention and health maintenance. Both infectious and not primarily infectious problems will be given consideration. A specific attention will be paid to the role of animal hygiene in a situation of crisis. Veterinary public health will be a major item of the congress and several sessions are scheduled on the purpose. Obviously food safety will have a place of choice in the programme but other aspects like environmental preservation and non-foodborne zoonoses won't be kept aside.

All-in-all the programme of the congress illustrates the broad spectrum of the scientific field of the ISAH. It clearly shows how critical most of the subjects are and not only to the animal production sector per se, but, at least for some, to our fellow citizens as well.

We cannot end this edito without mentioning the wonderful job made by the scientific and the organising committees. A special thank goes to the ladies of ISPAIA Ploufragan Zoopole for their dedication and their very professional contribution. We must also acknowledge the companies and the different organisations which gave us financial support. Needless to remind that they provided us with the means to organise our congress in an agreable place while keeping the attendees'cost affordable. We sincerely thank these sponsors for their commitment to animal hygiene.

Finally we must thank you all, participants, contributors, chairpersons, for your considerable input.

We wish you all a pleasant and interesting congress.

F Madec
Chairman

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LIVESTOCK SECTOR IN EUROPE: THE POLITICAL STANDPOINT

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Introduction:
At the beginning of the 21st century, livestock production in Europe is being faced with great challenges. This is due to the modified overall situation: on the one hand globalization is also progressing in this economic sector and the public demands on livestock production in Europe have also changed on the other hand. Both factors together generate stresses and strains so that extremely controversial debates are a foregone conclusion.

Initial situation:
Since the 1950s, the farming sector has made considerable progress in terms of the quantity and quality of products. Speaking from the experience of an economy characterized by constant shortages during and after the Second World War, the primary objective was to ensure the provision of the population with sufficient food at reasonable prices. This has also been a key goal of the common agricultural policy in the European Community right from the outset.

As far as livestock production is concerned, the performance ability of the animals has peaked today: the genetic potential had improved, feeding as well as veterinary care was optimized and the husbandry methods streamlined.

At the same time, we can observe an increasingly critical stance of large parts of the public towards farm animal husbandry as it is customary today.

State of play in animal husbandry today:
The enormous increase in productivity in livestock production has frequently caused problems relating to animal welfare and environmental conservation. A high stocking density with all the negative aspects that this involves, especially for the environment, can be found in lines of production that can be carried out off-land, first and foremost pig and poultry husbandry, to be specific. The efforts made in the Netherlands for many years now to achieve a cutback in nitrogen inputs by extensifying these lines of production are not coincidental.

This does not only impair the environment. The adaptability of the animals too is frequently overtaxed. This is less due to the large number of animals than to the frequently extremely barren housing environment that places a strain on pigs especially. For want of an alternative, the animals occupy themselves with their herd mates and become cannibals this way! Add to this that these animals are in an unstable health state that requires treatment with antibiotics-containing starter mixtures especially during the rearing period.

The price to be paid for the high productivity of the animals is mostly an impairment of the environment, animal welfare and animal health today. Over the past few years, these conditions became known to the general public because of various crises (BSE, FMD, avian influenza, hormone residues in animal feed) and entailed an extremely critical attitude!

Productivity and competitiveness of livestock production
All policy-makers who demand a change in the previous productivity gain strategy are accused of jeopardizing the competitiveness of German farming. This is all the more so as the ongoing WTO negotiations will result in a medium-term cutback in the protective measures for European production at the Community's external frontiers.

It's quite obvious that the competitive situation on the world market will engender accelerated structural changes in European livestock production. There will also be winners in the process, not only losers. Who these winners will be, is still unclear. If you analyze the previous trends in livestock production, you will note that farmers' incomes have actually tended downwards over the past decades despite all efforts to lower costs, and hence the competitiveness by international standards! The producers are caught in a spiral that points downwards. The animals clearly lose out at any rate because rationalization and mechanization are the decisive factors to increase productivity. This entails a barren environment, with the human care intensity diminishing in the face of ever larger and unstable livestock populations. This intensity of care is the key corrective factor whenever problems occur.

Options for future action
If you analyze the environmental problems caused by intensive animal husbandry and consider the increasingly disapproving position of the population, a differentiated picture of future animal husbandry emerges. The specific locational conditions must also be taken into account in the process.

Attention must be paid to the internationally agreed minimum standards in the environmental field at any rate. These tend to put regions rich in livestock at a disadvantage because a curbing of the environmental strain through air pollution caused by livestock is most urgently required here.
Furthermore, the internationally agreed minimum standards in animal welfare must also be respected.

Both factors are influenced by the public acceptance of the respective type of husbandry in particular cases. Hence, the resistance put up by the local population results in the closure of newly established livestock farms in Germany today. This does not only concern mass livestock production, but also outdoor husbandry systems, for example.

Irrespective of the other framework conditions, we must succeed in reconciling the targets of animal health, animal welfare and environmental conservation in

livestock husbandry in Europe. I am convinced that solving this challenge is actually the prerequisite for making livestock farming in Europe viable in the future!

I am also convinced that we can overcome potential conflicts of aims and that these are not as huge in actual fact as it is always assumed. What matters, after all, is which compromises one is ready to make in the various fields. These goal conflicts become insurmountable when all above-mentioned targets are to be implemented without making concessions in productivity! Animal welfare will most likely lose out in these cases.

We urgently require differentiated solutions for animal husbandry in Europe. This means that each region must allow for its location-specific factors and take the internationally agreed minimum requirements as a starting point.

As I see it, these location-specific conditions also encompass the social acceptance of specific types of animal husbandry alongside the production-specific circumstances. It can be noted in the process that a society mainly shaped by urbanity tends to accept compromises in environmental and animal protection to a lesser degree than a society that is more entrenched in its agricultural roots.
A further factor is the regional distribution of people and farm animals: where livestock farms are located in the vicinity of non-agricultural settlements, conflicts arise between the interests of animal producers and those of the residents.

The measures taken to reconcile the aspects of animal health, animal welfare and environmental protection vary from country to country: there are countries that bet on partnership and voluntariness (the Netherlands, United Kingdom and Denmark), while others set restrictive legal requirements (Germany, Austria). There are various gradations in between. Thus, mandatory requirements in the environmental field are frequently imposed first of all, with animal welfare being chosen as a restrictive factor only later on. Here, too, I wish to cite the Netherlands as an example.

Given all the existing differences, we can observe that Europe pursues the common aim of an animal-welfare oriented husbandry that is also eco-friendly. In this context, we cannot ensure our competitive capacity by just lowering the production costs to a maximum degree. I believe that a clearer differentiation of the markets will emerge in Europe in the future: alongside discount and organic food shops, other products will also draw customers. A wider range of production methods will therefore also evolve on the producer side: given a compliance with environmental and animal protection standards, some regions will due to other framework conditions (land and energy are cheap, low construction costs, favourable wage costs) achieve a kind of mass production that is also competitive on the world market, while other regions will focus on the production of so-called premium products to hold their ground on the market.

We are heading for exciting times marked by the recent agricultural reform. I very much hope that the animals will not again be made to suffer: we are all called upon to prevent this!

THE IMPACTS OF THE NEW EU DIRECTIVE FOR LAYING HEN HUSBANDRY ON THE PRODUCTION AND TRADE PATTERNS FOR EGGS AND EGG PRODUCTS IN THE EU

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INTRODUCTION

In June 1999 the Secretaries of Agriculture of the European Union (EU) passed a new directive for laying hen husbandry which will have far reaching impacts on the future sectoral and regional pattern of egg production in the EU itself but also in other parts of Europe, adjacent production regions, and the United States. EU member states play an important role in global egg trade. In 2002 about 56 % of all exported shell eggs for consumption had their origin in the EU and 59 % of all imported eggs were destined for an EU member state. Up to the present date about 89 % off all layers in the EU are kept in cages. The new directive prohibits the conventional layer cage from January 1st, 2012 on in all EU member states and the installation of such cages from January 1st, 2003.

If during the next round of the WTO negotiations no regulations can be found that prohibit the import of eggs and egg products into the EU from countries in which conventional layer cages are still permitted, the egg producing sector in the EU will no longer be competitive. What impacts this may have on the spatial pattern of egg production, egg trade and the poultry equipment supply industry will be discussed in this paper.

In October 2001 the German *Bundesrat* passed a directive for laying hen husbandry which states that from January 1st, 2007 on conventional cages will be prohibited and from January 1st, 2012 on also enriched cages. This directive became effective in Germany on March 13th, 2002. It will have far reaching impacts on the German egg and egg products industries and also on the global trade patterns of eggs and egg products as Germany is already now the leading importer of shell eggs. In 2002 more than 28 % of all shell eggs that were traded worldwide, were imported by this country. So it is not surprising that the egg producers in Germany started an initiative to alter the directive and to inform policy makers, consumers, and animal welfare organisations about the possible consequences of the directive. In November 2003 the German *Bundesrat* again discussed the directive. A majority of the state representatives voted for a modification. They suggested that all laying hen husbandry forms should be tested under animal welfare aspects, the permission to use conventional cages should be extended for two more years and that available husbandry systems for laying hens should be permitted for continuous use only after having been tested by an independent institution. The Secretary of Consumer Protection, Nutrition and Agriculture, Renate Kuenast, announced that she would not sign this decision because of its incompatability with a decision of the German Supreme Court (*Bundesverfassungsgericht*) which had decided that the old German directive for laying hen husbandry did not meet the standards of animal welfare and had demanded that a new directive would have to include several minimal standards with respect to trough length, the ability to move and rest etc.

However, after the publication of two empirical studies which compared laying hen husbandry in enriched cages and alternative husbandry systems (Kreienbrock 2004, FAL 2004), things began to change. The studies could show that enriched cages with much more space for the birds (750 cm^2), larger groups (up to 60 birds), perches, scratching areas etc. showed excellent results with respect to the health of the laying hens, mortality rates, laying rate, feed conversion in comparison with floor management and free range systems. On March 26th, 2004 the secretaries of agriculture of the German states discussed these results with the secretary of consumer protection, nutrition and agriculture. They unanimously agreed that *small aviaries* should be permitted in Germany. They also decided that regulations for the independent testing of laying hen husbandry forms should be passed by the end of 2004 so that based on the results of such a test the permission for the permanent use of the tested and approved systems will be granted. For the first time, the Secretary of Consumer Protection, Nutrition and Agriculture declared in a press conference that she supported a competitive egg production in Germany. Nevertheless, the decision of the *Bundesrat* of October 2001 is still effective.

In this paper the frame for further discussion of these topics will be set.

1. THE SETTING: REGIONAL PATTERNS OF EGG PRODUCTION AND EGG TRADE

The first step of this analysis will provide an overview of the regional patterns of egg production and egg trade in order to identify the major production centres and trade relations.

Global egg production rose from 35.2 to almost 56 million tonnes or by 59 % between 1990 and 2003 (table 1). A closer look at this development reveals marked regional differences. Whereas Europe had to face a decline in production by more than 1.2 million tonnes, production rose by 18.4 million tonnes in Asia, followed by North and Central America with 2.2 million tonnes and South America with 720,000 tonnes.

Table 1:
The development of global egg production between 1990 and 2003, data in 1,000 t
(Source: FAO-Database)

Region	1990	1995	2003	Change (%)
Africa	1,550	1,770	2,082	+ 34.3
N. a. C. America	5,698	6,411	7,951	+ 39.5
South America	2,233	2,641	2,951	+ 32.1
Asia	14,507	22,492	32,927	+ 127.0
Europe	11,125	9,514	9,886	- 11.1
Oceania	244	208	195	- 20.1
World	35,208	43,036	55,992	+ 59.0

These data are, however, too generalised to inform about changes in the centres of egg production. Therefore, in table 2, the ten leading egg producing countries in 1990 and 2003 are compared with respect of their ranking and the development of production.

Table 2 shows that in 2003 the ten leading countries accounted for 72.0 % of the global egg production, a massive increase from 1990 when they contributed only 55.8 %. This is mainly due to the enormous increase in China (+ 240.9) but also the United States, India, Mexico, and Brazil recorded impressive gains. On the other hand, egg production in Germany decreased by 10.7 %, and Ukraine is no longer in the top ranks. The regional concentration of egg production is very high. In 2003, the three leading countries alone contributed 53.6 % to the global egg production.

Table 2:
The ten leading countries in egg production in 1990 and 2003 data in 1,000 t
(Source: FAO-Database)

1990		2003	
Country	**Production**	**Country**	**Production**
China	6,559	China	22,362
USA	3,965	USA	5,141
Russia	2,641	Japan	2,500
Japan	2,419	India	2,200
India	1,282	Russia	2,040
Brazil	1,230	Mexico	1,925
Mexico	1,010	Brazil	1,580
Germany	985	France	1,000
Ukraine	944	Germany	880
France	887	United Kingdom	704
Total	20,692	Total	40,332
Share of world production (%)	55.8	Share of world production (%)	72.0

Total egg production in the EU has not changed very much between 1990 and 2003 as can be seen from table 3. The contribution of single countries to the overall production has changed considerably, however.

Table 3 shows that Germany and Sweden had to face the highest absolute decrease, the highest relative decrease Finland, Sweden, and Germany. In contrast to this, Portugal, Belgium, and France showed the highest relative increase, this changed the pattern of egg trade in the EU as will be shown later.

Table 3:
The development of egg production in the EU between 1990 and 2003, data in 1,000 t
(Source: FAO-Database)

Country	1990	1995	2003	Change (%)
France	886.8	1,024,6	1,000.0	+ 12.8
Germany	985.0	836.0	880.0	- 10.7
United Kingdom	622.3	625.2	704.2	+ 3.2
Spain	666.6	694.0	700.0	+ 5.0
Italy	655.9	721.0	698.7	+ 6.5
The Netherlands	648.0	602.0	653.0	+ 0.8
Belgium/Lux.	159.2	219.9	180.0	+ 13.1
Greece	116.0	116.3	110.0	- 5.2
Portugal	79.6	102.8	108.5	+ 36.3
Sweden	129.8	105.0	93.9	- 27.7
Austria	95.7	103.1	90.0	- 6.0
Denmark	82.4	94.8	81.6	- 1.0
Finland	76.4	74.7	53.0	- 30.6
Ireland	31.1	30.8	34.0	+ 9.3
EU (15)	5,234.8	5,350.6	5,386.9	+ 2.9
% of world egg production	14.9	12.4	9.8	-

Table 4:
World trade with shell eggs in 2002
(Source: FAO-Database)

Region	Exports		Imports	
	1.000 t	Share (%)	1.000 t	Share (%)
Africa	19.4	1.9	36.5	4.0
N. a. C. America	71.3	7.1	64.5	7.1
South America	14.6	1.5	6.2	0.7
Asia	260.3	25.9	202.5	22.4
Europe	639.2	63.5	595.0	65.7
Oceania	1.0	0.1	0.9	0.1
World	1,005.8	100.0	905.6	100.0

A closer look at the pattern of egg trade reveals that about 1 mill. t of shell eggs were exported in 2002, liquid eggs and egg products are excluded (table 4). In these figures, trade among EU member states are included. Europe and Asia hold a leading position in exports as well as in imports, followed by North and Central America.

As can be seen from table 5, several member states of the EU ranked among the leading export and import countries for shell eggs for consumption in 2002. The Netherlands were by far the most important export country, contributing 26.3 % of all exports, followed by Malaysia, Belgium, China, and Germany. If, however, the intra-EU trade would be omitted, Malaysia would be in a leading position. Malaysia and China are playing a considerable role on the egg market in East Asia, Iran in the Near East, which will have impacts on the exports of the Netherlands, as they are supplying the same countries. Germany has been the leading egg importing country for several years, with a market share of 28.5 % in 2002. The rate of self-sufficiency has been decreasing for years, in 2002 it was as low as 74 %. A per capita consumption of 217 eggs and a population of about 82 mill. people, made Germany the most attractive shell egg market in the world, as more than 4.0 billion eggs had to be imported annually in the 1990s. So it is not surprising that the adjacent countries tried to reach a high market share, not only for shell eggs but also for egg products. Changes in production cost as a consequence of new legal regulations in this country will therefore have far reaching impacts on egg production and egg trade, not only in the EU but world-wide.

Table 5:
The ten leading export and import countries for shell eggs in 2002, data in 1,000 t
(Source: FAO-Database)

Country	Export	Country	Import
Netherlands	264.6	Germany	257.8
Malaysia	115.2	China	82.0
Belgium	86.2	Italy	62,8
China	83.9	The Netherlands	61.4
Germany	68.5	United Kingdom	45.7
Spain	61.1	Canada	34.2
USA	60.9	Belgium	32.4
France	43,3	Singapore	26.5
Belarus	30.8	Switzerland	25.5
Iran	18.6	Austria	14.7
Total	833.1	Total	643.0
Share of world exports (%)	82.8	Share of world imports (%)	71.0

In order to better understand the statements in chapter 3, which will deal with possible impacts of the new EU directive and the new directive for laying hen husbandry in Germany, the export and import relations between Germany and the Netherlands will be studied in more detail.

Table 6 shows that Germany has been importing between 4.0 and 4.4 bill. eggs per year since the early 1990s. Whereas the Netherlands could more or less maintain their market position until 2000, then the impacts of the Avian Influenza outbreak and a new paradigm in agricultural policy which will deglomerate areas of intensive agricultural production, led to a massive reduction of the export volume. Belgium has lost market shares since the mid-1990s. In 2002, the Netherlands still contributed 85.7 % to Germany´s egg imports according to official German data, in 2003 only 69.4 %. Because of the dioxin crisis, imports from Belgium decreased by almost 90 % between 1996 and 2001, but recovered in 2003. Non-EU countries were of minor importance until 2002 as suppliers for the German egg market, then Poland became more important with an export volume of 141 mill. eggs. This trade pattern will further change, however, within the next years, definitely after 2007.

Table 6:
The development of Germany´s shell egg imports between 1992 and 2003, data in mill. pieces
(Source: ZMP Bilanz: Eier und Geflügel, various editions)

Exporting country	1992	1996	2000	2003
Netherlands	3,936.3	2,974.3	3,922.6	2,782.3
Spain	21.9	9.2	27.4	354.6
France	79.9	252.0	216.3	210.7
Belgium/Lux.	279.9	822.6	94.8	209.2
Italy	0.0	0.0	1.5	100.4
United Kingdom	1.1	6.6	11.8	7.9
Finland	16.3	10.8	2.7	0.0
EU total	4,366.5	4,158.5	4,323.4	3,780,6
Non EU countries	65.8	26.7	36.4	225.8
Total	4,432.3	4,185.2	4,359.8	4,006.3

From table 7 one can see that according to official Dutch data the exports to Germany in 2003 were a bit lower. This would mean that almost 77 % of all exports had Germany as their destiny. From a detailed data analysis it would become obvious that until 2002 the Dutch exporters tried to compensate their losses on the EU market by increasing the exports to non-EU countries. This is no longer true for 2003, as exports to these countries decreased by 60 %, total exports by almost one third. The industry has not yet recovered from the Avian Influenza outbreak.

Table 7:
The development of Dutch shell egg exports between 1992 and 2003, data in mill. pieces
(Source: ZMP Bilanz: Eier und Geflügel, various editions)

Importing Country	1992	1996	2000	2003
Germany	3,830.5	3,761.3	3,446.9	2,544.0
Belgium/Lux.	784.5	424.1	182.1	171.3
United Kingdom	171.6	204.1	234.9	123.8
EU Total	5,216.3	4,805.9	4,576.1	2,986.0
Switzerland	198.1	138.6	102.1	58.8
Unit. Arab. Emirates	123.1	39.9	210.5	2.7
Non-EU Countries Total	876.6	548.6	788.4	322.4
Total	6,092.9	5,354.5	5,364.5	3,308.3

2. THE NEW EU AND GERMAN DIRECTIVES FOR LAYING HEN HUSBANDRY

The invention of the layer cage and the combination with automatic water and feed supply systems as well as automatic egg collecting and sorting systems initiated a revolutionary change in egg production. The result was a safe and cheap animal product. When in the late 1960s and early 1970s such systems showed up in Europe and North America, a sectoral and regional concentration process began. On the one hand, egg production shifted from small farm flocks to vertically integrated agribusiness companies which combined parent stocks, hatcheries, feed mills, layer farms, and sometimes even egg products plants under one roof. On the other hand, such companies very often concentrated in favourable locations, so that these regions gained high market shares. Hybrid hens with laying rates that had not been thought possible before World War II, the improvement of the health status of the animals, and constantly increasing feed conversion rates mark the success story of industrialised egg production. Economic success, however, was only one aspect, the question if such a production system would also meet the regulations of animal protection laws was another. When vertically integrated companies originated and average flocks sizes increased, animal welfare groups started their crusade against this form of animal production, sometimes peaceful, sometimes militant. This is not the place to go into more detail, but one must not forget that the decision of the Secretaries of Agriculture of the EU member states does not only have an animal welfare but also a political aspect. A perhaps unexpected result was the fact that 13 of the 15 member states agreed to the new directive, only Spain abstained from voting and Austria voted against it as not being strict enough.

What are the regulations in *Directive 1999/74/EC (July 19th 1999)* and when will they become effective?

The directive distinguishes between regulations for alternative systems of laying hen husbandry, conventional cages and furnished or enriched cages. The main statements for conventional and furnished cages are:

Conventional cages:

- From January 1st, 2003 on for each hen a space of 550 cm^2 has to be supplied, also a trough length of 10 cm per animal. For 65 % of the cage base the height has to be at least 40 cm, no part of the cage may be lower than 35 cm.
- Conventional cages are not permitted after January 1st, 2012, from January 1st, 2003 on conventional cages may no longer be installed in layer farms.

Furnished or enriched cages:

- From January 1st, 2003 on for each hen a space of 750 cm^2 has to be supplied in cages of this type, of which 600 cm^2 have to be usable space. The base of a cage must not be smaller than 2,000 cm^2, outside the usable space the height of the cage has to be at least 20 cm.
- Cages have to be furnished with a nest, perches that offer at least 15 cm resting space for each hen, and a sand-bath (scratching area). For each hen a trough length of at least 12 cm has to be available.

How does the German directive differ from that of the EU?

On July 6th, 1999 the German Supreme Court passed a verdict that answered the question if the directive for laying hen husbandry (*Hennenhaltungsverordnung*, dated December 12th, 1987) was compatible with the Constitution (*Grundgesetz*) and the Animal Protection Law (*Tierschutzgesetz*, dated August 18th, 1986). This question had been asked by the State Government of Northrhine-Westphalia. The Supreme Court decided that:

- the directive for laying hen husbandry is not compatible with the Constitution and has to be modified by the federal government;
- layer farms can therefore no longer be permitted according to the directive of laying hen husbandry from 1986;
- a space of 450 cm^2 per hen and a trough length of 10 cm are not sufficient to allow an undisturbed resting and simultaneous feeding of the animals.

It is important to realise that the verdict of the Supreme Court demanded an immediate reaction of the federal government of Germany. After very controversial negotiations, the German *Bundesrat* passed the new directive for laying hen husbandry in October 2002 with a majority of only one vote, to the great surprise of the egg industry and perhaps even the Secretary of Consumer Protection, Nutrition, and Agriculture. The main regulations of the new directive are:

- From January 1st, 2003 on no cages may be installed, neither conventional nor enriched ones.
- From January 1st, 2007 on conventional cages and from January 1st, 2012 on enriched cages will be prohibited in Germany.
- From January 1st, 2003 on laying hens may only be kept in new facilities that are at least 2 m high and have a basic area of at least 2 m x 1.5 m.
- A single flock must not be larger that 6,000 hens.

The EU commission certified the new directive for laying hen husbandry in early March and it became effective on March 13th, 2002. From that date on it is prohibited to install any type of cage in a German egg farm. It can easily be seen that this new directive which is still effective, in spite of the decisions of the *Bundesrat* of November 2003 and March 2004, will have far reaching impacts on the German egg and egg products industries.

3. IMPACTS ON EGG PRODUCTION

Which impacts will the new EU and German directives for laying hen husbandry have on egg production in the EU and in Germany? At the present time it is almost impossible to give a reliable answer to this question as the transformation process is still in its initial phase. So only first results can be given, based on interviews with leading persons from poultry equipment suppliers, agribusiness companies, poultry associations, and scientific publications as well as own calculations.

What will be the impacts of the EU directive? From January 1st, 2003 on the guideline demands at least 550 cm^2 of space per hen. This means that one hen less can be kept in a standard cage, i.e. 4 instead of 5.

According to a study by Wolffram et. al. (2002) the following impacts of the EU-directive can be expected:

- Egg production in the EU will decrease by about 11 billion pieces.
- The rate of self-sufficiency will decrease from 103 % in 1999 to 96 % in 2012. This does not include the impacts of the new German directive for laying hen husbandry.
- The EU will become a net importer of shell eggs.
- About 5 to 6 billion € will be necessary until 2012 to fulfil the regulations of the directive.
- About 12.300 jobs will be lost.

The economic impacts of the new EU directive will be far reaching. Most of the egg producers in the EU are afraid that it will not be possible to reach a result during the next WTO-negotiations which prohibits the import of shell eggs and egg products from countries that still allow conventional cages. This would mean that the production cost within the EU would be much higher than in non-EU countries.

What will be the impacts of the German directive for laying hen husbandry?

Three scenarios for the possible development of egg production and egg trade in Germany will be presented. These scenarios are based on a study of this author (Windhorst 2004a).

The basis for the three scenarios is the year 2002. The structure of egg production and trade can be characterised in the following way:

- 40.8 mill. laying hens were kept in farms with 3,000 and more places for hens.
- 83.9 % of the hens were kept in conventional cages, the average laying rate was 285 eggs/hen and year.
- 8.6 % were kept in free-range systems, here the average laying rate was 250 eggs/hen and year,
- 6.6 % were kept in floor management systems with a laying rate of 260 eggs/hen and year,
- and 0.8 % in other systems with a laying rate of 240 eggs/hen and year.

Farms of this size produced 11.4 bill. eggs, this was a share of 86.4 % of the total egg production in Germany. About 4.1 billion eggs for human consumption had to be imported to cover the domestic demand.

Scenario 1: EU directive (1999/74/EC) becomes effective

On January 1st, 2003, the first step of the EU directive (1999/74/EC) became effective. For each hen a space of 550 cm^2 had to be supplied also a trough length of 10 cm. What were the impacts of this directive? Most of the installed conventional cages in Germany had a usable space of 2,300 cm^2 which made it possible to have 5 birds per cage. As the new directive demands 550 cm^2 one hen had to be removed from each cage. This resulted in:

- a reduction of the laying hen flock in farms with 3,000 and more places from 40.8 mill. laying hens to 35.7 mill. hens or by 13 %,
- a reduction of egg production from 11.4 bill. to 9.9 bill. eggs,
- a decrease in the value of primary egg production of 200 mill. € and of 100 mill. € in associated industries, such as feed mills or the egg products industry,
- a loss of 666 jobs,
- additional imports of 1.5 bill. eggs (total: 5.6 bill.),
- about 120 mill. € would have been needed to build new layer farms and to keep the production volume on the level of 2002. This, however, is a fictitious value as enriched cages are not

permitted and the market for eggs from alternative husbandry systems is more or less saturated.

Scenario 2: Banning of conventional cages from 2007 on

From January 1st, 2007 on conventional cages are no longer permitted in Germany according to the still effective directive of October 2001. As enriched cages are also not permitted, all eggs have to be produced in alternative husbandry systems. In the following scenario it is assumed that all farms with conventional cages will remain in production and either be transformed into floor management or free-range systems. The situation of the egg industry in Germany in 2007 can be characterised as follows:

- The number of laying hens in farms with 3,000 and more places will decrease from 35.7 mill. to 19.6 mill. or by 45.1 %. If the flocks of 2002 are taken as the basic value, by 52 %.
- Egg production will drop from 9.9 bill. to 5.0 bill. pieces or by 44.5 %.
- The value of primary egg production will decrease by another 500 mill. €, that of the associated industries by 400 mill. €.
- Egg farms and associated industries will lose at least 3,200 jobs.
- In order to supply the German market with shell eggs and to maintain a self-sufficiency rate of 74 %, another 4.9 bill. eggs have to be imported (total imports: 10.5 bill. eggs),
- As cages cannot be used any longer and the farms have to be transformed to alternative husbandry systems, about 950 mill. € will be necessary to switch to these systems.

Quite obviously, policy makers did not consider which problems would result from the banning of cages, especially in eastern Germany. Here, a large number of egg producers invested large amounts of money to build either new farms with state-of-the-art technology or installed new equipment in existing farm buildings. The federal and state governments supported their decision as necessary steps to be competitive in a globalising market. Now these egg farmers are forced to use their cages as long as possible because of the loans they received from the banks. The banks will not give the permission to install alternative husbandry systems before December 31st, 2006 and are not willing to give new loans to the farmers as many of them have not been able to pay off the old loans. The same is true for a considerable number of egg farms in western Germany. The consequence is that the transformation process will hardly begin before 2007 and then last for several years, as the companies which develop and produce the equipment will not be willing to pre-fabricate alternative husbandry systems for about 20 mill. laying hens, because they do not know how many of the farms will be transformed, how many egg farmers will quit egg production and how many large egg producers plan to build new facilities with enriched cages in Eastern Europe. The result will be that either the self-sufficiency rate will drop far below 35 % or the federal government will have to permit the use of conventional cages for several more years during the transformation period to alternative husbandry systems (c. f. Windhorst 2004a). One can only be astonished about the naivety with which policy makers passed such a directive.

Scenario 3: Enriched cages will be permitted in Germany

What will be the situation if the directive of October 2001 will be altered because of new insights in the disadvantages of alternative husbandry systems with respect to higher mortality, disease problems, the increasing risk for the introduction and dissemination of highly infectious diseases, egg quality, and environmental problems resulting from ammonia emission and the contamination of the soil and groundwater in free-range systems (c. f. Jacobs and Windhorst 2003, FAL 2004, Kreienbrock et al. 2004). If enriched cages or *small aviaries* will be permitted from 2012 on as in other EU member states, the situation of egg production and egg trade will be like this:

- The number of laying hens in farms with 3,000 and more places will decrease from 35.7 mill. to 28.9 mill. birds or by 19 %.
- Egg production will drop from 9.9 bill. to 7.9 bill. pieces or by almost 21 %.
- The value of primary egg production will decrease by another 200 mill. € compared to 2003, that of the associated industries also by another 200 mill. €.
- About 1,700 jobs will be lost on egg farms and in the associated industries.
- Another 1.9 bill. eggs (total imports: 7.5 bill. eggs) will have to be imported to meet the demand on the domestic market.
- About 820 mill. € will have to be invested to install enriched cages in farms which formerly used conventional cages.

This scenario shows that despite the permission of enriched cages Germany will have to import 3.5 bill. eggs more than in 2002 to meet the demand on the domestic market. As from 2012 on the EU will also be a net importing region for shell eggs if egg producers do not invest large amounts of money in new egg farms, there will be a shortage of eggs.

The willingness to invest in new egg farms will to a high degree depend on the development of production costs for eggs in the EU and non-EU countries. A study by van Horne and Bondt (2003) could show that the increase of production costs for eggs as a result of the EU directive (1999/74/EC) will result in the competitiveness of Polish producers on the German market. A further reduction of the import tariffs by 36 % and an increase of the exchange rate of the € by about 15 % will result in the competitiveness for producers from Ukraine and India on the German market. So these countries may become suppliers for the German consumers. What this does mean for

animal welfare, environmental protection, egg quality, and food safety will not be discussed here but should be considered by policy makers and the Secretary of Consumer Protection, Nutrition and Agriculture. It is one side of the medal to announce a shift in the paradigm of agricultural policy and another to deal with the consequences of such a shift.

It has to be assumed that the increase of production costs resulting from the banning of conventional cages in the EU and all cages in Germany will lead to higher egg costs for the egg products industry. If the industry will be able to adjust to this new situation is a still open question.

A very critical economic situation is also expected on the side of the poultry equipment suppliers. The EU guidelines and the decision of the German *Bundesrat* have led to an almost complete standstill in further investments in egg production in many of the EU member states. This phase could easily last until 2005, when the EU will finally decide about the equipment of enriched cages. Even though several prototypes of such cages are available, investors are very careful with their decisions as they cannot foresee the results of the WTO negotiations. According to our own investigations, poultry equipment suppliers could sell less than 1 mill. places for laying hens in enriched cages in EU member states until the end of 2003. This is about 0.4 % of the present hen population in cages. Especially in some of the future member states of the EU, cages that can be transformed to enriched cages were installed in 2003, exact numbers are, however, not available. It can be expected that some of the large German egg producing companies will build new facilities in Poland, Hungary, and other countries of Eastern Europe if the German directive will not be changed. This will lead to a further decrease of the German self-sufficiency rate.

4. DISCUSSION: FURTHER CHALLENGES FOR THE EGG AND EGG PRODUCTS INDUSTRIES

In addition to the changed legal framework, further challenges are at hand for the egg and egg products industries in Europe. They can be summarised as follows:

- The globalisation of the markets for agricultural products will offer new chances for non EU member states.
- Product safety and quality assurance will become the leading driving forces in the future development of markets for agricultural, especially animal products, and demand the implementation of supply chains.
- Aspects of animal welfare and environmental protection will become more important in future and ask for reactions.
- Biotechnology and gene-technology will open new ways in food design.

What impacts will this have on the egg and egg products industries? A first statement is that the egg as well as the egg products industries will be able to operate from a good position because most of the leading egg producing companies have already installed supply chains and can guarantee a high product quality and product safety. The most recent development in the EU with respect to keeping laying hens in cages or battery systems shows, however, that aspects of animal welfare will gain in importance and that the industry will have to adjust. In addition to that, environmental aspects as well as the permanent risk of the introduction and dissemination of highly infectious diseases in the centres of egg production in some EU member states will become more important during the next years and ask for reactions. The outbreaks of Avian Influenza in Italy and the Netherlands showed how far reaching the economic impacts can be.

A second statement is that in future only companies or production regions that are able to supply the market with high quality products and can prove that during the whole production process animal welfare and environmental protection have been cared for and legal regulations have been met, will be successful in the market. Those companies and regions that cannot meet these challenges will be the losers. Producers in non-EU countries that plan to export into the EU should adjust to these standards if they want to be successful in this attractive market in the long run.

5. CONCLUSIONS

It has become obvious from the preceding chapters that the Secretaries of Agriculture of the EU member states want to go a way of their own with respect to future systems in laying hen husbandry. Even if one takes into consideration that the aspects product safety, quality assurance, and animal welfare will gain in importance, at least in post-industrial societies, and that from this point of view the decision of the Secretaries of Agriculture and especially the German Secretary of Consumer Protection, Nutrition, and Agriculture will be met with sympathy in the broad public, one must not forget another fact. When asking the average buyer about her/his attitude towards keeping layers in cages, an overwhelming majority says that they dislike it, but nevertheless between 80 % and 90 % of the eggs bought in the EU stem from such farms. Quite obviously, the first attitude does not match with the buying behaviour. Could it be that without legal regulations and directives there would still be conventional cages in future because the consumer would decide this by his shopping behaviour? Another aspect that has not been discussed sufficiently is the aspect of product safety. Very often the average consumer concludes from his dislike of layer cages that shell egg produced in such systems are an unsafe product. The opposite is the case, as could easily be demonstrated (c.f. Jacobs and Windhorst 2003). Quite obviously, the industry has not been able so far to transmit this message. It will not be an easy task to convince the consumer once the new directive for laying hen husbandry will have become effective in Germany. Nevertheless the industry should try to go this way even if it will take a considerable amount of money and some hard years. A third aspect is the increasing risk of the introduction and dissemination of highly infectious diseases that will necessarily be a consequence of the increasing egg imports into the EU and

of the growing number of laying hens in free-range systems.

Because of the development during the last decade and the low growth rates it can be expected that organic eggs will remain a niche product for several more years in spite of the ongoing discussion about product quality, product safety, and animal welfare and supporting government programmes in some EU member states (Windhorst 2004b). The dissonance between the buying behaviour of the consumers and their statements as citizens about the food they prefer and plan to buy is quite obvious. In 2000, organic eggs contributed only 1.2 % to the total production volume of shell eggs in the EU, the same share was reached in human consumption of eggs.

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UNDERSTANDING LAY AND EXPERT RISK PERCEPTION IN THE LIGHT OF MORAL THEORY

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Introduction

It is well established that scientifically trained experts and those who lack such training, or lay people, tend to perceive risks differently, both in general (Slovic et al. 1985, Slovic 1987) and in connection with food-related risks (Fife-Schaw & Rowe 1996, Sparks & Shepherd 1994; see Hansen et al. 2003 for an overview). This expert-lay discrepancy can lead to real difficulty in setting agreed priorities in food risk management.

A case in point, and one that we shall discuss in this paper, is the present handling of Bovine Spongiform Encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD) in Denmark. Many scientifically trained experts believe that food hazards exist that are considerably more serious than BSE/vCJD on which money for precautionary measures would be better spent. They often imply that the present spending on BSE/vCJD only serves the political purpose of pandering to a disproportionate level of fear among lay people. On the other hand, findings on lay risk perception give us good reason to believe that the majority of people support strict regulatory measures against BSE/vCJD.

Disagreements of this sort tend to result in deadlock. Commonly, that is, experts continue to believe that lay priorities are irrational because they fail to reflect the true risks as measured by frequencies of adverse consequences. Their remedy is to 'get the numbers right' with the hope that lay people, once relieved of their 'knowledge deficit' and in put possession of correct information, will change their perceptions accordingly. However, the mounting empirical evidence from many controversial cases shows that this strategy generally does not work: Even in the face of credible numbers, lay people often do not adopt different attitudes.

The risk perception literature explains this phenomenon by showing that whereas experts judge risks in terms of annual fatalities (or the frequency of some other unit event), lay people operate with a much broader concept of risk, incorporating sensitivity to a wide range of hazard characteristics such as personal control, lethality, catastrophic potential and inequitable distribution. Lay priorities are different, then, simply because they are based on considerations other than the frequency and seriousness of consequences. This suggests that policy and risk communication will have to adapt to lay people's broader conception of risk. But it is important to see that this explanation does not resolve the disagreement. The deadlock is precisely that, regardless of whether we understand lay perceptions and attitudes as irrational, or as representing basic psychological facts, there appears to be no point in trying to change them.

Can this deadlock be broken? We believe that the risk perception literature contains two distinct lines of thought. One is the line just set out. It emphasises the finding that experts and lay people have different concepts of risk, adding that, although they can each be more or less systematically described and predicted, these concepts cannot be brought together in a reasoned discussion. The other line emphasises the finding that each party represents different but legitimate concerns. This line suggests that experts and lay people have different objectives in the face of risk, and – underlying these objectives – a different set of values.

The present paper follows the second line. It is motivated by the simple idea that, in order to move the debate on, we need identify these different value bases. In cases of disagreement, the expert and the layperson are both asking the same question, namely: What should we do? But they bring to this question rather different perspectives. If we can conceptualise these, experts and lay people ought to be able to gain more insight into one another's positions; and thus more in the way of mutual understanding. Their disagreements may not be fully resolved, but constructive dialogue will at least become possible.

The paper divides into two parts. The first part (comprising the next two sections) is empirical. In it we report a series of qualitative, semi-structured interviews with lay people and experts on food-mediated health risks. The interviews were conducted in Denmark in 2002. They focused on zoonotic risks. These interviews confirm that experts and lay people perceive and judge the risk of contracting zoonoses through food rather differently. However, the interviews also allow us – to some extent – to uncover the different contexts behind these differences in perception and priorities and to suggest a rational reconstruction of the values underlying each of them.

The second part of the paper (the following section) is philosophical in nature. In it we pick up of the some of more salient features of the interviews and try to interpret them in the light of ethical thought. The distinctive idea here – an idea we believe to be both new and instructive – is that a stylised conflict between the expert and the lay perspectives on zoonotic risks can be understood as a genuine moral conflict between legitimate concerns. In the final section we draw out some important consequences of this idea.

Interviews with Lay People

Eleven lay people (defined here as non-experts with no strong occupational or other links to the food sector) with diverse backgrounds in age, urbanisation, gender, role in providing food in the household, and occupation were selected for interview. The interviews were semi-structured; they began with enquiries about the everyday experience of food and moved on to questions about food safety issues raised by zoonoses. The aim was to obtain a qualitative, context-sensitive understanding of lay perceptions of these latter issues and to look at the personal strategies ordinary people adopt to manage food-mediated zoonotic risks.

Health risks associated with zoonoses were rarely mentioned by the interviewees when they were asked merely about their everyday experience of food. At this stage of the interviews, price, convenience and organoleptic quality were the dominant themes. This general feature is important for understanding the lay

perspective on zoonotic risks: People have a general expectation that food is safe, and their attention turns to hazards only when problems emerge or are brought up.

As the interviews progressed, the interviewees were asked to rank the zoonoses they knew out of a sample of the following nine according to how serious/dangerous/bad[1] they perceived them to be: BSE/vCJD and *Salmonella* (the infections on which the present paper chiefly focuses), *Campylobacter*, *Listeria*, *Yersinia*, botulism, 'Roskildesyge',[2] Trichinae and tuberculosis.[3] Here it emerged that the majority of participants had limited knowledge of zoonoses: All were aware of *Salmonella* and vCJD, and some recognised botulism, but few were familiar with *Campylobacter* and *Listeria*. Whereas the experts all understood the request to rank in the same way, the lay interviewees displayed different interpretations of this exercise, and quite a few in fact made two or more alternative rankings.

In a common interpretation the lay ranking was based on *how bad it is to have the relevant infection or condition.* The principles underlying this ranking of consequences seem to be that death is much worse than non-fatal disease, that certain death is worse than risk of death, that death after a dreadful deterioration (vCJD) is worse than instant death, and that availability of treatment and/or chance of recovery makes a disease less worse, whereas long term harm makes it worse. These principles all figure in quantitative risk perception studies as important hazard characteristics. Some of them are detectable in the following exchange:

> **Interviewer:** What is it that makes you place Creutzfeldt-Jakob on top?
>
> **Lay1:** Well, it is the deaths one has heard about… one has also heard that with the three others, that is *Salmonella*, tuberculosis and botulism… but Creutzfeldt-Jakob […] appears to be a disease one can build up over long time, and then suddenly it shows up with lethal effect… without particular warning, while the other three after all show some symptoms in the beginning, right?… which makes it possible to get treatment in time. And there is apparently no real treatment for Creutzfeldt-Jakob, so… that must make it the most dangerous…

According to this criterion, vCJD is consistently ranked as more serious than *Salmonella*. Some based this ranking on the belief that *Salmonella* is most often or always non-fatal, whereas vCJD is invariably fatal. Among these, some were reminded that *Salmonella* can be fatal, but this persuaded only one respondent to change his ranking so that *Salmonella* became "almost equal" with vCJD. The others, even after the reminder, continued to regard vCJD worse.

Only a few interviewees referred to frequencies, taking both the severity and frequency of zoonotic diseases into account. These respondents consistently ranked *Salmonella* as worse than vCJD. It is striking that only a few lay people appear to consider frequencies explicitly in this ranking exercise. However, as we shall see below, it cannot be concluded that lay people do not pay attention to frequencies (or probabilities). But as quantitative risk perception studies also show, they appear to be clearly sensitive to a range of distinctions between possible consequences.

In some cases, the respondents referred to certain characteristics influencing their personal perception of, or feelings about, the relevant risk. A few reported being influenced by experience, i.e. by having been infected themselves, or by having seen infected persons. Presumably, this had made them take the risk more seriously. A few ranked *Salmonella* as equally bad or worse than vCJD on this account; and interestingly, the only respondent who took botulism seriously referred to experiences from Africa.

A few others reported being influenced by media coverage. Since the media have reported a number of *Salmonella* outbreaks in Denmark, whereas there have been no confirmed cases of vCJD, media coverage makes it easy to identify with the victims of *Salmonella* infection.[4] By contrast, vCJD in Denmark is experienced as a remote danger. Accordingly, these respondents ranked the former as worse.

Finally, a few referred to the criterion of whether or not it is possible to reduce the risk through personal effort (ranking vCJD as worse in this regard than *Salmonella*). An example:

> **Lay2:** So that one [BSE/vCJD] is probably the worst one, because it is something in the meat you can't avoid. *Salmonella* I can avoid by cooking the meat properly and sterilise the things when I have been working with the meat, and the eggs… I can also… abstain from eating soft-boiled eggs.

This consideration, which is also dominant in quantitative risk perception studies, appeared to be important for many respondents when the interviews moved on to a more personal perspective.

The lay interviewees were asked to describe their personal concerns and priorities vis-à-vis food safety. At this point, implicit beliefs about probabilities of personal exposure became more evident. It turned out that, in general, the interviewees did not feel zoonoses to be personally threatening – which explains why zoonoses were not mentioned initially when the respondents were talking about their everyday experience of food.

[1] The exact wording of the question varied from one case to another depending on the context.

[2] The Danish vernacular term for mild diarrhoea.

[3] We recognise that, strictly speaking, a zoonosis is a *disease* (transmissible to human beings from animals in ordinary circumstances) and not the agent which causes the disease. However, in the lay interviews, we followed common usage in order to use the names most familiar to lay people. Event though this usage intermix pathogens with diseases, we have chosen to keep it in the paper.

[4] One interviewee reported being vividly affected by his knowledge of a spectacular case where a father and son died from eating a heavily *Salmonella* infected cake.

However, if we compare *Salmonella* and BSE/vCJD, it is clear that, even though people felt safe from both, they did so for very different reasons.

Thus one interviewee said:

> **Lay3:** It [vCJD] is not something I am at all afraid of getting myself… If I went out a lot, for instance, and got food from catering companies, then I would be more concerned about botulism, for example, and *Salmonella* probably… be more worried about that.

Here there appears to be little or no concern about exposure to BSE/vCJD, but the picture is different with *Salmonella*. Although massive campaigns to control *Salmonella* have been run at all levels of the food-production chain, only a few of the interviewees believed food to be free of genuinely threatening levels of it. The existence of official control programmes was often regarded as mere confirmation that *Salmonella* is not presently under control.

This notwithstanding, there was a relatively relaxed attitude to *Salmonella*. This attitude seems to be based on the belief that *personal* coping strategies, involving, among other things, the maintenance of high levels of kitchen hygiene and the avoidance of high-risk dishes or ingredients, are generally effective against infection:

> **Lay4:** *Campylobacter* and *Salmonella* I can do something about myself. Here [pointing] I feel powerless – that is the lottery with *Listeria* and botulism… BSE, I certainly hope that one is in control, but otherwise I do not feel very affected by it. [5]

This confirms the importance of personal control, or at least the feeling that one has the ability to control one's exposure to a disease. In addition, most respondents were quite familiar with *Salmonella*, the risk of becoming infected, the symptoms of salmonellosis and the availability of treatment. Where vCJD was concerned, a rather different picture emerged. Although a few respondents mentioned personal strategies,[6] most seemed to believe that these are not as effective against vCJD as they are against *Salmonella*. And clearly, there was no familiarity with BSE/vCJD. Again, however, vCJD was not in general seen as a personal threat in everyday life.

As the last sentence in the Lay4 excerpt makes clear, this belief may depend very much on trust in the control systems.[7] Unlike with *Salmonella*, the widely shared attitude appears to involve fundamental trust in public control of BSE. This trust might be the upshot of the resolute reaction of the Minister of Food, Agriculture and Fisheries to the first confirmed case of BSE in Denmark. Apart from immediately imposing the strict regulation required by the EU, she recalled beef-cuts with backbone from retail outlets and encouraged consumers to discard any similar beef they already owned.

Finally, the interviews touched on the issue of responsibility for food safety. Here, a further notable difference between BSE/vCJD and *Salmonella* emerged. Some respondents argued that some incidents of *Salmonella* infection are unavoidable and natural, even if the present number of incidents is particularly high:

> **Lay5:** The *Salmonella*, yes… we all know where that comes from and how you risk getting it… most people after all also know how to avoid it… but there are still many cases of it… There will be bacteria in the food regardless of how you jump and leap, so… Somehow or other, I believe they have been there all the time… people have not always known what they died from, though… something else got the blame.

A few interviewees added that a natural level of 'background contamination' in *Salmonella* might even strengthen our immune systems. By contrast, vCJD can be seen as solely man-made, and more specifically as something imposed on us by agriculture and the food industry.

Interviews with Experts

Thirteen experts on zoonoses (defined here as people who deal professionally with zoonoses), most of whom had been involved more or less directly in the discussion and formulation of food policy, were selected for interview. Four came from industrial associations in primary production, one from retail business, two from regional government agencies, three from government research institutes, one from a clinical hospital department, one from a university and one from a consumer NGO.

The interviews were again semi-structured. They focused on the experts' roles in their organisations; their professional assessment of the nine zoonotic risks presented in the lay interviews; their attitudes to lay perceptions of these same risks; and their views on risk communication in the field of zoonoses.

Among the experts in our sample, there was wide agreement over many factual questions about zoonoses in Denmark. And as remarked in passing above, when asked to rank the nine zoonoses, all the experts understood the task in the same way – as a matter of describing their perception of the actual health threats. Some showed a clear awareness that this 'scientific' approach was likely to be different from the lay approach:

> **Exp1:** First, I shall have to ask you: What does 'risky' mean? Is it my personal scientific attitude to what one really should be concerned about, or is it what people believe is dangerous – the ordinary citizen?

[5] Interestingly, the kitchen strategies adopted by the interviewees to combat *Salmonella* were often (though not entirely in this quotation) assumed to be effective against other bacterial agents. In this sense *Salmonella* functioned as a 'headline' proxy for all bacterial zoonotic agents.

[6] The minority of respondents referred to here said that they could buy organic, buy local or just buy Danish. It was also mentioned that one could avoid cuts like T-bone steaks or (in one case) rely on proper cooking.

[7] This point is well described by Wynne (1996).

In their approach, the experts all took into account both the frequency and seriousness of zoonotic infection; they tried to assess, for each zoonosis, how many instances of human infection are there, and how serious these infections are. In summing up this information, the experts' implicitly assumed a weighting of the possible different health consequences, ranging from diarrhoea to death. Here is a typical example of how this approach works:

> **Interviewer:** If we speak broadly about zoonoses, which ones do you then consider most risky?[8]
> **Exp2:** Well, it is probably still *Salmonella*, because certainly there has been some success the last year or two in reducing the number of diagnosed cases,… but it is indeed the more aggressive form compared with *Campylobacter*, which is the other main problem… It is very seldom that *Campylobacter* infections become what is called invasive and possibly end up with blood poisoning, complications and death. So you can certainly say that in terms of numbers, they take up a lot of space. And if you add up days lost through sickness and other economic effects, then they take up the most space right now. But the *Salmonella* infections are the ones that potentially involve the greatest risk and complications, and in the worst case death, so it is still these that ought to be in focus…
>
> CJD, or mad cow disease, as it is also called, on which there has been a tremendous focus for quite a few years, is absolutely disproportionate… misjudged as a big threat…

Overwhelmingly, the experts ranked *Campylobacter* and *Salmonella* as the most serious zoonotic food safety problems presently being faced in Denmark. Below these[9] they placed *Listeria* and *Yersinia*. Many ranked Roskildesyge, which they interpreted as a viral infection, quite high.[10] A few thought that more attention should be given to botulism, because of the many new production forms. Trichinae and tuberculosis were ranked quite low, and BSE/vCJD was ranked generally lowest, although uncertainty about the prevalence of BSE and vCJD caused some experts to rank it somewhat higher, to a midway position at most.

It was on *policy implications* that the experts, representing different interests, found themselves in a certain amount of disagreement. However, most experts adopt a policy objective that takes the frequency and seriousness of human health consequences as its point of departure. This assumption is evident, when Exp2 above says:

> But the *Salmonella* infections are the ones that potentially involve the greatest risk and complications […] so it is still these that *ought* to be in focus…

Note that Exp2 here, from a description of the greatest risk, almost imperceptibly moves to a value judgement about what ought to be done. It is reasonable to interpret this value judgement as the view that resources spent on any efforts to control and prevent zoonoses should be used in proportion to the risk they pose so that reduction of health risk per money unit is maximised. Thus, after having called the recent focus on vCJD "absolutely disproportionate", Exp2 goes on:

> As far as I know, it is something like 500 million Kroner [roughly equivalent to €67 million] that we spend in Denmark alone on combating BSE, and theoretically the chance of ever seeing one single human case is about 0.1%. Thus, we are typically driven by the media and communication problems and always end too far out… when this money could have been returned many times if we had concentrated on the real risks. It is a very instructive story.

The notion that resources ought to be spent in proportion to the risk goes unidentified as a value judgement. It appears to be considered part of professional or scientific judgement:

> **Exp3:** vCJD has to my knowledge not been confirmed in Denmark. And I consider it a political problem. This is what we professionals have difficulty finding reasonable – that so many resources are spent on that risk.

On the other hand, objectives involving considerations other than the proportionate reduction of health risks are clearly identified as political in nature. For one thing, this means that they are clearly identified as value judgements; but often, being perceived as disproportionate, they are also considered unjustified:

> **Exp1:** …BSE, which I find is a very huge political disease. We have had two million cattle in England infected by clinical outbreak of BSE. We have had approximately one hundred persons who became ill. The evaluation is wildly exaggerated, if one looks at it from a strictly scientific point of view… And I know that all the scientific colleagues I have spoken with, who are working on this, totally agree.

The adoption of this 'professional' view does not mean that the experts are unaware of the political reality or do not recognise the legitimacy of other policy objectives. It is, however, a view that is readily adopted or referred to by the experts; and they seem to use it implicitly as a standard against which they compare and judge other

[8] In introducing the ranking exercise to the experts, the word 'risky' was often used.

[9] Interestingly, the experts placed *E. Coli* O157 immediately below *Campylobacter* and *Salmonella*, although they were not asked about this infection.

[10] Most experts protested that food borne viral infections are not strictly zoonoses; some observed that other members of the nine zoonotic risks in our sample need not be zoonotic in nature.

objectives. As we have seen, a key example of a political priority – one explicitly mentioned by most of the experts – is the present spending on BSE/vCJD. Most experts recognised, however, that the measures adopted in response to BSE/vCJD were politically necessary, given EU regulations; and many of them also recognised that the measures have succeeded in closing the issue as far as consumer trust is concerned.

It is worth mentioning that uncertainty about the spread, or potential spread, of BSE/vCJD complicates matters here. A number of experts said that even though the risk presented by BSE/vCJD appears to be small, uncertainty about its true size justifies a precautionary approach. Some saw this precaution as a political necessity. Thus Exp4 conceded, "a politician nowadays cannot act otherwise". Others advocated precaution on a professional/scientific basis.[11] One expert said that the effort to combat BSE/vCJD had been warranted, but that too little was being done about *Campylobacter* and *Salmonella* by comparison.

Reconstruction of Underlying Values

We now move to an analysis of some of the dominant perspectives in terms of moral theory. This will necessarily be somewhat schematic. We shall concentrate on the stylised problem of how to prioritise between *Salmonella* and BSE/vCJD. We start by summarising the expert and the lay perspectives.

As we have seen from the interviews, the experts appear to perceive it as their duty to present a professional view on zoonoses. One part of this duty will be to get the numbers right: The main tools are here epidemiological analyses and risk assessments. However, the experts will also see it as a their duty to present a professional corrective to any political priority, namely that we ought to make the highest possible reduction in health risk per cost unit. This corrective typically involves an implicit critique of other priorities which we reconstruct along the following lines: Resources are limited, and any disproportionate effort necessarily involves costs in terms of negative health consequences that could have been avoided. For instance, the present very costly policy on BSE necessarily draws away resources from other tasks, such as *Salmonella*. It is not necessarily implied that other (political) considerations are illegitimate or wrong; but at least their costs should be recognised openly.

The lay interviews present a less homogenous picture. Lay people appear to think about food risks in many different ways and express no clearly shared priorities. As a point of departure, however, a shared perspective seems to be the expectation that food ought to be reasonably safe to eat. For the fulfilment of this expectation, we are all obviously very dependent on the food industry and its regulation. If for some reason we begin to loose trust in these, some of the differences between *Salmonella* and BSE/vCJD will appear significant.

For one thing, the fact that, in the case of *Salmonella*, there is still room for reducing the risk by a personal risk management effort makes this risk far less frightening than being powerlessly exposed to "the lottery" of BSE/vCJD (as one interviewee expressed it).[12] Moreover, in this kind of lottery, it is far more frightening that the risk is one of getting an invariably fatal and very dreadful disease. (Of course, *Salmonella*-infection by comparison is usually non-fatal and has an effective treatment available.) For these reasons, we believe that a change in priorities along the lines suggested by many experts – moving resources from BSE/vCJD to *Salmonella* – would be opposed by many lay people. At least, it would if they began to lose trust in beef and perceived even a very small probability of personal exposure.

If we look at these conflicting perspectives from a moral point of view, two important points emerge. First, whereas the lay perspective appears to be primarily personal, the experts take an impartial point of view on society as such. In their view, the objective should be minimising most cost effectively the *total* of expected negative health consequences. This objective is compatible with imposing costs on the individual for the sake of the common good. For instance, by giving priority to *Salmonella* over BSE/vCJD, an individual can be forced to accept a small risk of *death* for the sake of reducing the total number of human *Salmonella* infections (most of them non-fatal). But from the individual layperson's point of view, this is probably perceived as unacceptable. The individual may have no personal interest in accepting this kind of trade-off.

From a moral point of view, the question here is whether the individual has a right not to have risks placed upon him without his consent. It is generally recognised that a person has a right not to be harmed by others. It is more controversial if a person also has a right not to have a *risk* of harm imposed on him by others, even if the risk is very small. We believe, however, that a case can be made for a right of this kind in the case of BSE/vCJD. Thus, Danish Law forbids selling food that by normal use may be assumed to transmit or cause disease. If the purpose is to protect the individual, then, since even a very small risk of death might be frightening, it might be assumed to cover the case of BSE/vCJD.

In principle, there would then be a similar right not to have the risk of *Salmonella* imposed on one. However, this risk might not be perceived as equally frightening, in view of the probably non-fatal consequences. Moreover, the possibility of personal control makes it possible for the individual to decide himself whether or not he wants to run the risk. Finally, if some prevalence of *Salmonella* is natural and hence unavoidable, the picture is different. Because of the perceived benefits, people might generally be content to run the risk of *Salmonella* infection, and they would still have some personal control.

The conflict between the objective of making outcomes as good as possible from the impartial point of view of society, on the one hand, and respect for individual rights and personal autonomy, on the other hand, is well known from political philosophy.[13] If there are reasons from the

[11] One respondent who took this line recognised that hers was a minority view among professionals.

[12] Of course, one could reduce the risk from BSE by simply abstaining from eating beef altogether. However, the general expectation is that it should not be necessary to resort to such drastic measures in order to feel safe about food.

[13] A clear statement of the individual perspective is given by Nozick (1974). Kagan (1989) provides a thorough defence of an impartial

point of view of society to change priorities in the direction of *Salmonella*, but people have a right not to have a risk of death placed upon them, what can then be done about this moral conflict? The simple answer is that experts can respect the lay right by involving the general public in the decision. If the objective of reducing total health costs is so important, it ought to be possible to convince people about it, thereby persuading them waive their right.

Another important point is that lay people appear to consider the consequence 'death' as substantially more serious than the consequence 'illness', whereas experts are willing to make a 'proportional' trade-off between these types of consequence. The experts' professional view implies that lay people attach too much weight to the risk of death. The fact that lay people take a primarily personal point of view, whereas experts take an impartial point of view probably again plays a role here. However, it is well known that it is a controversial moral question how to assess the value of avoiding untimely death (See e.g., McMahan (2002) for an overview).

This kind of discussion has been pursued for some years within medical ethics. The measurement of health benefits in terms of QALYs (Quality Adjusted Life Years), for instance, assumes that the value of avoiding untimely death equals no more, and no less, than the value (quality) of the extra years the person enjoys. The implication is that the benefit of a life-prolonging treatment such as heart transplantation can be compared with the benefit of a treatment like hip replacement, which does not prolong life but makes the quality of the remaining life years better. However, critics claim that the value of prolonging life might be incomparable with enhancing the quality of life; or that there is more to the value of prolonging life than simply the value of the extra years. It might be difficult to reach consensus on this question. But clearly, there is a substantial moral issue worth discussing. For an overview, see Bell & Mendus (1988) and Broome (1993).

Finally, we should like to add that the motives behind risky activities appear to play a role for lay people. Thus, the fact that food industry is perceived as governed by a profit motive seems to make food risks more unacceptable than they would have been if food production had been governed by more noble motives. We suggest that organic production could be perceived in this perspective, even to the extent that lay people might accept greater zoonotic risks from organic products because those risks are perceived, perhaps, as foreseeable but unintended consequences of the ideal behind organic production. Kant (1959) is well known for stressing the importance of motives in moral thought.

Conclusion

If we consider the conflicting perspectives in the light of socio-psychological descriptions, debate between them seems bound to be at cross-purposes. Disclosure of the values underlying the conflicting perspectives makes those perspectives open for reasonable discussion. Once the moral nature of this kind of debate is recognised, a new set of concepts becomes available for rational discourse. We cannot be sure that a moral dialogue will lead to consensus. However, given the bleak prospects of the presently used strategies in risk communication, it should certainly be worth trying.

The experts' distinction between 'professional' and 'political' views seems to signal that whereas the latter involve value judgements, the former does not. However, it is important for experts to recognise that the professional priority involves a value judgement as well. More precisely, the view that resources should be spent so as to reduce negative health effects most cost effectively is value judgement. This judgement may seem self-evidently correct to experts. Still, it is a value judgement all the same. And this has important consequences for risk communication.

Experts have no professional authority to make value judgements, although they do of course have authority in presenting the facts, and among these facts are the consequences of not pursuing a given objective. Equally, experts may be endowed with some authority by their organisation in holding a particular value judgement. For instance, an agency may have assigned an objective to it by the government, and from this assignment some authority flows. But the experts have no authority to make a value judgement simply because they are experts.

Arguing for a value judgement is very different from arguing for facts. Scientific training is very useful in factual argument. But no value judgement follows from a report of scientific findings. In order to convince someone about the validity of a value judgement, it is necessary to appeal to the values he or she already holds. But this is only possible if the appeal itself is presented as a value judgement. If it is presented in a value-free guise as a 'professional' or 'scientific' judgement, communication becomes distorted and therefore runs the risk of being at cross-purposes. We all tend to react with resistance when someone tries to change our value judgements by appealing to his authority in the matter – as if it was a simple matter of expert opinion. We only engage with others, and consider our own value judgements, when the latter are respected from the outset. This requires an open dialogue.

It may be that experts, in an open dialogue, will be able to convince the public that it should accept a small risk of death for the sake of the common good in terms of the total number of human infections. But they are only likely to succeed if they take seriously the individual's legitimate interests. Arrogance in this matter clearly will not help. It is not a matter merely of 'getting the numbers right'. Delicate moral discussion of how far we, as individuals, are obliged to accept risks of death being forced upon us for the sake of reducing general illness in society is required. However, as the interviews showed, most experts actually do understand the lay perspective on food safety quite well, just as lay people are not necessarily insensitive to frequencies and do not generally believe in food that is 100% safe. So the conflicting perspectives should be able to meet.

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consequentialist perspective. For the issue of risk in this connection, see Jensen (2002).

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ETHICS: THE NEW CHALLENGE FOR LIVESTOCK PRODUCTION

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Changes in the animal production sector

During the last 150 years, in Europe and North America, the agricultural world experienced dramatic changes. Thanks to the concepts of experimental science and of domination of humankind on the nature pioneered by Francis Bacon (1561-1626), and following the age of Enlightenment, the agricultural revolution, along with the industrial revolution, led during the 19th century to a rationalisation of the agricultural production, including animal production. The last 60 years saw the progress of the industrialisation of this sector, together with a transition from a rural to an urban social structure, disconnecting the major part of the population from the agricultural production process. During this period, progress has been done in food security and self sufficiency in terms of quantity, in costs for the consumer, in security of the products of animal origin, and in their physical quality (such as standardization, marketability).

The increased intensification of animal production, together with the accelerated introduction of new biotechnologies during the last two decades, led to increased detrimental environmental impact, deep alimentary and sanitary crises (such as BSE, FMD, contaminants in meat), and distrust of the population (Hodges, 2003). As a consequence, a new demand emerged, centred on what we could name "subjective quality", stressing on ethical and sustainable sides of livestock production.

Ethical issues and concepts

Ethics can be viewed as rules of action set inside a given Society, in accordance to its beliefs, for an harmonious development, or as the eye that any individual turns on his/her action. In terms of human-animal relationship, stewardship and domination of humankind on nature, denying any moral value to animals, prevailed for long (Burgat, 1997). In the Duty ethics advocated by Immanuel Kant (1724-1804), if moral value is denied to animals, an indirect moral duty towards them is commended, for the respect of the categorical imperative. Meanwhile, utilitarianism, following Jeremy Bentham (1748-1832), developed the paradigm of optimisation of happiness inside the Society, including all sentient beings, of which animals, in the interested parties ("the question is not can they reason? nor can they talk? but, can they suffer?"). This view led to the creation of the Royal Society for the Prevention of Cruelty to Animals in Great Britain in 1824, and respect for animal welfare became the main ethical component considered in animal production.

Animal welfare

Definitions of animal welfare are numerous, and depend upon the components taken into consideration. Biological functions (Broom, 1991) may be seek as "objective" indicators: productivity, behaviour, physiological parameters (hearth rate, respiratory rate, stress indicators,…), anatomy, health can be used for that purpose. A second way is to consider the affective states of the animals: feelings, pain, suffering (Duncan and Fraser, 1997); a third way is relative to living conditions respecting the "natural" conditions of a given species and allowing species specific behaviour to be experienced (Rollin, 1993). As a result, two families of methods for assessing animal welfare (Broom, 1997; Vessier *et al*, 1999; Johnsen *et al*, 2001) coexist: those based on the breeding environment and conditions (Bartussek, 1999), and those based on observation of the animals (Capdeville and Vessier, 2001). The diversity of scientific approaches to animal welfare, which can lead to different solutions for one problem, as pointed by Fraser (2004), requires a multidisciplinary process, and a balance of science with philosophical components; in that sense, animal welfare is a mixture of science and values. In fact, for operational reasons, the concept of the five freedoms (freedom from thirst, hunger and malnutrition; freedom from discomfort; freedom from pain, injury and disease; freedom to express normal behaviour; freedom from fear and distress), set up by the Brambell Committee in 1965 (FAWC, 1992), is the most popular.

With animal becoming a commodity in an industrialised production system, efficiency developed at the expense of the human-animal link or of the care for animal well-being. In this context, situations in which animal welfare concerns are high are numerous. For example, high producing dairy cows show higher prevalence of mastitis, metabolic diseases, or lameness. Veal production in individual crates deprives calves of solid food, of iron, reduces the possibilities of moving and of social contact, with pathological and ethological consequences, breaching all five freedoms. Confinement, convenience surgery (beak trimming, teeth clipping, tail docking or castration) are sources of concern in intensive breeding of pigs or poultry, and affect even the breeders themselves (Larrère and Larrère, 2000; Porcher, 2004). More traditional ways of breeding animal, and organic farming, can also lead to drawbacks such as insufficient medical care or exposure to hazards (such as predators). Animal transport, inside an integrated production process, or from farm to slaughterhouse, may result in discomfort, or elevated death rate. Slaughter can be a source of stress and pain. (Webster, 1994; Burgat, 2001; Denis, 2001)

If animal scientists focused mainly on animal welfare, this field does not cover all ethical aspects of animal production (Fraser, 1999, Christiansen and Sandøe, 2000).

Integrity, intrinsic value

Selection is an age-old method used by breeders in order to create breeds, or to improve their characteristics. The rate of genetic progress increased dramatically during the last decades with the use of biotechnologies such as artificial insemination, then embryo transfer (Schroten, 1992), and more recently marker-assisted selection. Without mentioning cases such as featherless broiler chicken or genetically blind laying hens, these traditional but enhanced ways of modifying the nature of individuals have had quick and deep consequences on performances and health. Now, new biotechnologies, more invasive,

such as cloning and transgenesis, are developed and may soon arrive on the market of animal production. By their action on the genome they have the potential to create and perpetuate new forms of life. Even if this would have no consequence on the welfare itself of the individuals (but we know that, at this time, hard consequences in terms of suffering, abnormalities and death are associated with these techniques), the induced modifications affect animal integrity. Rutgers and Heeger (1999) defined animal integrity as "the wholeness and completeness of the animal and the species-specific balance of the creature". Breach of animal integrity, in a biocentric ethical point of view, is a morally relevant fact. The Swiss constitution recognizes the "dignity of creatures" in relation to transgenesis, genetic engineering of non-human beings being allowed only if their own good is not impaired (Balzer *et al*, 2000; Brom, 2000b).

The Animal Health and Welfare Act (1992), in Netherlands, supports the concept of intrinsic value of animal life (Verhoog, 1992). For Taylor (1984), animals and all other living beings have inherent worth: they develop, grow and maintain their life, and, as such, are due moral consideration. For Rollin (1992), animals have interests (needs, wants, goals) which matter for them. If they have some conscious awareness of these interests, of their *telos*, then humans have duties towards them. These zoocentric views go beyond the pathocentric, utilitarian position (Heeger and Brom, 2001).

Sustainability

Hans Jonas (1979), observing that "Modern technology, informed by an ever deeper penetration of nature and propelled by the forces of market and politics, has enhanced human power beyond anything known or even dreamt of before. It is a power over matter, over life on earth, and over man himself; and it keeps growing at an accelerated pace.", concluded "Act so that the effects of your action are compatible with the permanence of genuine human life", setting, in his Imperative of Responsibility, the basement of a sustainable development.

Livestock production has direct consequences on environment, biodiversity (in livestock and wildlife spheres), landscape, sociological structure, and (micro- and macro-) economics: as such, it covers the three pillars of sustainability: ecological (agro-environmental), social and economical, and shares ethical principles, particularly, responsibility towards Society and future generations. This relationship is now more and more acknowledged (Gibon et al, 1999; Thompson and Nardone, 1999; McGlone, 2001).

Driving forces

Among new priorities and values set by the Society are ethics and sustainability. In Europe, improvement and harmonization of regulations, through the Council of Europe and European Union, is in progress. The new Common Agricultural Policy (Agenda 2000) links now direct payments to farmers with high standards of animal welfare (Winter *et al*., 1998). Research in this field increased notably, and higher education developed new curricula, for example in Europe (Lund, 1997, Marie *et al*, 2003).

Driving forces for implementation of this ethical agenda can be upstream, or downstream. In a social market economy, upstream regulations (Mellor and Bayvel, 2004) may force further progress, the additional cost being supported by the community, as in Switzerland or in EU. At this day, the attempts of the EU to have its high animal welfare recognized by the WTO, and by this way extended, failed (Hobbs *et al.*, 2002, Chatellier et *al.*, 2003). The recently set-up OIE Working Group on animal welfare (Bayvel, 2004) may offer an opportunity to establish internationally recognized welfare standards, taking account of the globalisation of animal production.

On the other hand, in a liberal free trade economy, ethical concerns are endorsed, on a voluntary basis, by food industries and retailers, through labels or schemes, and financed by the consumer.

A necessary progress for acceptability

Progress towards a better taking account of the ethical issues in animal production is a condition of product acceptability in the future (van Genderen, and de Vriend, 1999; Brom, 2000a; Bennett et al; 2002).

Furthermore, it is also a condition of social acceptability of the animal production sector, which remains an important (central) element of rural development.

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AUTOMATIC ON-LINE MONITORING OF ANIMALS BY PRECISION LIVESTOCK FARMING

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1. Objectives of Precision Livestock Farming in monitoring

Livestock production today is no longer limited to obtaining economic goals. Modern society is concerned about food safety and quality, efficient and sustainable animal farming, healthy animals, guaranteed animal well being and acceptable environmental impact of livestock production. As a consequence, there is a growing need to monitor many variables during the entire production process in order to satisfy these targets. In the past, livestock management decisions have been based almost entirely on the observation, judgment and experience of the farmer (Frost et al., 2003). However, together with the increasing scale of the farms and the corresponding high number of animals, this evolution has resulted in an increasing administrative, technical, organisational and logistic workload for the farmer and has limited the possibilities of the same farmer to monitor his animals by himself.

Observation by ethologists is needed for research purposes, but is very expensive for practical application and has the disadvantage of limited time period of observation. Today, automatic monitoring and controlling techniques are becoming more and more important to support the farmer in managing the production process. Although biological processes involving living organisms have always been considered as too complex to be monitored and controlled in an automatic way, today new emerging technologies offer possibilities to develop full automatic on-line monitoring and control of many of these processes.

One of the objectives of Precision Livestock Farming (PLF) in the field of monitoring is to develop on-line tools to monitor farm animals continuously during their life, in a fully automatic way, with objective measures and criteria calculated on-line from collected data and without imposing additional stress to the animals. The aim of these technical tools is not to replace but to support the farmer who always remains the crucial factor in good animal management. Besides on-line automatic monitoring, PLF offers also interesting possibilities in automatic control for supporting the management of such complex biological production processes (e.g. feeding strategies, growth rate control, activity control, see Morag et al., 2001; Halachmi et al., 2002; Aerts et al., 2003a, b; Kristensen et al., accepted).

2. Basic principles of Precision Livestock Farming

The PLF approach starts from the observation that the animal is the most crucial part in the biological production process in an animal house. Despite this fact, in most modern livestock houses worldwide farmers use control equipment (e.g. climate control, feeding supply etc) that does not measure anything on the most important part of the process: the animal.

Animals, as all living organisms, are *complex*, *individually different* and *time-variant* (meaning that they respond differently at different moments of time). Therefore, we say that animals are CIT systems (Complex, Individual and Time-variant).

A starting point in PLF is the recognition that each individual animal is such a CIT system. This contrasts with more classical approaches where animals are considered as "an average of a population and due to its complexity as a steady state system".

For monitoring and control of livestock production processes, the PLF approach makes use of modern monitoring and control theory. To achieve favourable monitoring and control of such processes, *three conditions must be fulfilled.*

The *first condition* to be fulfilled is that *animal variables must be measured continuously* and this information is analysed continuously. "Animal variables" can be very different such as weight, activity, behaviour, drinking and feeding behaviour, feed intake, sound production, physiological variables (body temperature, respiration frequency, blood variables,). What "continuously" means is depending on the measured variable such as 25 times a second when monitoring on-line animal activity from video images or a sample every day when monitoring animal weight.

A *second condition* to realise accurate animal monitoring and management is that at every moment *a reliable prediction (expectation) must be available* on how the animal variables will vary or how the animal will respond to environmental changes. By environment we mean the whole of all variables that are not genetically defined. It is the continuous comparison between this prediction (in the past the experience of the farmer and now for example a mathematical model) and the actual measured values that allows to identify animal activities and to judge when something abnormal is happening.

The *third condition* is that this prediction together with the on-line measurements are integrated in an analysing algorithm (a number of mathematical equations implemented in a microchip) to monitor or manage the animals automatically and to achieve on-line monitoring of animal health, welfare, or take control actions (climate control, feeding strategies,). A schematic overview of the three conditions is shown in Fig. 1.

Since the animal is acting as a complex, individual and time varying system we need to apply this PLF approach in an appropriate way. The best way to handle this time-variant character of all the complex individual animal responses is by applying continuous measurements and predictions and by using predictions and applying mathematical data-analyses in an on-line or real time way and, if possible, on individual animals. The required technology is available. The key to realise this application is novel and innovative multidisciplinary research.

3. Sensors and sensing techniques

The last years, many research and development efforts have been done all over the world to develop new sensors and sensing techniques to acquire on-line information from animals and to collect different animal variables. For cows, pigs and chicken several sensors, sensing principles and sensing techniques have been described in literature. As shown before (Berckmans, 2003) we have found in recent literature 11 papers to measure eating behaviour, respiration rate, non destructive chewing behaviour, stress responses, etc. for pigs (e.g. Eigenberg et al., 2000). For cows, 29 sensors are described in recent literature to measure deep body temperature, body weight, udder health, oestrus, breath emissions, biting rate in grazing cows and others (e.g. Velasco-Garcia and Mottram, 2001). For chicken's recent literature gives 9 papers describing sensors to measure body temperature with radio transmitters, biosensors to detect pathogenic bacteria at very low levels, heat stress and others (e.g. Lacey et al., 2000). Twenty papers were found about vocalisations of pigs to measure variables such as: pigs need for supplemental heat, peripheral endocrine stress responses, behavioural responses to separation, on-line detection of infection of the respiration system (e.g. Marchant et al., 2001).

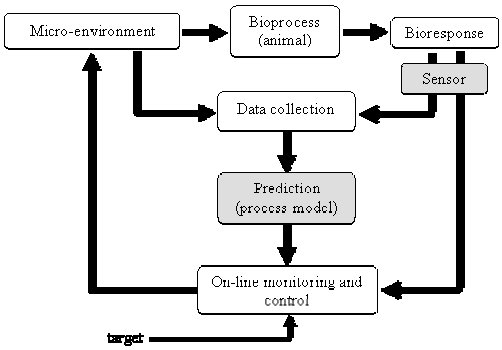

Figure 1. Precision livestock farming (PLF) by integration of measured bioresponses together with a predictive process model into a model-based monitoring or control algorithm: a schematic overview (Aerts et al., 2003a).

Twelve papers were found about the vocalization of cow sounds to measure animal's condition, effects of separation on behavioural responses, milk production, and identification of individual cows (e.g. Weary and Chua, 2000). Eleven papers were found on vocalization of chicken describing: an increasing number of gakel-calls with an increasing hunger state, stress dependence of chicks call qualities, capacity to emit food calls and quantification of stress (e.g. Zimmerman et al., 2003). Nineteen papers describe image analysis of pigs to measure: location of pigs in scenes, stress conditions, tracking of piglets, relation of outside 3 dimensional body conformation and lean-fat ratio, on-line monitoring of pig weight (e.g. Onyango et al., 1995). Twenty seven papers describe image analysis of chicken for real time disease detection, behavioural responses, non-destructive prediction for yolk-albumen ratio in chicken eggs, feeding behaviour, animal distribution and activity and automatic identification of activities related to animal welfare, animal weight (e.g. De Wet et al., 2003).

It can be concluded that several efforts are done to develop sensors and sensing techniques for animal variables and this is just a beginning stage. Many new sensing systems will be developed in near future (sensors at the micro- and nano-scale, biosensors, telemetry, etc.).

Today, the availability of reliable and accurate sensors still is the main bottleneck to apply PLF in practice. The price of this new technology is not a main problem since as shown by many examples (CD player, mobile phone, GPS) the number of produced units is the main factor influencing the price.

4. Exemplar 1: Real time sound analysis to detect health status in pigs

A first example of PLF in monitoring animals is a system to detect infections in fattening pigs by on-line analysis of their produced sound. The basic idea is that the respiration system is producing a sound when coughing. When the animal is infected by a respiratory disease, the characteristics of the respiratory system, such as the cell of the air pipes, are changing. Consequently, the characteristics of the energy in the sound signal that is produced when air is pulsed through this system, when coughing, will be different as well. If this difference in sound signal can be detected fast enough after infection, on-line monitoring of pig's coughs and other animal sounds could be useful as a biomarker for infection and improve disease management (resulting in, among others, reduced antibiotics usage) (see also Fig. 2).

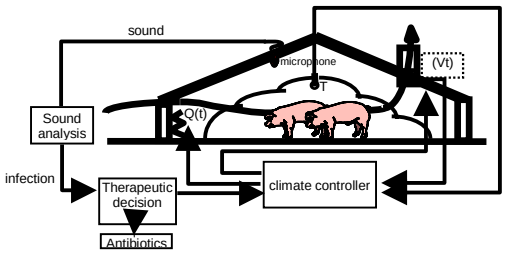

Figure 2. Use of on-line sound analysis as a basis for health monitoring in pigs.

Algorithms have been developed to detect coughs of pigs out of a raw sound signal (Van Hirtum et al., 1999; Chedad et al., 2001). In a first step, these algorithms make a distinction between a sound and no sound. Secondly, the sounds are classified in coughs and no coughs (other sounds) and finally also a distinction can be made from healthy and sick coughs (Van Hirtum and Berckmans, 2002) (cf. Fig. 3).

Based on laboratory experiments, it could be demonstrated that pig's coughs could be classified correctly in 94% of the cases (Van Hirtum and Berckmans, 2003). Testing of the developed algorithms in practice, showed that in pig houses in the field the coughs could be correctly classified in 86% of the cases (Guarino et al., in press; Jans et al., accepted).

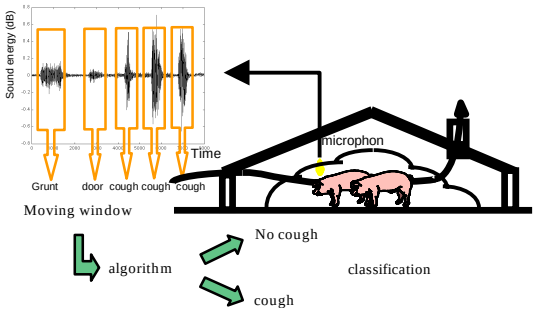

Figure 3. Classification of pig coughs.

5. Exemplar 2: Real time identification of the behaviour of laying hens to monitor animal welfare

A second example of PLF in monitoring animals, is a system to monitor fully automatically the activities of laying hens to score their welfare and to use this information for better managing the production process environment (cf. Fig. 4).

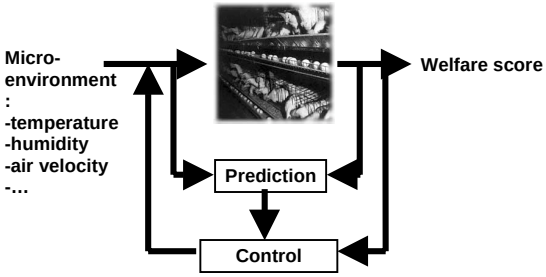

Figure 4. Integration of on-line monitored welfare score in the management of production processes for laying hens.

Behavioural characteristics are usually evaluated by audio-visual observation done by a human observer present on the scene. This method is time consuming, expensive and cannot be done continuously during the life time of the animal. Automated objective surveillance, by means of cheap cameras and image-processing techniques, has the ability to generate data providing a continuous measure of behaviour, without disturbing the animals. A fully automatic on-line image-processing technique was developed to quantify the behaviour of laying hens as opposed to the current human visual observation. The classification of the hen's behaviour was performed by dynamical analysis of a set of measurable parameters, calculated from the images using image processing techniques. A first implementation of the system allowed identifying three different types of behaviour (standing, walking and scratching) (Leroy et al., 2003). In Fig. 5, an example is shown of automatic scratching detection based on the developed algorithm (see also *http://www.labr.be*).

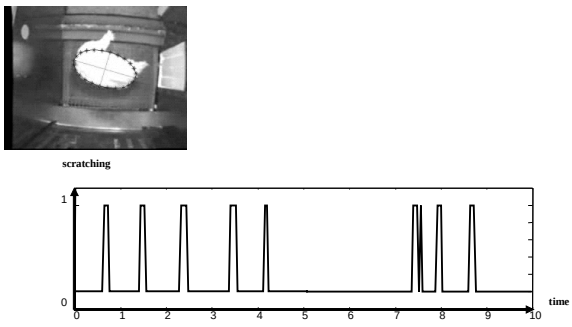

Figure 5. Automatic detection of scratching behaviour for laying hens.

6. Conclusions

Precision Livestock Farming involves the measurements, predictions and data-analyses of animal variables. PLF offers totally new possibilities to collect and analyse data from farm animals in a continuous and fully automatic way. We cannot only replace the farmers "eyes and ears" to each individual animal as in the past, but several other variables (infections, physiological variables, stress, etc) will soon be measurable in practice. The bottleneck to apply this technique is in the availability of reliable sensors and sensing systems, since it has been shown that the required mathematical algorithms can be developed.

The application of this technology offers new possibilities to realise food safety and quality, efficient and sustainable animal farming, healthy animals, guaranteed animal well being and acceptable environmental impact of livestock production.

Therefore, efforts should be increased for bringing this challenging approach of Precision Livestock Farming to practice. This is only possible when teams from different research disciplines, such as physiology, ethology, nutrition, hygiene, engineering, etc. join their research efforts.

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Animal Welfare, Focus on transport / slaughter / euthanasia

Oral Communications

ANIMAL WELFARE DURING LONG DISTANCE TRANSPORT OF CATTLE - FACTS AND PUBLIC PERCEPTION

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Introduction

Animal transport gives cause for concern for several reasons: (1) Transport can cause severe stress in animals entailing poor welfare. (2) Stressful transports may have a negative effect on meat quality. (3) There is the risk of spread of infectious diseases over large distances (HARTUNG et al., 2003). Particularly long distance transports of animals are one of the most emotionally discussed topics in the field of animal protection today, and the transport of slaughter cattle to the Near East Region touched the nerve of the public after some television programmes showed cruelties against animals during unloading in the harbours at destination.

The European Union was challenged to act and issued the Council Directive 91/628/EEC of 19th November 1991 on the protection of animals during transport (1991) followed by several regulations such as the criteria for staging points and the route plan (1997) and standards for long distance road vehicles (1998). The most important details for long distance transports (longer than 8 h in special vehicles) refer to the transport time and the unloading rule for the 24 h resting period. The transport times for cattle are 14 h transport followed by 1 h rest and a second period of 14 h transport. Thereafter, the animals have to be unloaded in the resting facilities of a staging post for 24 h. After reloading the journey can continue in the same pattern.

This paper investigates whether these resting and driving times really protect the animals from poor welfare and meet their physiological needs. Examples are given for heart rate and energy metabolism of transported cattle.

Stressful situations during transport

There is no doubt that transport is an unknown procedure for cattle which can be irritating and aversive. The most aversive factors are loading and unloading, bad handling, inappropriate driving, poor road conditions, too hot or too cold climate, insufficient ventilation, high stocking densities, mixing of unfamiliar groups, deck height, lack of water and food, vibration, vehicle motion and length of the journey. Levels of stress in animals may be measured by physiological (e.g. heart rate, body temperature), biochemical (e.g. cortisol, catecholamines, lactate, creatine kinase) and behavioural (video observations) indicators (BROOM, 2003).

Loading and unloading: In **Figure 1** the mean of the heart rate responses of 12 bulls on a journey of 60 h is shown. High heart frequencies can indicate a status of reduced welfare. High heart rates of more than 150 bpm (beats per minute) were observed during loading and unloading, weighing, shortly after the start of the transport and during blood sampling. During the transport journey the heart rate slowed down to 70 and 80 bpm on the average (MARAHRENS et al., 2003). Therefore the repeated unloading and loading during long distance transports should be avoided. This will also help to reduce the incidence of transport injuries which frequently happen when loading and unloading. Resting, feeding and watering should take place on the vehicle in properly equipped supply stations. It seems to be more adequate to realise welfare than stressful loading procedures.

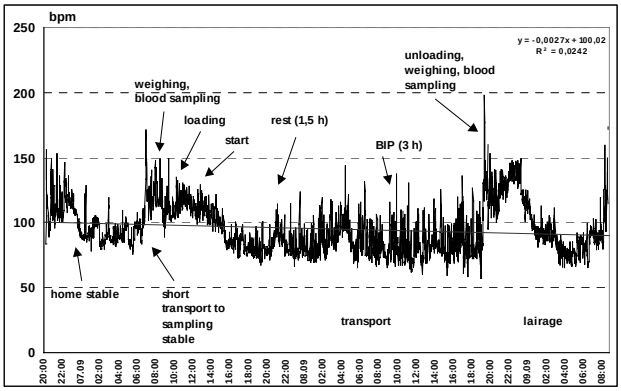

Fig. 1: Means heart rate curve of 12 bulls over 60 hours in home pen, during short term and during long distance transport (from MARAHRENS et al., 2003)

Cortisol is one of the often measured stress indicators. In **Figure 2** cortisol levels in the blood plasma of bulls, steers and heifers before and after transport and after resting time at lairage are given. During collection, weighing and loading the cortisol concentrations increased in the blood of the bulls and steers by a factor of 4 to 5 (40 – 50 ng/ml) above basal values (less than 10 ng/ml in the blood plasma of the male cattle). In heifers cortisol increased by a factor of two only. Steers are usually reared on pastures where they have sufficient physical exercise but not much contact to unknown

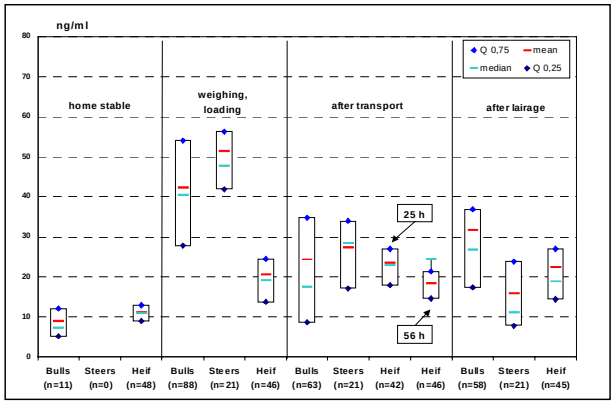

Fig. 2: Cortisol (50 % confidence interval, median, mean) in blood plasma of bulls, steers and heifers before and after transport and after resting time in the lairage

people and strange situations such as handling, weighing and loading. It seems that the increase of cortisol may be an indication of an emotional stress reaction rather than a

physical one. This is supported by the fact that the heart rates were relatively low during loading compared to bulls and heifers (MARAHRENS, 2003, data not shown). After transport and after lairage time the cortisol levels in bulls and steers decreased distinctly. In some steers the concentrations were back to normal ranges. The smallest increase in cortisol was seen in heifers. It looks that heifers become acquainted to the transport with the length of the journey as far as cortisol is concerned. After a transport of 56 h the concentration was lower than after a 25 h transport. The bulls showed the highest concentrations at all sampling points. The high values at the beginning may also be due to the fact that they were transported for 1 to 2 hours from the home farm to the
sampling and loading point in small vehicles.

Resting times: Transport is always a burden and can be energy consuming for the animals. The resting times are designed to give sufficient time to recover. In **Figure 3** the plasma concentrations of non-esterified fatty acids (NEFA) of bulls, steers and heifers before and after a long distance road transport and after the 24 h resting period are shown. NEFA is an indicator of lipomobilisation. In the home pen and during collection, weighing and loading the plasma levels of NEFA in bulls and steers reach about 400 µmol/l, in many of the heifers 700 and 800 µmol/l. In cattle, concentrations of NEFA up to 600 µmol/l are considered "normal". The lipomobilisation in the heifers is distinctly higher. The differences may reflect different feeding regimes in the home farm or high energy expenditure or an insufficient supply during the transport. Fattening bulls are usually fed ad libitum, their energy supply is high and their fat tissue is involved in energy metabolism only to a small

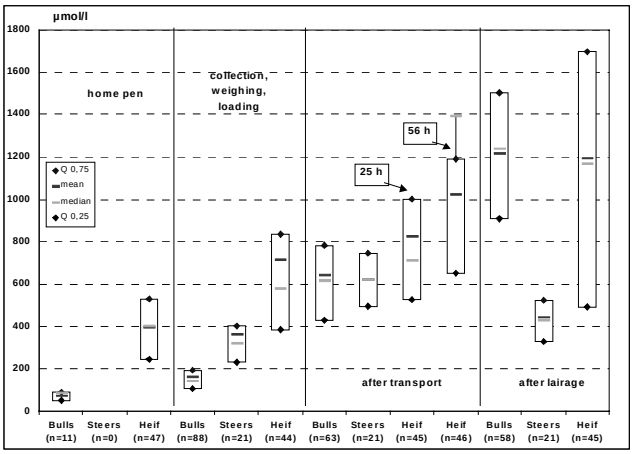

Fig. 3: NEFA (50 % confidence interval, median, mean) in blood plasma of bulls, steers and heifers before and after transport and after resting time in the lairage

extent. The same is more or less true for steers. Pregnant heifers, however, are fed restrictively, especially in the last months of pregnancy, to avoid parturition paresis. In this case the energy reserves (carbohydrates) in the muscles are low and more energy is mobilised from the fat tissues. The longer the journey lasts the higher is the mobilisation rate in heifers. The bulls and steers seem to have quickly available sufficient energy reserves. The picture changes after the resting period of 24 h. Some of the heifers recover but a large number tends to a ketotic metabolism which is also confirmed by high plasma levels of ß-hydroxybutyrate (MARAHRENS et al., 2003, data not shown). Similar high energy expenditure is observed in bulls when kept in resting areas for 24 h without mounting prevention. The steers seem to be able to recover quickly. It was observed that, in contrast to the bulls, they calmed down and consumed roughage and water soon after penned in the lairage.

Conclusions and Recommendations

These few results show that transport of cattle need not to be a serious stressful experience for the animals if the nature and needs of the animals are sufficiently taken into account. For this purpose the present transport directive should be amended according to the needs of the animals.

Loading and unloading in staging posts is stressful for all cattle and should be abolished. During the transport journey stress indicators such as heart frequency are tending towards normal values. However, the welfare can become poorer as journey length increases particularly when the food energy supply is insufficient. Heifers in particular tend to develop an energy deficit in long distance transports. Therefore, it seems useful to supply some energy rich feed during the breaks. For this purpose, the resting time after the first 14 h transport period should be extended from 1 to at least 3 h to give sufficient time for feeding and watering.

For bulls transport is less stressful than resting in lairages when fighting and mounting cannot be avoided. In that case, bulls should be transported as gently and as fast as possible to their destination avoiding long breaks.

Steers recover very quickly after transport when given the opportunity to eat.

The welfare of bulls, steers and heifers is limited by their needs not by a fixed maximum transport time, if vehicle and transport conditions are appropriate. The adaptation of transport schemes to the needs of the animals is necessary. Further improvements should also include an intensified education of handlers and drivers in animal welfare (pay for gentle driving not for speeding), better monitor systems for driving conditions, climate etc. and a more comfortable suspension and vehicle body design.

Last but not least is it necessary to inform the public about the progress made in animal friendly transport schemes and ask for their support.

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TRANSPORT AND OTHER POTENTIAL STRESS FACTORS AT SLAUGHTER: EFFECTS OF GENETIC AND REARING BACKGROUND.

Studies on pigs

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Introduction

The slaughter practice involves several potentially stressful procedures, such as mixing of pigs, transport, manipulation and food restriction. Stress reactions at slaughter may be due to physical (food restriction; fatigue; pain due to slaps, shocks or fights) and psychological discomfort (disruption of the social group; fear, due to novelty and human presence). Behavioural stress reactions involve running or on the contrary immobilisation of the animals, making moving them more difficult. Physiological reactions involve increase in heart rate and secretion of stress hormones such as cortisol and catecholamines (adrenaline and noradrenaline).

Meat quality is influenced by behavioural and physiological status of the animals before slaughter. Meat quality depends on speed and amplitude of post-mortem muscle pH decline. Amplitude depends mainly on muscle glycogen reserves, speed on muscle metabolic activity, mainly ATPase activity, at slaughter (4). Physical effort increases muscle metabolism, leading to net glycogen loss. Physical effort and psychological stress increase secretion of hormones that exacerbate effects of muscular activity on muscular glycogen depletion (1,2).

The way the pig responds to mixing, transport and slaughter stress in terms of physiology and behaviour, is influenced by its genetic background and earlier experience. Various experiments have been conducted to assess responses of pigs of different background to different stages of the slaughter procedure.

Material and Methods

Exp. 1. *Effect of loading, transport and unloading on adrenaline and/or heart rate of pigs of different genetic background.* Part I. Thirteen Large White and 12 Duroc crossbreeds (LW x LR dams, PIC sires) were reared indoors (6.3 m^2 pens with slatted floors) or outdoors (600 m^2 fields with huts) in a 2 x 2 factorial design. At 110 kg, pigs were slaughtered in an abattoir on the farm, after 24 h of food withdrawal and 10 min transport. Heart rate was measured for 10 min in the home pens and huts, and subsequently during loading (3 min), transport (5 min) and waiting (10 to 30 min) until slaughter (no unloading occurred during this experiment). Post-slaughter, technological meat quality measurements (pH, temperature, colour, glycogen and its metabolites contents) were taken (15, 45 min, 24 h post-mortem) on *Longissimus lumborum* (LL) and *Semimembranosus* (SM) muscles. Part II. Forty-eight pigs were reared individually in 1.5 x 1.5 m straw-bedded pens. Sixteen of them were Pietrain pigs, heterozygous for the halothane gene (Nn), the remaining 32 were homozygous non-carriers of the halothane gene: 16 of these were Large White and 16 Pietrain pigs. Urine was collected at spontaneous micturition, 1 wk before slaughter, in the morning while the pigs woke up. At 110 kg, half of each genetic type was mixed overnight, and the following morning transported for 3 h, then physically restrained for 1 min and immediately slaughtered (high stress). The other pigs remained in their home pens. The next morning they were individually transported for 7 min and immediately slaughtered upon arrival (low stress). For all pigs, duration of food withdrawal until slaughter was 20 h. Heart rate was measured over the 3 h (low stress group) or 30 min (high stress group) preceding slaughter. Post-slaughter, urine was collected for catecholamine assays, and various technological meat quality measurements (pH, temperature, colour, contents of glycogen and its metabolites) were taken (1, 45 min, 24 h post-mortem) on LL, *Adductor femoris* (AF) and SM muscles.

Exp. 2. Effect of handling training on reactivity at slaughter; consequences for meat quality. Forty-two Large White pigs were reared in groups of 7 pigs in straw-bedded pens. Pigs received different handling treatments for 40 d until slaughter age. Fourteen pigs were assigned to the Human Interaction group (HI), 14 pigs to the Refusal of Contact group (RC), 14 pigs to the control group. Pigs of the groups Human Interaction (HI) and Refusal of Contact (RC) were individually introduced into a pen each day where they remained for 3 min in presence of a squatted handler. The handler tried to increase progressively physical reciprocal interactions with the HI pigs. In contrast, RC pigs were pushed away when they touched the handler. Control pigs remained in their home pens. Pigs were slaughtered in a commercial abattoir, either in presence or absence of their handler, after 16 h of food withdrawal and overnight mixing. Behaviour during mixing was recorded. Technological meat quality measurements (pH, temperature, glycogen and lactate contents) were taken (1, 45 min, 24 h post-mortem) on LL, SM and *Semispinalis capitis.*

Exp. 3. Effect of breed on reactivity to stress and consequences for meat quality. Twenty-one pure-bred Durocs and 21 pure-bred Large White pigs were reared in groups of 7 pigs in straw-bedded pens. Over the 2-months-period preceding slaughter, pigs were subjected to two tests: exposure to a non-familiar object (traffic cone) and exposure to a non-familiar human, each lasting a total of 20 min, including 10 min habituation to the test situation. Behavioural reactions and heart rate were recorded. Order of testing was organised in a balanced design. At 110 kg, pigs were slaughtered, either after 34 h of food withdrawal, 5 h of mixing, 3 h of transport and 12 h of lairage (high stress) or after 14 h of food withdrawal and only 15 min of transport (no mixing: individual slaughter; low stress). Technological meat quality measurements (pH, temperature, colour, glycogen and lactate contents) were taken (1, 45 min, 24 h post-mortem) on LL, *Biceps femoris (*BF*)*, and AF.

Results

Exp. 1, Part I. Pre-loading heart rate was 109.9 ± 2.4 beats per minute (bpm). It rose to 153. 7 ± 3.6 during

loading, then reduced to 133.0 ± 2.3 and 121.2 ± 2.6 during transport and waiting, respectively ($p<0.0001$). Duroc and Large White pigs showed similar tendencies ($p=0.15$) as did indoor and outdoor reared pigs ($p=0.45$). No correlations were found between heart rate responses and meat quality data. *Exp. 1, Part II*. Resting heart rate in the home pen was 89.1 ± 2.8 beats per min. Loading and unloading were associated with significant increases (e.g. unmixed pigs: 176.5 ± 3.2 and 175.6 ± 4.8, for loading and unloading, respectively) with an average peak value of 210.2 ± 4.0 bpm. Genotype did not have any effect. Heart rate was high during initial transport (unmixed pigs, 7 min of transport: 137.1 ± 3.4 bpm). At the end of 3 h transport heart rate was reduced to 121.8 ± 3.6, which is significantly lower than initial ($p<0.05$) but still much higher than resting values ($p<0.0001$). Urinary adrenaline was higher after slaughter compared to resting levels in the home pen ($p=0.06$). Only Pietrain Nn pigs showed a stronger increase after high stress than after low stress slaughter conditions ($p<0.05$). For pigs of the high stress group, heart rate during the 1-min physical restraint was negatively correlated with initial pH of the LL ($r=-0.46$; $p<0.05$). Post-mortem glycogen contents were negatively correlated with post-mortem urinary adrenaline content (e.g. glycogen immediately after slaughter, $r=-0.60$; $p<0.01$). *Exp. 2.* Prior handling experience did not in itself influence ultimate meat quality, but the presence of the negative handler (RC pigs) at slaughter caused lower pre-slaughter LL glycogen content. Fighting behaviour during mixing explained between 14 and 52 % of the variability of lightness of the LL, BF and SM ($p<0.05$). Multiple regression analyses including visual contact with the handler at the start of the handling training and number of fights initiated during mixing explained between 31 and 42 % of the variability of ultimate pH of the studied muscles ($p<0.05$). *Exp. 3.* Durocs touched the person significantly more often than Large Whites ($p<0.01$). Frequency of contact and heart rate were positively correlated for Durocs ($r=0.48$; $p<0.05$) and Large Whites ($r=0.61$; $p<0.01$), explaining higher heart rates of Durocs ($p<0.02$). No differences were found for frequency to touch the novel object and associated heart rate ($p=0.68$). Breed and slaughter effects were significant ($p<0.05$) for ultimate pH and meat colour for most muscles, and drip loss (LL). Breed x slaughter condition interactions showed that slaughter effects were mostly due to the larger sensitivity of muscles of the Large Whites to slaughter conditions. For Large Whites slaughtered in the industrial plant, ultimate pH of AF, BF and SM muscles were significantly higher compared to experimentally slaughtered Large Whites ($p<0.05$). Yellowness scores of AF, SM and LL of these same animals were lower ($p<0.05$). For Durocs, ultimate pH and colour of these same muscles were not influenced by slaughter conditions. For Large Whites of the high stress slaughter group, a negative correlation was found between frequency of touching the human during the test, and initial LL and BF temperature (e.g. LL: $r=-0.86$; $p<0.01$).

Discussion

Loading, transport and unloading caused significant increases in heart rate that are similar for pigs of different rearing and genetic background. The mere manipulation and 10 min transport of pigs caused increases in urinary adrenaline similar to overnight mixing and 3 h transport for non carriers of the halothane gene. Pietrains heterozygous for the gene showed an increased adrenaline response to these high stress slaughter conditions. Presence of the halothane gene appears thus to influence catecholamine secretion as earlier suggested (3). Adrenaline production and heart rate are both under the control of the autonomous nervous system. Their correlations with early post-mortem muscle metabolic activity illustrates that its activity during the hours or minutes preceding slaughter may have measurable consequences for meat quality.

Positive and mildly negative handling training modified behaviour towards the handler (5), but this had only a small effect on pre-slaughter glycogen meat metabolism and only if the negative handler was present during slaughter. In contrast, tendency to fight with other pigs and pre-training reactivity to humans determine part of the variability in ultimate pH and meat lightness.

Duroc and Large White pigs evaluated differently presence of man. The results show that Durocs were less fearful and/or were more motivated to touch the person. The meat quality results may indicate that in contrast to Large Whites, behavioural and physiological status of Durocs was little influenced by slaughter conditions. However, the stress reactivity tests had found a similar (non-familiar object) or increased reactivity (human exposure) of Durocs. It is therefore likely that Durocs did respond behaviourally and physiologically to slaughter conditions, but that these responses had little effect on post-mortem muscle metabolism. Increased approach to humans during the test was associated with higher pre-slaughter metabolism, but only for Large Whites. The results suggest therefore, that the impact of stress responses on meat quality is breed dependent.

Conclusion

At slaughter, loading and transport, fighting during mixing and reactivity to humans caused physiological and metabolic changes that explain part of the variability in pork. In the above studies, breeds did not influence heart rate and adrenaline responses to loading and transport, but did influence reactivity to humans, and its correlation with post-mortem metabolism. Although handling experience modified behaviour towards the handler, correlations between reactivity to humans and meat quality were not influenced by prior handling training.

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EFFECTIVE LOGISTICS TO IMPROVE ANIMAL WELFARE IN THE PRODUCTION CHAIN, WITH SPECIAL EMPHASIS ON FARM-ABATTOIR SYSTEM

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Introduction

In relation to global marketing system and structural adjustment, transport of animals for slaughter and breeding is increasing. During transport and handling animals are subjected to unfavorable conditions that compromises their welfare and the meat quality. Beside this, spread of infectious disease and the associated environmental questions are becoming the main societal concern. Therefore, eeffective logistics and control system could be the key issue for the improvement of welfare, meat quality and environment.

The objectives of the current work were to map out the logistics system of meat production chain and develop a system to monitor the environmental conditions in the vehicle.

Material and Methods

The logistics chain mapping study was based on interviews, measurements and observations of activities during animal transport and slaughter operations. Detailed data collection was made at four levels; (a) truck driver interviews, (b) transport route on-board activity registration,´(c) delivery point activity registration (including vehicle and animal activities), and (d) slaughter chain activity registration.

The registered data were analysed to determine the impact of frequency of arrival and duration of activities, on queues, capacity utilisation and other system constraints. 22 routes were registered, all with start and stop at the abattoir. The routes were the second or third route of the day for the drivers involved.

Development of data collection and data transfer system: The development was made at two phases. In the first phase, the data logging was made using a PC system. In the second phase, sensors, data logging and data transfer is made using one unit. The unit is developed in cooperation with a company that may produce the unit for commercial purposes in the future. Data collection and data transfer experiments were made using the unit. The measured and transferred parameters were environmental conditions (temperature, relative humidity, and vibration) in the loading compartments of vehicles, geographical positions (GPS) and speed of vehicles and transferred data continuously to stationary or mobile database stations using GSM.

Results

Description of operation: The logistics chain was divided into four components: (a) Ordering and planning cycle, (b) Vehicle route activities, (c) Animal reception activities at the abattoir (unloading, lairage), (d) Slaughter chain activities (from stunning to cooling room)

Planning:. Cattle were delivered to slaughter for three reasons; slaughter weight reached for meat production cattle, milking cows removed from production due to lameness and other diseases, or the entire production at a farm closed down**.** The detailed planning of transport operations including scheduling and transport routes was done by transport operators after receiving order from the abattoir on a weekly basis. The suppliers and the animals to be delivered were scheduled at the abattoir in order to meet the market demand and production capacity, and the arrival of each delivery preliminary scheduled.

Documentation on the identity of animals, and transport operation, followed each delivery, to enable traceability in the meat production chain and to be used as the basis of payment to producer and transporter. All documentations were carried on paper, although all animals were already registered in the meat cooperative's database of production. Changes in time of arrival and loaded animals were communicated by telephone between driver and abattoir.

Vehicle (DL1 - Transport activity chain (vs abattoir activity chain) activity chain: According to the drivers, transport between farms was limited and the abattoir was the destination of 98% of their transport routes. The working day started in the early morning, at around 5 a.m. and involved 1-3 routes. If routes could not be finished within normal working hours, the driver was replaced during the route. Collection routes normally involved loading at 5 (from 1 to 9 occurred) farms, 3 (1–15) cattle at each farm.

Loading at farms normally required slightly less than 30 min per farm, with variations from 10 to 180 min. Major factors determining loading times were (in order of rated importance): vehicle design, drivers' behaviour, number of animals, farmer's behaviour, farm design and penning system, and the animals' reactions. According to observations of 22 routes involving 90 collection stops at farms, the effective loading time was 13 min, while the time required for preparations before and after loading (i.e. parking and preparing the vehicle and contacting the farmer), was 7 min. Drivers' estimations of unloading time at the abattoir, when there were no queues, varied between 15-60 min, with an average of 24 min. The drivers interviewed regarded queues as a principal problem of current transport operations. Queues were said to occur at around 20%, or less, of the deliveries at the abattoir, and likewise before vehicle wash. Waiting times in case of queues, were estimated by the drivers to on average 22 min (max 80) before unloading and 30 min (max 80) before washing. Important factors behind the build-up of queues were said to be that vehicles arrived before or after the assigned/expected arrival time, and that the limited stable capacity of 350 pigs; marginally more than one vehicle load. Except for queues, major factors determining the unloading time were, according to the

drivers (in order of rated importance): abattoir design, behaviour of abattoir staffs and driver, vehicle design and the animals' reactions. After unloading, washing the vehicle took 60 min (30-150). Observations of unloading included 59 deliveries (during observation at the abattoir and some of the route observations). The duration of unloading, including waiting time and preparation, varied between 7 to 98 minutes, with an average of 23 minutes. The effective unloading time (waiting time and preparation excluded) was 17 min, distributed as indicated in Figure 3.19. Waiting and preparations before unloading took on average 6 min, but delays of up to 85 min were observed.

Route optimisation: The average route registered involved 4 collection points, 2:58 hours driving time and a total distance of 161,5 km. Departure time varied from 8:22 to 12:00, and arrival time at the abattoir from 11:30 to 19:02. The differences between registered and calculated driving times for the 19 routes were approximately normal distributed around an average of 6.4% of registered driving time. The analysis revealed potential savings for individual routes of up to 23%.

Animals' activity chain at the abattoir: After the unloading the cattle were moved by the abattoir's staffs, either directly to the stunning station, or to lairage boxes. Moving cattle after unloading at the abattoir, from the vehicle to a lairage box, normally took about 30 seconds, but observations of up to 4 minutes were made. The mean value from observations of movement was 1.3 minutes.
The waiting times in the lairage box for 55 observed animals were on average 45 min. For 15 of the same animals (27%), lairage time exceeded 1 hour (maximum was 2.2 hours). During a technical breakdown in the slaughter chain (which was considered a very exceptional incident), which temporarily stopped all production, lairage times for 17 observed animals increased to on average 3.8 hours; minimum 2.2 hours and maximum 6.5 hours was observed.

Data recording and transfer system: Continuous recording and data transfer from vehicles during transport to a stationary database is important for information monitoring system for surveillance of animal welfare. In Sweden, development of the recording and transfer of data system has been made at two phases. The first phase system has been developed to record relevant parameters and transfer to the stationary database using GSM-system. An instrumentation system was developed to carry out the measurements of the parameters mentioned earlier and additional parameters simultaneously and continuously during transport from the farms to the abattoir. The instrumentation may be classified into four groups. Instrumentation for measuring: animal behaviour (digital video), heart rate, transport route, geographical location, vibration sensors mounted both on vehicle and animals, climatic conditions (temperature and humidity), emissions, and information transmission from vehicle to stationary database.
All instrumentation groups were monitored using on-board portable computers from the cabin of the vehicle.
A compact unit has been manufactured by Mobitron for monitoring of environmental data . The unit system is composed of measuring sensors for temperature; relative humidity and vibration, storage, and data analysis and data transfer to mobile or stationary stations. The measurements are initiated and analysed by means of a PC / Windows program. The user can easily, by menu run procedures, start the measurements and then read and evaluate the measuring results. Once carried out, the measurements can easily be analysed for every minute of the measuring period.

Discussion
Frequent queues, at the abattoir's delivery point and especially at the vehicle wash facility, was one of the major problems reported by the drivers. At times, queues extended unloading and washing with more than one hour. Queues at the abattoir's delivery point frequently cause problems for drivers and for the abattoir staffs, creating a stressful work environment, which could also negatively affect the animals' conditions. Another problem was the extended waiting times for animals before slaughter. Animals were frequently kept for more than one hour and occasionally more than two hours in the lairage box. In order to effectively utilise the conveyed slaughter chain's capacity, a smooth flow of animals is essential. Uneven supply to the chain would result in costly idle times transplanted throughout the slaughter chain, and/or queues of animals waiting for slaughter. In the observed logistics chain, the lairage box functioned as a buffer, in order to ensure continuous supply to utilise slaughter capacity. The uneven distribution of arrivals of deliveries affects the handling of animals at the delivery point.
The smart system that has been developed for measuring, storing and transfer of data performed satisfactory. However, further development is required to include video pictures, and to improve the speed of wireless data transmission from the moving in the field to any mobile or stationary stations.

Conclusion
Effective logistics could be the major key solutions to reduce stress inducing factors emanating from road conditions, transport time, environmental conditions in the vehicles, stops related to traffic conditions, queuing at the abattoirs, at border controls etc. Queues at the abattoir's delivery point frequently cause problems for drivers and for the abattoir staffs, creating a stressful work environment, which could also negatively affect the animals' conditions. With the surveillance system it could be possible to trace all animals from the farm to the abattoir while animal welfare can be monitored by measuring transport performance and animal conditions gives an additional guarantee to the consumer.

Acknowledgements
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AN INVESTIGATIVE STUDY OF 2 PIG ABATTOIRS IN SWEDEN WITH REGARD TO $C0_2$ CONCENTRATION, $C0_2$ EXPOSURE TIME, STUN GROUP SIZE, STUN TO STICK INTERVAL, AND STUN EFFECT.

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Introduction

Preslaughter stunning has the prime aim to ensure the animal is insensible to exsanguination and post slaughter procedures. The stun itself should be painless, close to instantaneous in its effect, and should provide a duration of insensibility which ensures that death from subsequent slaughter intervenes before recovery of sensibility (Cook et al., 1999).

The Swedish Board of Agriculture have set down regulations for $C0_2$ stunning of pigs in abattoirs, and stipulate that the time interval between the last $C0_2$ exposure and exsanguination (stun to stick interval) should be within 60 seconds. These regulations are based on EU guidelines made in 1991 for $C0_2$ stunning in abattoirs.

In recent years group CO_2 stunning procedures have been implemented in Sweden based on Danish designs. The concept is comprised of three main elements:

- an area where groups of approximately 20 pigs are divided into smaller groups
- automatic transfer of these smaller groups to and through the stunning equipment utilizing their flock behaviour
- a system for presenting the stunned pigs for shackling and sticking.

After the end of $C0_2$ exposure under some conditions pigs can begin to regain consciousness and can recover completely (Forslid, 1987; Holst,.1998). Also, due to biological variations, some pigs will never recover from the stunning conditions while others will be reversibly stunned and show indications of regaining consciousness within a given time after the end of the $C0_2$ exposure. Therefore to ensure good animal welfare during post stun handling of pigs, $C0_2$ stunning should always induce unconsciousness of a sufficient duration which should include not only the stun stun-stick interval but also the time taken for the animal to become insensible due to debleeding (Holst, 1999). According to EU regulations (Council Directive 93/119/EC) pigs must be exposed to $C0_2$ for long enough to ensure they remain unconscious until they have been killed. Furthermore, bleeding (sticking) must be started as soon as possible after stunning, and in any event carried out before the animal regains consciousness.

To ensure good stunning practice under $C0_2$ stunning i.e. no pigs regain consciousness during post-stun handling and bleeding, the safe depth of anaesthesia at the time of sticking can be evaluated by the following criteria:

No pigs shall show deep or regular respiration except for irregular abdominal gasping

No pigs shall show signs of excitation or kicking apart from for slow movements of legs

No pigs shall have spontaneous blinking of the eye

Maximum of 5% pigs can have corneal reflex

Corneal reflex is a good criterion for assessing consciousness because when there is no corneal reflex, it indicates deep anaesthesia or death of the animal (Holst, 1999).

Aim

This study aimed to investigate in 2 different $C0_2$ group-stunning abattoirs (A and B):

Actual group size in the stun boxes in abattoirs A and B

Actual stun to stick time intervals for each pig in the stun groups

Stun effect when stun boxes are in $C0_2$ concentrations not less than 70% in the first stop and not less than 90% in the bottom stop at exposure duration of 210 seconds in abattoir A and 150 seconds in abattoir B.

Material and Method

Observational studies and data recordings were made at 2 abattoirs A and B for stun group size, stun to stick interval and stun effect. These parameters were recorded for 502 stun groups (3444 pigs) over a 2 day period for abattoir A, and 553 stun groups (2325 pigs) over a 3 day period for abattoir B. The results for each abattoir were analysed separately.

The stun effect was assessed for every stun group during the study period starting after the 2nd pig of each group. This was done by a person trained and experienced in assessing pigs for consciousness. Any information indicating poor stunning was recorded. When there were stops in the slaughter line and the last pigs in a stun group had extended stun to stick intervals, extra care was taken to check for signs on these pigs for regaining conciousness. The same person conducted all consciousness assessments throughout the study.

Results

Abattoir A: In abattoir A the stun group sizes varied between 3 and 10 pigs, with majority between 6 and 8. The most common stun group size however was 7. The rotation times for the boxes to pass through the stunning system varied. Thus the $C0_2$ gas exposure times for each group of pigs in the stunning system varied. The $C0_2$ exposure times for stun groups were estimated from 46 box rotation times and making a calculation to estimate the $C0_2$ gas exposure times when pigs were in $C0_2$ gas concentrations above 70%. The average $C0_2$ exposure was estimated at 282 seconds. There were no pigs out of a total of 3444 that showed signs of regaining conciousness.

Abattoir B: In abattoir – B the stun group sizes varied between 2 and 6 pigs with the majority between 4 and 5. The most common stun group size however was 4. 79% of all pigs in the study were stuck after 60 seconds, i.e. 21% with a stun to stick interval within 60 seconds. The fastest stun to stick interval was 41 seconds and the longest 145 seconds. The $C0_2$ exposure times for stun groups were estimated from 28 box rotation times and making a calculation to estimate the $C0_2$ gas exposure times when pigs were in $C0_2$ gas concentrations above 70%. The average $C0_2$ exposure was estimated at 238 seconds. There was one pig out of a total of 2325 pigs that showed spontaneous blinking of the eye and corneal reflex.

Discussion

This study investigated stunning procedures in 2 different abattoirs with similar $C0_2$ pig group stunning practices. In total, 5769 pigs and 1055 stun groups were observed for the purposes of assessing animal welfare after stunning. The stunning procedure was observed to assess how good the stunning practice was overall. The stunning systems varied in that abattoir A operated with 7 boxes rotating through a $C0_2$ chamber 10 meters deep. Abattoir B operated with 6 stunning boxes rotating through a $C0_2$ chamber 9 meters deep. The maximum slaughter capacity for abattoir A is 720 pigs per hour, and in abattoir B, the slaughter line has the capacity for 300 pigs per hour.

In both abattoirs the $C0_2$ gas concentrations exceeded the minimum recommended by the manufacturer of the system i.e. 70% at the upper level, and 90% at the lower level. In both abattoirs the $C0_2$ gas concentrations exceeded the minimum recommended by the manufacturer of the system (EU legislation requires a minimum of 70 %). In abattoir A the average $C0_2$ exposure time was 286 seconds, and the minimum time 218 seconds. In abattoir B the average $C0_2$ exposure time was 238 seconds, and the minimum time was 193 seconds. There is variation in stun exposure times and box rotation times due to factors that occur during the division of the immediate preslaughter groups.

The stun to stick intervals for each pig in a stun group varied by 20 to 30 seconds for both abattoirs. Stun to stick intervals varied due to many factors other than group size. Stops occurring in the systems caused most of the delays in sticking time. In abattoir A stops occured due to the derailment of shackles holding pigs as they were conveyed around a hook bend just before being stuck. Occasionally shackled pigs were derailed completely and fell off the line onto the abattoir floor before sticking. There were also quite a few stops just after sticking where shackled pigs passed through a narrow gap between 2 walls. However most causes of the stops could not be seen, as they occurred far down the slaughter line past sticking.

The stun effect in this study was considered to be 100% effective in abattoir A and B. One pig (abattoir B) of a total of 5769 pigs (0.043%) stunned showed corneal reflex and spontaneous blinking of the eye. It was the 3rd pig in a group of 4. The stun to stick interval was 80 seconds, which was the average interval for every 3rd pig of a group for this abattoir and it showed no other symptoms.

Conclusions

The results of this study have shown that the minimum to maximum stun to stick intervals are 46 to 160 seconds for abattoir A, and 41 to129 seconds for abattoir B and that these intervals allow for an acceptable stunning. Therefore the recommended stun to stick interval can be reviewed.

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A SHORT OVERVIEW OF THE WELFARE IMPLICATIONS OF PRE-SLAUGHTER STUNNING IN POULTRY

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Introduction

In poultry, as in any other animal species, it is a legal requirement that the animals must be rendered unconscious, *i.e.* insensible to pain, before cutting the neck vessels and remain so until death supervenes from blood loss. From an ethical point of view, the following conditions are required : i) the induction of insensibility must be effective in all the animals, ii) the induction of insensibility must be painless and iii) the duration of insensibility must be long enough to allow the birds dying from bleeding without recovering consciousness.

Appropriate neurophysiological measurements, *i.e.* electroencephalogram (EEG) recordings, must be used for an objective and unequivocal assessment of stunning efficiency. In red meat species, the induction of 'grand mal epilepsy' in the brain, is recognised as a sign of stunning-induced unconsciousness (for a review see Raj, 2003). In poultry however, the changes in EEG following the application of a stunning current differ from those observed in mammals. Abolition of evoked potentials in the brain has been therefore used as an unequivocal indicator of unconsciousness (loss of brain function) in poultry (Raj & O'Callaghan, 2001; Raj et al., 1992). We recently showed that the power spectra of the EEG, obtained by Fast Fourier Transformation could be used as an indicator of stunning efficiency in ducks (Beyssen *et al.*, 2004).

From a technological point of view, stunning aims to reduce birds movements in order to facilitate bleeding, especially on automatic slaughter lines. In addition, the reduction of birds movements during bleeding, including wing flapping, reduces the incidence of carcass appearance defects.

Electrical stunning and Controlled Atmosphere Stunning (CAS) are the two main methods used in poultry slaughter plants. In the following, the ethical implications of both methods will be shortly discussed.

Stunning or stunning/killing?

The interest of killing the animals by the stunning method is still a matter of debate. From the point of view of animal protection, killing the animal at this point is the best way to ensure that it will not recover consciousness during bleeding. This aspect is particularly relevant to gas stunning since Raj & Gregory (1990) stated that the return of consciousness after a non-lethal gas stunning was by far to quick to ensure that birds would die from bleeding while being still unconscious.

Electrical stunning

Electrical stunning in a water-bath is the most common method in Europe and was until recently, in France, the only technique encountered in commercial slaughter plants. This method is based on the application of a current flow through the body of the birds which are hanged head-downwards on a moving shackle. The systems are designed to flow from the live water electrode to earth trough the bird. Research works carried out under controlled laboratory conditions and based on EEG recordings have allowed to determine the minimum current intensity required to induce an efficient stun in various poultry species (table 1).

Table 1- Minimum currents required to induce an efficient stun in the water-bath stunning system

Species	Minimum current (mA)
Turkey	150
Chicken	100
Goose	130
Duck	130
Quail	50

It is important to note that these recommendations correspond to the values which must be applied to individual birds. Under practical situations, several birds are present simultaneously in the water-bath and generate a parallel resistances pathway. A very rough estimation would lead to the fact that the intensity of the current delivered on the circuit must be at least equal to the minimum current required/bird x number of birds present simultaneously in the water-bath. This is however not sufficient to ensure that each bird receives the accurate amount of current (Wotton & Gregory, 1991). The recommendations are actually based on the most common current waveform commercially available : a sinusoidal alternating current (AC) at 50 Hz. In turkey, we demonstrated that the application of a 50 Hz, 150 mA AC induced cardiac arrest in 100 % of the birds. Increasing the current frequency from 50 to 600 Hz decreased both the incidence of cardiac arrest (0 % at 600 Hz) and the duration of stunning-induced unconsciousness (Mouchonière *et al.*, 2000). Therefore, the minimum current required to stun the birds need to be re-evaluated for each current frequency. Apart from the difficulty to ensure under practical situations that all the birds receive an adequate current, electrical stunning in a water-bath poses some other welfare problems:

- this method implies the shackling of conscious birds head downwards, and this may last several minutes in poorly designed slaughter plants,
- when turkeys are shackled head downwards, their wings hang lower than the head and may therefore touch the water-bath first, thus leading to a painful electric shock. Because of anatomical differences, this problem is much less important in broiler chickens,
- some birds may lift up their head and thus avoid the water-bath. In that case, they may be fully conscious at time of neck cutting.

Gas stunning

The use of modified or controlled atmospheres for stunning birds (CAS) has continually developed since the

end of the 1980s. A great deal of laboratory work has been carried out mainly in England and in the Netherlands. CAS is considered as an alternative to electrical stunning since it is thought to eliminate the welfare and meat quality problems encountered with the water-bath method. One of its most important advantage is that it avoids shackling conscious birds, either they are stunned in their transport crates or on a supply conveyor. This is an obvious advantage for bird welfare but also for the welfare of the staff involved in shackling. In practice, only mixtures of gases that occur naturally in air, such as carbon dioxide (CO_2), nitrogen (N_2), oxygen (O_2), and argon (Ar), are used in different combinations and proportions. Different CAS methods can be identified (Barton-Gade *et al.*, 2001) :

- anoxia by displacing air with an inert gas such as Ar,
- combined effects of anoxia (Ar) and anaesthetic effect of CO_2 (hypercapnic anoxia),
- anaesthetic effect of CO_2 at high concentration in air (hypercapnic hypoxia)
- increased concentration of O_2 and CO_2 (hypercapnic hyperoxygenation),
- the bi-phase system use a first exposure to increased O_2 (30 %) and CO_2 (40 %) in air as an induction phase to reduce the aversiveness of CO_2, followed by a stunning/killing phase into high CO_2 concentration after the birds have lost consciousness in the CO_2/O_2 mixture.

Anoxia induces i) a depression of activity in the brain which extend progressively from the telencephalon to the mesencephalon, ii) a suppression of the rostral reticular formation leading to a loss of consciousness and iii), a suppression of the caudal reticular formation triggering the onset of convulsions (Lambooij & Pieterse, 1997). When exposed to high CO_2 concentrations, the saturation of tissues with CO_2 leads to an impairment of cell function which induces on the EEG a decrease in amplitude and frequency and a desynchronisation of activity, preceding an isoelectric state (Bauer, 1982).

Behavioural reactions of the birds when exposed to the various gas mixtures have been used to appreciate the aversiveness of the different atmospheres. When exposed to anoxia, hypercapnic anoxia or hypercapnic hypoxia, the behavioural reactions usually follow the pattern : gasps (light and/or severe), head shaking, wing flapping, convulsions and loss of posture. Based on these observations and on the EEG recordings, the fastest stunning is obtained with Ar/CO_2 mixture (Barton-Gade *et al.*, 2001 for a report of different studies). The behavioural reactions seems to be of lower intensity in the CO_2/O_2 mixture but in the other hand, the time to loss of consciousness is longer. Some of these results are shown in table 2. Whether the severe behavioural reactions seen during gas exposure (wing flapping and convulsions) are unequivocal signs of pain and distress is still not clear. Recently, Coenen *et al.* (2003) demonstrated that the signs of agitation and distress during exposure of chickens to oxygen/carbon dioxide conditions occurred at a time where consciousness could not be fully excluded from the EEG recordings. Under such circumstances, it seems more acceptable to promote a method where the behavioural reactions are the less severe. This is the case in the CO_2/O_2 mixture which induces unconsciousness within 60 s of exposure. Then, the aversive reactions to the following exposure to high CO_2 concentration (the second and 'finishing' step of the stunning/killing) are suppressed.

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Table 2 - Behavioural responses of broiler chickens to different gas mixtures (Barton-Gade et al., 2001)

	Number entering the room	Number with light gasps	Number with head shaking	Number with convulsions	Time to loss of posture (s)
Negative control	17	2	7	0	-
Positive control	18	2	18	0	-
> 90 % Ar	9	0	4	9	21
70% Ar + 30% CO_2	15	14	14	15	12
60 % CO_2	12	12	11	12	17
40% CO_2 + 30% O_2	19	19	19	1	30

Negative control = no air circulation; positive control = air circulated at the same rate as in the other gas mixtures

EUTHANASIA IN DANISH DAIRY HERDS

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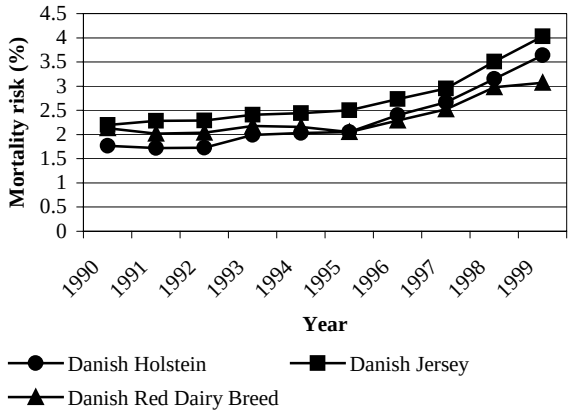
Introduction

Mortality among dairy cows constitutes a problem both in terms of financial losses (value of dead cows, decreased production and cost of replacement cows) and compromised animal welfare (suffering before unassisted death or euthanasia).

Mortality risk (unassisted death or euthanasia) among Danish dairy cows has increased from 2 % in 1990 to 3.5 % in 1999 (Figure 1).

Figure 1. Breed-specific mortality risk among Danish dairy cows 1990 – 1999 (7).



The proportion of euthanised cows among dead cows has been examined in a questionnaire survey among 196 Danish dairy farmers in 2002. Replies from the survey showed that 58 % of the dead dairy cows were euthanised. Furthermore, the replies indicated that the proportion of euthanised cows has increased in the past five years. More than half of the farmers stated, that they euthanise relatively more cows now than five years ago (7).

Decreasing average profits per cow, decreasing value of the individual cow, increasing labour costs and increasing veterinary expenses (4) might have affected the farmer's decision-making concerning treatment versus euthanasia. Thus, the farmer's interest in intensive treatment of seriously ill cows might have decreased, resulting in more euthanasia and a decrease in expensive treatments. The increase in mortality risk seen during the last decade may therefore predominantly be due to an increasing number of euthanised cows.

Results from the questionnaire survey indicated that 77 % of the euthanised cows was shot by the farmer or a veterinarian. 23 % were euthanised by an overdose of an anaesthetic. According to Danish legislation exsanguination of shot cattle is mandatory (2). Our objective was to study whether this exsanguination was conducted properly.

Materials and Methods

Cows and calves shot (penetrating captive bolt) were examined at an incineration plant, where the majority of dead Danish cattle (including all adult cattle) are processed. The animals were sampled by systematic random sampling. It was noted whether the animals were both shot and exsanguinated or shot without subsequent exsanguination.

Results

The study at the incineration plant showed that out of the examined cows that were shot only 24 % were also exsanguinated (95 % confidence interval: 8 – 40 %). Among calves shot only 4 % were exsanguinated (95 % confidence interval: 0 – 12 %).

Discussion

An increase in the number of cows dying unassisted constitutes an animal welfare problem (suffering before death). The situation concerning euthanasia is more complex. An increase in the number of euthanised cows might be due to an increase in the number of seriously ill cows. This situation also has negative impacts on animal welfare. If, on the other hand, the increase in the number of euthanised cows is not a consequence of increased morbidity, but caused by an altered threshold for euthanasia among farmers, it might have a positive impact on animal welfare. More seriously ill cows might be euthanised and thus not put through a (perhaps long) period of suffering associated with disease and treatment. Euthanasia has been defined as rapid, painless death (1,5,8). Euthanasia in itself is not an animal welfare problem, if it is performed quickly and without suffering for the cow. This might be accomplished by an overdose of an anaesthetic (e.g. a barbiturate) or by shooting (penetrating captive bolt) followed by exsanguination (1,3,8). Exsanguination subsequent to shooting is needed to ensure the death of the animal. Some authors do not emphasize this fact (e.g. (3)) whereas others do (e.g. (1,8)). However, Grandin (6) has shown that 1.2 % of shot cows and bulls returned to sensibility after shooting. Thus, shooting without subsequent exsanguinations is not an acceptable method of euthanasia. Failure to exsanguinate shot cows or calves constitutes a problem both legally and in relation to animal welfare (1,2,6,8).

Conclusion

We find euthanasia acceptable in relation to animal welfare if it is performed properly. However, in our study the proportion of shot cows and calves that were also exsanguinated were very low. Both veterinarians and farmers need to pay further attention to this problem in the future. An information campaign emphasizing the need for exsanguination might reduce the proportion of shot cattle, which are not exsanguinated.

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COMPARISON OF DIFFERENT PIG EUTHANASIA METHODS AVAILABLE TO THE FARMERS

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Introduction

Pig farmers are interested to learn euthanasia methods that are authorised in France and Europe and which they could adapt to their farms. The ITP has conducted a study aimed at evaluating the various methods legally authorised under European regulations (Directive 93/119/EC on the protection of animals at the time of slaughter or killing) for four categories of animal: sucking piglets, piglets from 8 to 25 kg, grower pigs, and sows.

Material and Methods

The possible euthanasia techniques that we have assessed include:
- electrical stunning (electrodes applied to the head for 5 seconds) followed by electrocution (electrodes applied to the heart for 15 seconds) for pigs over 25 kg (piglets from 8 to 25 kg, grower pigs, sows)
- prolonged exposure to 80% CO_2 gas for piglets below 8 kg
- captive or penetrating bolt pistol, either with or without sticking, for all 4 categories of pig
- trauma to the head (using a 0.5 kg to 1.5 kg hammer) for piglets below 25 kg and 8 kg.

Ten to 15 animals from each category were euthanized.

The target parameters for assessing the efficiency of the methods studied break down into pain assessment methods (vocalisation) and methods for evaluating state of anaesthesia and death (instantaneous and permanent collapse of the animal, immediate and permanent mydriasis, convulsion, limb reflex responses and lack of movement, measurement of heart beat and cardiac arrest, corneal reflex, urination and defecation, and gaps or spasm). The observations were carried out every 30 seconds from the beginning of euthanasia to cardiac arrest.

Results – Discussion

1) Euthanasia of piglets under 8 kg

Carbon dioxide

This method proved particularly efficient, as all piglets presented cardiac arrest in under 6 minutes, and without having to resort to exsanguination. However, loss of consciousness was not immediate (no instant collapse and immediate mydriasis), and 4 out of 5 pigs vocalised within the first 30 seconds of exposure to the gas.

The CO_2 inhalation phase appears to cause pigs distress and pain. Piglets became motionless in less than 1 min 30 s.

Blunt trauma to the head with a 0.5 kg instrument

After the blow, loss of consciousness is immediate (mydriasis, collapse, no vocalisation), and the piglet shows no signs of pain. When this method is properly performed, the animal becomes motionless in less than 1 minute 30 s, with onset of cardiac arrest occurring in less than 10 minutes. The blow causes more or less heavy local haemorrhaging (nose, mouth, ears). This unaesthetic method is quick and effective.

2) Euthanasia of piglets from 8 to 25 kg

Captive bolt pistol without sticking

This is a painless technique causing immediate loss of consciousness. Animals become motionless between 30 seconds and 1 min 30 s depending on the animal. Local haemorrhaging (nose, mouth, ears) is routinely observed. Cardiac arrest is reached in less than 6 minutes. Relatively violent spasms and paddling episodes are evidence of a complete loss of function of the central nervous system. This very efficient method causes convulsion episodes that are liable to distress an outside public.

Blunt trauma to the head with a 1.5 kg instrument

This is an efficient and painless technique. Time to immobility varies between 1 min 30 s and 4 minutes. Cardiac arrest occurs after 7 to 9 minutes, i.e. a slightly longer period than with the captive bolt pistol. The difference between these two methods can be explained by blood flow at the wound not being systematic with the knocking procedure.

3) Euthanasia of growing pigs

Euthanasia by electrocution without sticking

This is a very efficient method that is painless for the animal. Electrodes applied to the head (eye-to-eye, eye-to-ear, ear-to-ear) for a minimum of 5 seconds cause instantaneous loss of consciousness (collapse, immediate mydriasis, no vocalisation). Electrocution by applying the electrodes to the heart (min. 15 seconds) results in cardiac arrest within one minute, with the animal being immobilised in less than 30 seconds.

Euthanasia by captive bolt pistol with or without sticking

This method is painless for the animal, loss of consciousness is immediate and regaining consciousness impossible, regardless of whether knife sticking is performed. Frequent unconscious and relatively violent movements can be observed during the first 2 minutes 30 s, together with convulsions over a maximum period of 4 minutes 30 s. Sticking shortens the interval to cardiac arrest (1 minute 30 s to 2 minutes with sticking compared to between 4 and 7 minutes without sticking).

In contrast to animals less than 25 kg, there is heavier bleeding through the nose or mouth at the point of impact. Sticking reduces episodes of convulsion and unconscious spasms, and shortens the time to cardiac arrest.

4) Euthanasia of sows

Electrical euthanasia without sticking

As with growing pigs, when properly performed this method is highly effective and painless for the animal. Cardiac arrest was, on average, obtained within 1 minute 30 s.

Euthanasia by captive bolt pistol with or without sticking
This is an efficient technique that sufficiently punctures and injures the brain, provided that a very high-power cartridge is used. If sticking is not performed, then - as is the case with growing pigs - potentially violent spasms and more prolonged convulsion occur, with onset of cardiac arrest after 5 to 7 minutes (compared to 2 to 8 minutes with sticking). The quality of the sticking, if used, is an important factor: bleeding should be heavy and free-flowing. The recommended technique is to bleed the heart using a dagger, as this leads to a much faster onset of cardiac arrest without blood flow into the surrounding environment.

Conclusion
This study has demonstrated that, given the efficacy, feasibility and cost of the various methods tested, the recommended method for piglets over 8 kg, growing pigs and breeding animals is now the captive bolt pistol. This technique is painless for the animal and causes immediate loss of consciousness together with irreversible brain injury and death, irrespective of whether sticking is performed. This method frequently causes relatively violent convulsion reflex and spasms, although none of this should be attributed to any conscious agony or pain whatsoever for the animal. Sticking reduces the convulsion reflex response and quickens the onset of cardiac arrest. Sticking is performed either by cutting the animal's throat, or better still, by a dagger blow to the heart triggering internal haemorrhaging, which does not pollute the surrounding environment.
The most effective techniques for suckling piglets (less than 8 kg) are the cranial blunt trauma methods. This unaesthetic method is quick and effective.
Electrical euthanasia provides a highly effective method for animals over 25 kg. Unfortunately, the high cost of approved electrical equipment means that this technique remains an unrealistic option for implementation in farms at the present time.
Euthanasia by exposure to CO_2 gas is a fast and effective method, but is not without pain for the animal in the first 20 to 30 seconds following inhalation. The costs involved and its limited applicability in farms (the method can only be applied to piglets) mean that it is difficult to implement this technique in farm structures.
Setting up guidelines for good practice in on-farm animal euthanasia would represent a positive step towards helping farmers design appropriate euthanasia action plans and the welfare of serious sick and injured pigs.

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THE EFFECT OF THE REARING ENVIRONMENT ON THE PREVALENCE OF BEHAVIOUR RELATED DISEASES IN LOOSE HOUSED LAYING HENS

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Introduction

The importance of early experiences for a normal development of the individuals has been demonstrated in different animal species, including the jungle fowl and the domestic hen. When studying health and behaviour of the laying hen, it is therefore also crucial to consider the rearing period and its effects on the adult hen. There are indications that failure in the normal development of the individual hen may jeopardise appropriate utilisation of resources, even if the hen has a need for the resource.

This paper will present some previous work on the early access to perches influenced the prevalence of floor eggs and cloacal cannibalism in laying hens, in relation to the development of spatial cognition of the birds (Gunnarsson et al., 1999; Gunnarsson et al., 2000). The first aim was to investigate the effect of early rearing conditions on the prevalence of floor eggs, cloacal cannibalism, and feather pecking in commercial flocks of laying hens in loose housing systems. The second aim was to investigate the spatial skills in laying hens that had been reared with and without early access to perches.

Material and Methods

Epidemiological study

A retrospective epidemiological investigation were performed in a database created within the Swedish National Board of Agriculture pre-testing programme for new technique for laying hens (Gunnarsson, 2000a). The bird health was studied at clinical examinations, performed on a representative sample of 100 birds from each flock, and at post-mortem examinations of a representative sample of dead or culled birds. The individual farmers were recording the egg production daily.

The database contained information about egg production, clinical health, and post-mortem examinations from 59 flocks housed in either OLI Free or Vencomatic aviary at 21 different commercial farms in Sweden. The total study population was about 120,000 laying hens of seven different hybrids.

Three different subsets of data used for modelling of floor eggs, cloacal cannibalism and feather pecking were used. To test for rearing effects on the dependent variables and correct the results for interaction effects and confounders, multiple regression analyses were performed, using stepwise linear logistic regression. These separate stepwise logistic regression models were applied to estimate predictors for the dependent variables; floor eggs, cloacal cannibalism and feather pecking, respectively.

Experimental study

Thirty, day-old chicks (Hisex brown hybrid) were randomly allocated into 2 equal groups and reared in litter pens, one with access to perches (P+) and one without (P-). At 8 weeks of age all birds were given access to perches and by 15 weeks all birds were using perches for roosting at night.

At 16 weeks, 10 birds from each group were tested in pens where food was presented on a wire mesh tier 40 cm above the ground. Three consecutive tests, with increasing difficulty for the bird to reach the food, were then performed. Firstly the food was presented at 80 cm above the ground but with the tier at 40 cm still present, secondly food was present on the tier at 80 cm and then, finally, with the food on a 160 cm high tier with the tier at 80 cm still present. All birds were food deprived for 15 h before each test and the time from the bird entering the pen until reaching the food was recorded.

Wilcoxon rank sum tests were used to analyse the jump scores, as well as time to reach the food.

Results

Epidemiological study

In the epidemiological study of commercial aviary farms, it was found that access to perches from not later than the fourth week of age decreased the prevalence of floor eggs during the period from start-of-lay until 35 weeks of age, (Odds Ratio=0.30; $P<0.001$). Furthermore, there was a significant effect of early access to perches which decreased the prevalence of cloacal cannibalism during the production period (OR=0.46; P=0.03). No other factor studied, such as strain of birds, stocking density or nesting space, was found to have significant effect on floor eggs and cloacal cannibalism. Early access to litter did not reduce the prevalence of feather pecking.

Experimental study

In the experiment there was no difference in the time to reach the food between birds reared with early access to perches and birds that had late access to perches in the test where the food was placed on a tier 40 cm off the ground (p=0.82). However, when the food was placed on a tier at 80 cm with the tier at 40 cm still present, birds reared with perches (P+) were faster in reaching the food (p=0.04). In two consecutive tests, where the food was placed on a single tier at 80 cm and on a tier at 160 cm with the tier at 80 cm still present, the differences in time to reach the food between birds reared with and without perches were even larger ($p<0.01$).

Discussion

Epidemiological methods are excellent to use when investigating relationships between different factors within databases created from commercial farms. Thus, it is possible to study more factors than in one experiment. Furthermore, it is possible to investigate diseases that can not be studied in experiments as they are hard to predict or ethically questionable to induce, such as cloacal cannibalism. Epidemiologically studies have been successfully used to study feather pecking and

cannibalism in commercial laying hens (Huber-Eicher B. & F. Sebö, 2001; Nicol et al., 2003; Pötzsch et al., 2001).

However, it was not possible from the epidemiological study to draw any conclusions about, why birds reared without early access to perches have a higher risk for laying floor eggs and being cannibalised. Therefore, this had to be investigated further in an experiment. A possible explanation for why hens that had early access to perches have a lower prevalence of cloacal cannibalism and floor eggs during the production period, compared to hens that had been reared without early access to perches, may be that the former have enhanced spatial skills. Therefore, these hens might be more likely to escape cannibalistic attacks and may more easily find nest boxes.

Thus, perches or tiers seem to be an important resource to prevent problems with cloacal cannibalism and floor eggs. No difference in the time to reach the food was found between P+ and P- birds in the T40 test. But, as the difficulty of the task increased, the difference between the P+ and P- birds became significant, with the P- birds taking a longer time to reach the food or not reaching it at all. Since there was no difference between P+ and P- in the T40 test, it seems reasonable to suppose that the later differences did not depend on differences in physical ability. Therefore, the results may imply that rearing without early access to perches in some way impairs the spatial cognitive skills of the domestic hen.

Yngvesson (2002) found experimentally that birds reared with early access to perches had a reduced latency to jump onto a perch when exposed to simulated cannibalistic attacks. However, there was a large individual variation in the birds' reaction to cannibalism, which may partly be related to other individual characteristics than previous experiences, e.g. Yngvesson & Keeling (2001) found that both cannibals and victims were more asymmetrical than control birds.

The importance of the two-dimensional features of the rearing environment has been investigated by Freire and coworkers (2004). They found that the spatial memory in occlusion-experienced domestic chicks had a dramatic effect on how the birds performed a detour test at ten days of age, where chicks reared in a barren environment had less developed spatial memory. Furthermore, these chick also had a less developed brain functions associated with spatial memory.

The majority of layer chicks in Europe today are reared in brooding cages or on litter floors without perches. An implication from the results of the present study is that the chick should be reared with access to perches before 4 weeks of age, in order to obtain good adaptability to loose housing systemsin order to avoid cloacal cannibalism and floor eggs.

Conclusion

Access to perches by four weeks of age decreased the prevalence for floor eggs and cloacal cannibalism in commercial flocks of laying hens housed in loose housing systems.

Rearing without early access to perches impairs the cognitive spatial skills of the domestic hen and the effect is both pronounced and long lasting. It affects how easily birds move about in an aviary system and this, in turn, has practical and welfare implications.

In order to make aviaries and other loose housing systems, that will replace battery cage in Europe within the next decades, it appears to be crucial for animal health and welfare that the rearing of the pullets is done in a way that prepare the birds for the housing system they will be housed in as adults (Gunnarsson, 2000b).

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Animal Welfare, Focus on transport / slaughter / euthanasia

Posters

STUDIES CONCERNING SHEEP WELFARE DURING ROAD TRANSPORTATION

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Introduction

Studying the effects during road transportation on sheep welfare has imposed like a necessity in the new context of harmonizing the legislation of our country with the European Union. The study has followed animal behaviour during the travel and then measuring some physiological and biochemical indicators.

Material and Methods

The study was made on an adult sheep, transported autumn with vehicles for animal transportation from a farm in the north of the country to an export base in south east of the country. The journey last to 16 hours. Before embarkment the animals were identified, clinic examined, then were weighing, thermometer and was measured the skin thickness. Each sheep was applied intravenous catheter that maintained 24 hours. For putting in evidence the metabolic and physiological modifications that appeared during transport, were cropped blood proofs 2 hours before embarkment then after departing at 2, 4, 8, 12 hours at the destination (16 hours) and at 24 hours from departing. In all this time was followed the evolution of some physiological and biochemical indicators like the cardiac frequency, the body temperature, the animal weight, the size of the skin thickness, plasmatic cortisol, free fatty acids, urea and creatine phosphokinase. The remaking of proofs was made at destination by the RIA method, optic test, Novak colorimetric method and urea method.

Results and Discussions

Sheep transport with vehicles in optimal conditions, represents a factor of moderate stress comparative with shearing that is considerate a major stress to these.

In this use the searching has followed the animal answer like a reaction of adaptation at transport determined by the measuring of the plasmatic cortisol. The high level of this was at 2 hours from embarkment that shows the fact that this operation is stressing for sheep's causing the increasing of plasmatic cortisol till 54µg/l instead of 27,6µg/l measured before embarkment. After 2 hours since departure the level of plasmatic cortical reached the maximum value after this register a progressive diminution of this maintaining then constantly (fig. 1). This thing proves that after a period of adaptation of sheep's at the transport condition, it becomes a moderate stressor (>30µg/l).

Also, was noticed that because of no foddering during 16 hours of travel, there is an acceleration of protein catabolism that has as result the increasing of urea (fig.2).

The metabolic changing at sheep's during transport is significant and constant on a large period of time. This can have at least important causes stress, no foddering and limited animal movement. Till 80% of metabolic energy necessary to sheep's is from free fatty acids made at rumen level.

These are represented by acetate, propionate and butyrate. Propionate is transported at liver level and converted in oxaloacetat and glucose. From metabolic energy approximately 20% is obtained by oxidation of the acetate a part of it is stored in glucose. If there is no foddering fatty acids volatile don't produce anymore and using of acetate like energy source is about 2%. The continuation of glucose that is still used like an energy source until the existent store in the shape of hepatic glycogen is flat after 24 hours in general. In these conditions the plasmatic glucose, plasmatic acetate and plasmatic free fatty acids are used like an energy source for musculature. Free fatty acids come near glycerol by plasmatic trigliceride of lionize phenomena. If hunger is extensional and oxaloacetat and betahidroxibutirat has limited quantity appears ketogenesis followed by the increasing of plasmatic level of acetoacetat and betahidroxibutirat.

Conclusions

1.The plasmatic cortisol level touches the maximum value after 2 hours from embarkment and maintains constantly reason that sheep's journey can be considered a moderate stressor.

2.The level of some free fatty acidic and the plasmatic urea increased because of the animal hunger and metabolic energy resources limited.

3.During sheep transport with vehicles 16 hours time there is noticed the increasing of cardiac frequency as well as the corporal temperature.

4.Because of the dehydration, there was noticed a diminution of the thickness.

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Fig.1. The plasmatic cortisol evolution during transport of sheep

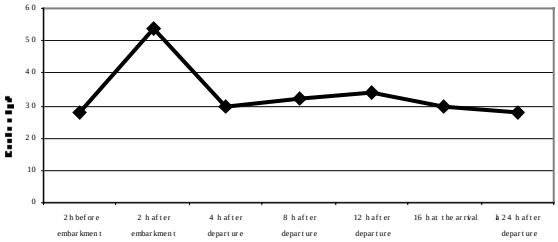
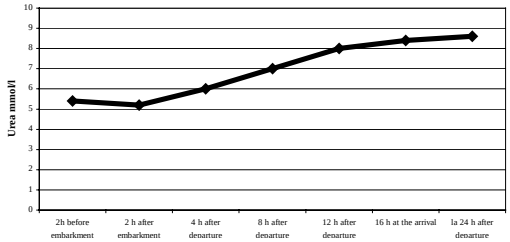


Fig.2. The urea evolution during transport of sheep



INFLUENCE OF FLOOR TYPE ON THE INCIDENCE AND SEVERITY OF LEG WEAKNESS SYNDROME (LWS) AND OF ARTICULAR OSTEOCHONDROSIS (OC) IN ITALIAN HEAVY PIG.

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Introduction

The condition known as "Leg Weakness Syndrome" (LWS) is one of the most important cause of culling in growing and fattening pigs, causing severe productive and economic loss. This syndrome, characterized by limb deformities and locomotor dysfunction, is a multi-factorial disease with a polymorphous symptomatology and is widespread in all types of genetic and pig husbandry. Osteochondrosis (OC), osteocondritis dissecans (OCD) and degenerative joint disease (DJD) are the most frequent articular lesions associated with leg weakness (Reiland, 1978). The floor type, i.e. too firm or too slippery or with reduced support, can add further mechanical stress and may be a predisposing condition for increased foot and leg problems.

The aim of this study was to examine the influence of floor type on the incidence of LWS and on the frequency and severity of articular osteochondrotic lesions in fattening pigs.

Material and Methods

The study was carried out in an experimental facility in Northern Italy. The experiment took place over two successive trials in 2002. In both trials, 264 commercial hybrid pigs 170-180 days old and with an average live weight of 90 kg were used. Only animals with normal limb conformation were selected. Both trials lasted from the day of selection (T0) to the day the animals were slaughtered at approximately 270 days of age. The experimental building was divided into 3 different rooms with different floor types for a total of 22 pens: room 1 (a concrete solid floor with a slatted area for dunging 1m wide under the windows); room 2 (a concrete fully slatted floor); room 3 (a concrete solid floor with an outdoor dunging slatted area 140 cm wide and as long as the pen). Where present, the gaps consisted of 2 centimetre spaces between 8 cm wide cement slates. The population density of the pig was at least $1m^2$/pig. In both trials, the animals were randomly distributed in the three rooms as follows: room 1: 72 animals; room 2: 96 animals; room 3: 96 animals. Two days before slaughter a clinical study was carried out to find any signs of lameness and gait changes for all animals. The clinical signs were classified according to an arbitrary scheme based on a severity score of lameness (from 1 to 4). A macroscopical study of the front leg joints of 120 animals in the first trial and 118 in the second one was carried out. The scapulo-humeral and radio-humeral joints of all animals were analysed considering the following articulation areas: the glenoid cavity of the scapula; the central area of the humeral head; the medial humeral condyle. The severity of lesions in fore legs was measured according to Borghetti et al., 1991. In order to study the association between symptomatology and joint lesions, the animals were divided into two distinct categories: animals without lesions or with grade 1 and 2 lesions (sub-clinical OC); animals with grade 3 and 4 lesions (clinical OC). This choice was made for physiopathological reasons given that 3 and especially 4 grade lesions showed damage [raising and loss of cartilage (OCD), osteoarthritis (OA) and bone exposure, acute synovitis] able to induce pain and so to be clinically evident.

Results

The results of the clinical examination are reported in table 1 and of the anatomopathological examination of the front limb joints in table 2.

Table 1. Clinical examination (trial 1 plus trial 2)

	Healthy	Healthy (%)	With Clinical Symptoms	With Clinical Symptoms (%)
Room 1	121	85	21^{a}*	15
Room 2	136	71	56^{b}*	29
Room 3	181	96	7^{c}*	4

*With different letters $p < 0{\cdot}02$ (statistically significant difference)

Table 2. Results of the anatomopathological examination of the front leg joints

	Trial 1		*Trial 2*	
	Animals with OC lesion			
	Clinical	Subclinical	Clinical	Subclinical
Room 1	6	18	3	21
Room 2	5	42	14	34
Room 3	15	33	11	37

No significant statistical differences in the anatomopathological examination of the fore limb joints were found.

Discussion and Conclusion

The significant clinical difference which was found in both trials, indicated that the slatted floor (room 2) is more critical than the other two examined for LWS incidence and severity. Unlike the clinical results, the anatomopathological exams, that evaluated their potential ability to cause symptoms, did not show any significant difference between the different floor types. Our results can be explained considering that the appearance of clinical signs can be influenced by very different situations not only related to the severity of articular cartilage damage. We think that the fully slatted floor by increasing the static overload, muscle development and reducing the locomotory ability, could influence more drastically the incidence of abnormal leg conformations. Furthermore, it could induce a structural muscular weakness not directly related to a greater occurrence of severe OC lesions.

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THE STRESS RESPONSE IS STRESSOR-SPECIFIC: COMPARISON OF RESPONSES TO SOCIAL AND INFLAMMATORY CHALLENGES IN PIGLETS

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Introduction

The aim of the present experiment was to characterise the stress response in weaned pigs at endocrine, immune and behavioural levels. For this purpose, the responses to psychic and immune stressors were compared.

Material and Methods

At twenty eight-days, piglets were moved to a weaning room and housed with their litter-mates. Stress treatments were administered five days after weaning (day 0), when most of the acute physiological adjustments of weaning have disappeared. Piglets were either separated from their litter and moved in a new group until the end of the experiment (mixed (M) group, n=8), either injected by intra-peritoneal route with 50 µg/kg lipopolysaccharide (LPS group, n=8) or remained undisturbed in their litter pen (control group, n=8). Responses to stressors were assessed by measuring salivary cortisol, blood lymphocyte proliferation and behavioural activity from day -1 (one day before stress) until day 3 (three days after stress). In mixed pens, an index of dominance, assessing the final social rank of each cage-mate, was determined by direct observation of agonistic interactions.

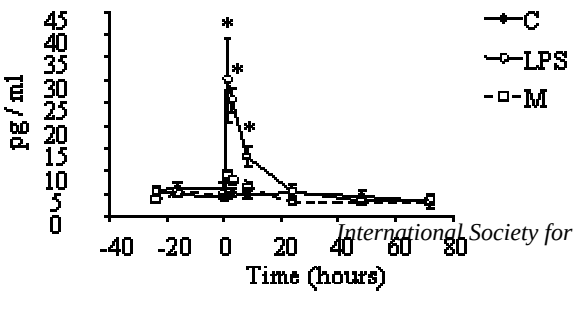
Results and discussion

LPS induced a transient increase in salivary cortisol (figure 1) and inhibited the phytohemagglutinin-induced proliferation of lymphocytes (figure 2). Mixed pigs fought during the first thirty minutes after regrouping. Mixing induced a smaller increase in salivary cortisol than LPS administration and lymphocyte proliferation was not affected.

Consequences of mixing on behaviour were very different from the response to LPS (figures 3 and 4). LPS challenged pigs displayed sickness behaviour (decreased locomotor activity and feeding behaviour). A transient increase in the frequency of activities that were not synchronised with the other group members on day 0 reflects the higher frequency of resting activity in LPS pigs.

In mixed pigs, the frequency of desynchronised activities was increased until day 2 while locomotor activity and feeding behaviour were not affected. This behavioural desynchronisation could be interpreted as an attempt to avoid other pigs at the feeder or at the water nipple. Interestingly, salivary cortisol and locomotor activity were correlated with social rank (figure 5).

Figure 1: *salivary cortisol in control (C), LPS-challenged (LPS) and mixed (M) pigs,*P<0.05.*



Conclusion

These results show that mixing young pigs is stressful and that the amplitude of the stress response depends on social rank. Furthermore, they demonstrate that animals develop stressor-specific endocrine, immune and behavioural responses to adapt the situation

*Figure 2: Blood lymphocyte proliferation in response to increasing doses of phytohemagglutin in control (C), LPS-challenged (LPS) and mixed (M) pigs, *P<0.05.*

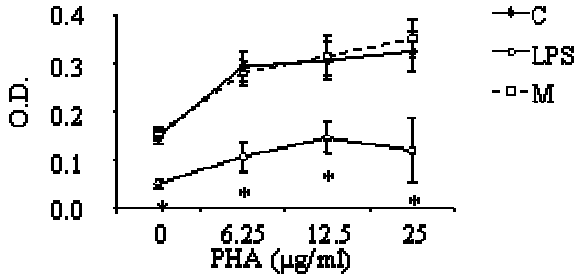


*Figure 3: Locomotor activity from day -3 until day 3 after challenge (day 0) in control (C), LPS-challenged (LPS) and mixed (M) pigs, *P<0.05.*

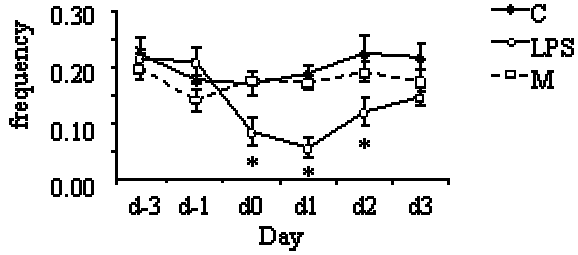


*Figure 4: Behavioural desynchronization of control (C), LPS-challenged (LPS) and mixed (M) pigs with their group, *P<0.05.*

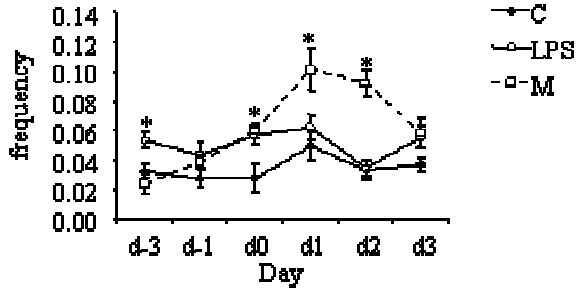
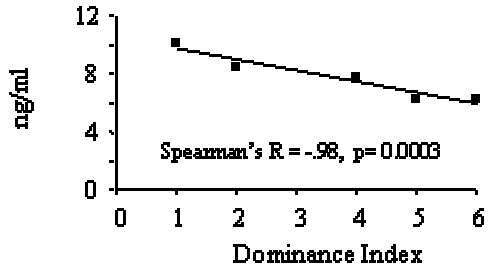


Figure 5: Correlation between dominance index and salivary cortisol 3 hours after mixing.



Integration of animal farming systems in the environment

Oral Communications

CONTRIBUTION OF HERBIVORES TO ENVIRONMENTAL MANAGEMENT AND CONSERVATION OF BIODIVERSITY

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Livestock farming uses nearly 60% of French agricultural land, and so is a major factor in the management of the countryside, especially in sensitive rural areas. The term 'management' has different implications according to whether the emphasis is on production, biodiversity conservation, landscape preservation or recreational use. Livestock farmers use their land first to feed their animals, while ensuring the persistence and quality of their forage resources. For environmentalists and the public authorities, livestock farming should preserve, and sometimes restore, local biological and heritage assets, and in certain areas it should conserve open areas, in particular to reduce fire risks. Lastly there is a social demand for the preservation of attractive landscapes for the pleasure of the local population and recreational use by city dwellers and holidaymakers. Besides these 'service' functions, a 'well-kept' countryside is an important cultural asset and forms a part of local identity. Rural features and patterns of use are a source of income through the diversity of landscape and the quality of local produce from livestock.

In what follows, 'biodiversity' means the diversity of the biosphere at the different biological scales (genes, species, ecosystems), with their specific functional and ecological variables, and forms one component of the environment. We address the issue of environment management by livestock farming in terms of the underlying processes in play. We explain trends in plant cover due to farming practice, and in particular to pasture management, in terms of both vegetation dynamics and animal grazing. We focus on grassland use, rangelands being not examined.

1. Effect of management practices on the evolution of permanent grasslands

Permanent grassland is composed of a mixture of between 10 and more than 100 different species sharing the 'same' environment and competing with each other for light, water and nutriments. The combination of management practice and soil and climate factors determines which species will become dominant or be maintained in each particular case. At any given level of fertilization and intensity of use of a plot, a species abundance profile can thus be established that is a measure of plant diversity (Fig. 1). Moderately extensive farming, combining low fertilization with late cutting, favours weakly competitive species with low growth rates and an aptitude for internal nutrient recycling, and so favours a broadly diverse flora. Conversely, intensive farming (high fertilization, early cutting) selects highly competitive species with a high capacity for nutrient capture and high growth rate, which eliminates most of the other species and so leads to an impoverishment of the flora (Cruz *et al.*, 2002). The entire grassland ecosystem and landscape can then become modified. For example, in uplands, the development of silage and bale silage practices, together with higher nitrogen fertilization makes it possible to start cutting at least one month earlier than previously, well before the plants have flowered, which in the medium term gradually narrows flora diversity by the reduced production of seeds (Carrère *et al.*, 2001). This in turn impacts on nectar-seeking and pollinating insects, especially butterflies and bees, which are deprived of the resources offered by flowers. Finally, the gradual disappearance of the colourful early summer flowering of the grasslands makes the landscape more uniform and so less attractive.

Hence, while it is possible through careful land use to combine area productivity with reduced releases into groundwater and the atmosphere, it is more difficult, and sometimes impossible, to combine high productivity and high plant diversity at plot scale. However, area productivity is not all. Extensively used grassland with diverse flora can however present characteristics that are valuable to the farmer, in particular a steadier nutritional value over the year due to the diversity of the species present and the staggering of their phenological cycle.

Concerning the dynamics of vegetation under very extensive farming, we observe that the plants in this type of environments include species of larger size, longer leaf life, and lower nutritional value owing to a greater proportion of supporting tissue. If grazing intensity is further lowered by a reduced stocking rate, then shrub and tree species can develop, gradually leading to landscape closure. Thus species diversity first enters a phase of enrichment through the coexistence of grassland and shrub species, and then narrows as grassland species are displaced. Grazing herbivores are thus necessary to keep the landscape open and preserve the biodiversity of grasslands.

2. Impact of dietary choices of herbivores on the evolution of grasslands

Herbivores make dietary choices in all types of pasture, but diet selection becomes increasingly important as vegetation becomes more heterogeneous and grazing pressure is reduced. An animal that has access to diverse resources and a quantity of grass in excess of what it can graze will tend to feed selectively on its preferred species and leave others ungrazed. An understanding of how the animal's choice is determined is thus an important factor in predicting the vegetation dynamics. A lot of the choice differences between different herbivore types (species, breed, physiological stage, age, etc.) can be explained by differences in energy requirements, intake capacity, dental and digestive anatomy (Rook *et al.*, 2004). Thus small ruminants, which require more energy relative to their gut capacity than large ruminants, tend to select higher quality foods. The shape of their dental arcades and their mobile lips enable them to sort plants and vegetative organs in the plant cover. In contrast, the larger muzzles of cows make them less able to sort plant items and to graze on short swards, where they no longer meet their requirements. On the other hand they are better

able to digest rough forages because of their longer residence time in the rumen. This is why cattle switch to poor quality grass sooner than sheep as the height of high quality swards is reduced (Dumont *et al*., 1995). These choice differences can result in a different evolution of pasture grazed by these two species. The proportion of *Nardus* within a good *Agrostis-Festuca* sward fell from 55% to 30% after 5 years grazing by cattle, whereas with the same stocking rate it increased to cover 80% of the sward when grazed by sheep (Grant & Hodgson, 1986).

The choice made by animals is also influenced by how familiar they have become with different foods by individual learning, and even more efficiently by social transmission. Early grazing experience of harsh vegetation at a young age prepares the animals to deal with these resources later on.

How animals graze a plot also depends on the distribution of preferred food resources. Sheep and cattle use their spatial memory to efficiently return to preferred feeding sites they have previously grazed. As it is easier to remember a few large patches than many small ones, they graze a preferred resource more readily when it is concentrated in a few large patches rather than disseminated throughout the plot. (Dumont *et al.*, 2002). This can favour the persistence of disseminated species of low abundance in the sward.

The positioning of points of attraction such as shelters, salt blocks and drinking troughs can improve how herbivores distribute within large and extensively-grazed pastures. Not installing all these points of attraction in the same location reduces damage to sward by trampling, provides a more even distribution of excreta, and induces the animals to move on from one point to another, and to exploit vegetation along their grazing journey.

3. Management and preservation of biodiversity at the farm scale

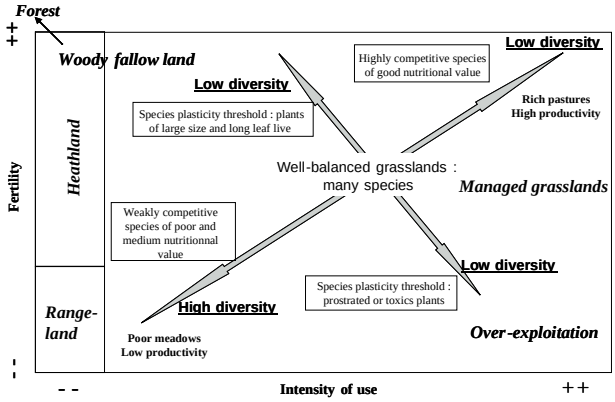
Environmental management and preservation of biodiversity cannot be conceived solely at the plot level. They have to be considered at larger scales, such as the whole farm or rural area. Within a livestock farm there is a great diversity of land use, from intensive use in early-cut plots (silage or bale silage) or plots near farm buildings, to a very extensive use for the least productive or least accessible plots, often left for grazing by animals with lower requirements. This diversity of plot management creates a mosaic of vegetation states conducive to an increased plant biodiversity at farm level through the juxtaposition of different plant communities (Fig. 1), and which also favours grassland microfauna. Orthoptera and lepidoptera will be favoured by the presence of extensively grazed plots, where they can find shelter and food, while coprophagous insects and predaceous ground beetles will be more numerous where plots are grazed intensively (WallisDeVries *et al.*, 2004). In addition to cut or grazed plots, a farm has hedges, isolated trees, stumps, stone heaps, ponds and ditches, the maintenance and preservation of which also contributes to the diversity of the flora and fauna.

Conclusion

Livestock farming, whether grazing is extensive or intensive, contributes to environmental management by maintaining open landscapes. However, the more intensive production systems reduce biodiversity for the sake of higher productivity, although the mosaic of differently managed plots at the farm level helps to preserve a certain overall biodiversity.

The income of livestock farmers in grassland areas depends closely on European funding linked to production or to agri-environmental measures. If the aim is to preserve biological diversity, whether for environmental protection or to conserve the specific features of local produce, then it is important to continue providing financial incentives to help livestock farmers maintaining extensive management practices (late cutting, low fertilization and stocking rates) on part of or the entire farm.

Figure 1 : Relationship between intensity of use, fertility, and biodiversity level in grasslands



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EXPERIMENTAL EVALUATION OF TWO HUSBANDRY METHODS FOR GROWING-FINISHING PIGS

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Introduction

A rejection of the conventional pig production system by the society has been occuring for few years, because it is generally associated with a negative environmental impact (problems of pollution and offensive odours), a poor animal welfare (due to high animal densities and bad housing conditions) and could be involved in a reduced meat quality. This feeling is observed in areas of high pig production density and also in areas showing a low pig farm density but a high potential for the development of pig production. Thus, in the near future, the pork chain has to propose different types of pig production systems that satisfy the consumer and citizen demands : lower environmental impact, better animal welfare and meat quality. The present study is set in this general objective, and aims to evaluate the effects of two contrasted pig husbandry methods on animal performance, welfare, health, environmental impact, and meat quality.

Material and Methods

Animals and husbandry. The experiment included a total of 120 synthetic line x (Large White x Landrace) pigs (castrated males, CM and females, F), all free of the n and RN^- alleles. At an average live weight (LW) of 35 kg, littermates were allocated to either a conventional (totally slatted floor, 0.65 m²/pig, controlled ambient temperature at 22°C considered as control, **C**), or a alternative system (**O**) : sawdust-shave bedding (1.3 m²/pig, fluctuating ambient temperature) with free access to an outdoor area (concrete floor, 1.1 m²/pig). Pigs were fed *ad libitum* growing (up to 70 kg) and finishing diets, and had free access to water. Trials were undertaken in spring, summer and winter, each involving 2 pens of 10 pigs (5 CM and 5 F) per system. Pigs were reared in two different rooms (one per system) of the same building.

Pig behaviour. At the average LW of 70 kg, the different activities and number of pigs implicated were evaluated every 10 min from video tapes recorded continuously over 24 hours. Time-budget (%) from 8 am to 4 pm were established for each husbandry method (see Lebret *et al* 2004 for more details on materials and methods).

Evaluation of environmental impact. At the end of each replicate, effluents were collected, weighted and analysed for dry matter (DM), nitrogen, phosphorus, potassium, copper and zinc. In each room, air flow was measured 4 times a week, and a determined sample of extracted air was continuously collected in sulfuric acid for subsequent ammonia determination. Four samples of extracted air were taken (replicates 1 and 2) for determination of dust level and odour concentration (olfactometry).

Slaughter and carcass traits. Pigs were slaughtered at around 114 kg LW, by groups of 5 pigs per system and slaughter date. After overnight fasting, transportation (2 hrs) and lairage (3hrs), pigs were slaughtered by electrical stunning and exsanguination. Blood was collected for determinations of plasma lactate, cortisol and ACTH (RIA). Severity scores of nasal cavities, lungs and stomach were evaluated to determine the occurrence of respiratory tract pathologies and ulcers, respectively. Carcass weight, mean back fat depth and lean meat content (calculated from linear measurements) were measured on the day of slaughter.

Meat quality. pH_1, pH_u, colour (L*a*b*) and lipid content were determined on *Longissimus lumborum* (LL), *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles. LL drip losses were evaluated at 4 days p.m. Loins were kept at 4°C for 4 days, put under vacuum and frozen (-20°C) until sensory analyses. After thawing at ambient temperature, chops were grilled (double contact grill, 280°C, 6 min.) and assessed for odour, tenderness, juiciness and flavour on a scale from 0 (absent) to 10 (high) by a 10-member trained taste panel.

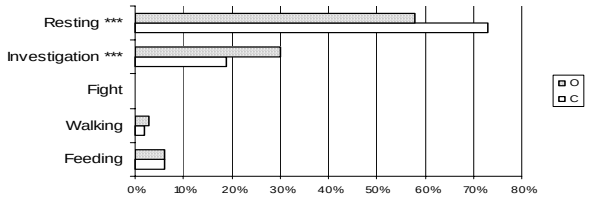
Statistical analyses. Data were submitted to an analysis of variance (GLM procedure, SAS), including the effects of husbandry method, season and sex. Slaughter date intra-replicate was added to the model for the analysis of stress at slaughter and meat quality variables. Time-budgets were compared using the χ^2 test.

Results and Discussion

Housing conditions. In the C system, the average ambient temperature was 23.5 ($\pm$1.0)°C. It was lower with higher fluctuations in O system : 12.0 ($\pm$4.0)°C and 18.8 ($\pm$2.7)°C in outdoor and indoor areas, respectively. Temperature differences between the C and outdoor and indoor areas of the O system were the highest in winter, the lowest in summer, and intermediate in spring.

Behavioural observations. The O pigs spent on average 24% of time outdoor. During daytime they exhibited a higher exploratory behaviour than C pigs, and spent less time resting (Fig. 1). Although pen wall and floor were the main structures investigated by the pigs in both systems, the O pigs spent 25% of their exploratory time manipulating the bedding, and exhibited less exploration behaviour towards others (25% *vs* 43% of time for O and C pigs, resp^{ly}., $p<.05$). These results, in agreement with Lyons *et al* (1995) and Beattie *et al* (2000), suggest that the O system would improve pig welfare.

Figure 1. Time-budget (%) during daytime (8 am-16 pm)



Environmental impact : effluents, air quality. Around 199kg bedding/pig (44.7% DM) and 228kg slurry/pig (outdoor area) were collected in the O system, compared to 366kg slurry/pig (9% DM) in the C system. The respective levels of potassium (mainly released by urine) and phosphorus, copper and zinc (mainly released by

faeces) in the slurry from indoor and outdoor areas of the O system indicated that about 61% of urine and 44% of faeces were excreted in the indoor area, whereas pigs spent there about 76% of time. Air dust level and ammonia volatilization were similar, whereas the level of offensive odours was strongly decreased in the O compared with C system (Tab. 1).

Table 1. Air quality in indoor areas of C and O systems

	O	C
Dusts, mg/m^3	2.0 ± 0.6	1.5 ± 0.8
Ammonia, g/pig/d	10.8 ± 3.6	12.6 ± 5.0
Odours, U/pig/d	5.6 10^5 ± 4.5 10^5	19.0 10^5 ± 13 10^5

Growth performance. Compared to the C, O pigs had higher feed intake, growth rate and were heavier at slaughter at 155d, but mean feed conversion ratio did not differ between groups (Tab. 2). In the O system, the lower average ambient temperature may explain the higher feed intake and, consequently, the higher growth rate although the decreased competition among pigs (resulting from the increased space allowance), may also have been involved. The higher growth performance of O pigs agrees with results of Lyons *et al* (1995) and Beattie *et al* (2000).

Table 2. Effect of husbandry method on growth, carcass traits, animal health and performance meat quality traits

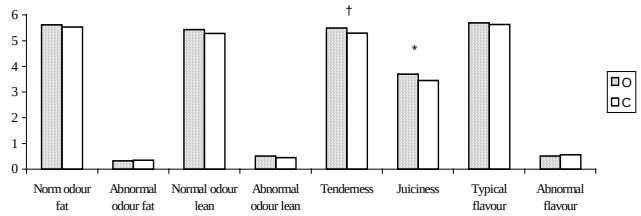
	O	C	Sign.
Number of animals	120	120	
Growth performance			
Final LW, kg	119.0	110.6	***
Feed intake, kg/d	2.94	2.71	**
Growth rate, g/d	1045	960	***
Feed conversion ratio, kg/kg	2.82	2.83	ns
Carcass traits			
Mean back fat depth, mm	20.9	18.5	**
Lean meat content, %	59.2	61.2	***
Health evaluation			
Nasal cavities (note/14)	0.7	2.0	***
Lungs (note/28)	2.5	3.5	ns
Stomach (note/7)	1.3	1.7	ns
Meat quality traits (LL)			
pH_1	6.37	6.42	ns
pH_u	5.50	5.49	ns
Colour L*	55.2	54.2	ns
a*	5.8	5.5	ns
b*	5.7	5.0	**
Drip loss, %	5.7	4.6	**
Lipid, %	1.68	1.44	**

Carcass traits and health at slaughter. The O system gave fatter carcasses (Tab. 2), in particular for CM compared to F pigs (p=.12). When adjusted to the same slaughter weight, differences in carcass traits between groups remained high and highly significant, suggesting that our observations are not the consequence of the heavier LW of the O pigs at slaughter, but resulted from a direct effect of the husbandry method. These findings are in agreement with Beattie *et al* (2000), whereas Van der Wal *et al* (1993) and Lyons *et al* (1995) did not report any significant effect of husbandry method on carcass fatness.

At each season, O pigs had lower severity scores of nasal cavities (Tab. 2) and we observed more uninjured O than C pigs (62 *vs* 38%). On average, lung scores were similar but they were lower for the O pigs at the winter replicate (3.6 *vs* 7.1/28, p<.05). Occurrence of stomach ulcers was low and similar in both groups. Altogether, this shows that, in our experimental conditions with a good health status, the O husbandry method led to less respiratory problems, in particular in the upper respiratory tract.

Reactivity of pigs to stress at slaughter and meat quality. Plasma ACTH, cortisol and lactate levels (not shown) and pH_1 of the 3 muscles (Tab. 2; SM and BF not shown) were similar between groups, suggesting that in our conditions, the husbandry method did not influence reactivity of pigs to stress at slaughter. The O rearing system had no effect on pH_u, meat lightness (L*) and redness (a*), but increased yellowness (b*) and drip losses in the LL. By contrast, we noticed a lower pH_u in the SM (5.50 *vs* 5.57, P<.001) and BF (5.49 *vs* 5.52, P<.05) muscles from O than from C pigs, suggesting that the effects of husbandry method on muscle metabolic traits are muscle-specific. In all 3 muscles, lipid content was higher in O than C pigs, in particular for CM. Meat from the two groups exhibited higher normal flavour score and did not show any abnormal flavour (Fig. 2). The O husbandry method increased loin juiciness and tended to increase tenderness (p=.08), however differences were small and may not be noticeable in a domestic situation. The other eating quality traits were not influenced by the husbandry method. Van der Wal *et al* (1993) did not report any significant effect of pig housing system on meat eating quality.

Figure 2. Effect of husbandry method on eating quality.



Conclusion

Compared to the conventional, the alternative husbandry method evaluated here led to : 1) an improvement in animal welfare as evaluated by animal behaviour and health, 2) decreased level of offensive odours 3) higher growth performance and improved loin eating quality, but fatter carcasses and lower pH_u in ham muscles.

Acknowledgements

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FARM-LEVEL MEASURES TO REDUCE AMMONIA EMISSION FROM TIED DAIRY CATTLE COMPATIBLE WITH IMPROVED ANIMAL HEALTH AND PRODUCTIVITY

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Introduction

Ammonia is a water-soluble gas, which can travel over great distances in the atmosphere and deposit as rain. Ammonia deposition affects the environment through eutrophication and acidification of the ground and water. The presence of ammonia in the atmosphere is a result of human activities; all handling of manure causes ammonia emission and farming causes 90% of all emissions. Efforts are made to reduce ammonia emissions at an international level, e.g. through conventions like CLRTAP from 1979 (10) and the National Emissions Ceiling Directive of 2001 (3).

However, farm-level efforts to minimize ammonia emission are not necessarily accompanied by improved animal health and welfare. For instance, reduced ventilation of a cowshed would reduce emission from the building but increase indoor gas concentrations. Likewise, manure-draining flooring may be uncomfortable for the animals when walking and resting. Ammonia paralyses the cilia of the upper respiratory tract and presumably increases the risk of respiratory disease (4, 8). According to the Swedish animal-welfare regulations, indoor air in buildings for domestic animals shall not have higher concentrations of ammonia than 10 ppm. Poor hygienic conditions in animal houses – related to high ammonia emission – reduces livestock welfare through itch, pain, burns, infections and a reduced heat-insulating capacity of the haircoat. Both hoof lesions and mastitis are to a great extent caused by poor hygiene (1, 2, 6, 9).

The objective of the present study was to describe cow health, fertility and milk production in connection with technical steps to reduce ammonia emission from a dairy farm.

Material and Methods

At a university farm with 42 tied dairy cattle of the Swedish Red and White Breed and 92 ha of arable land in southwest Sweden, methods and techniques to reduce the ammonia emission in milk production were studied and demonstrated during two winter seasons, as part of a large 4-year EU-financed project with cooperation of major agricultural organizations and companies.

At the start of the study, the cows were kept in a traditional Swedish cowshed, together with heifer calves to 1 yr of age. The cows were tied in 220-cm (long) stalls with lockable stanchions and rubber or EVA mats. Half of the stalls were equipped with rubber-slatted flooring (Fritz Foderstyrning AB, Nässjö, Sweden) in the rear part. Chopped straw was used as bedding material. The gutters lacked a well-functioning urine drainage system. The building was ventilated by a fan in the ceiling, with a maximum capacity of 273 m^3/cow-h. The cows were fed grass/clover silage, hay, grains (oats and wheat) and protein concentrates. The animals were managed by a full-time herdsman. Cleaning of stalls was done several times a day (whenever needed) and the cows were groomed manually twice daily. Claw trimming was done twice a year.

In connection with rebuilding of the cowshed in the summer of 2000, changes were made regarding feeding, housing and indoor manure handling, including:

- lowered dietary crude protein concentration (from approx. 170 to 160 g/kg dry-matter in early lactation), paying attention to recommended levels of rumenally degradable and undegradable proteins,
- 181-cm (short) tie-stalls with indivídually adjustable fronts and rubber-covered slatted flooring,
- wood shavings used as litter material,
- improved gutters with a 3% slope towards a urine drainage channel equipped with a rotating auger,
- cooling of gutters by incoming water,
- main ventilation by four wall fans, giving a maximum capacity of 457 m^3/cow-h, and
- manure-gas ventilation from the urine channels and manure culvert.

During rebuilding, from May to September 2000, the cows were kept on another farm. Cow cleanness and performance were recorded continuously or at regular intervals before and after rebuilding. Clinical diseases were recorded by veterinarians.

Results

The technical changes made reduced the ammonia emission through the exhaust air by 30%, from 24 to 18 g/cow-d, or from 7.2 to 5.0 kg/cow-year. Average indoor ammonia concentration dropped from 7.9 to 3.2 ppm. Average indoor temperature dropped from 16.5 to 13.5 °C and the relative humidity remained unchanged at 62-64%.

The changes made influenced feed costs and milk revenues only marginally. Feed consumtion increased with almost 2 kg dry-matter. Milk yield dropped during rebuilding, but then increased by approx. 2.4 kg energy-corrected milk per cow-d, from 30.6 kg before to 33.0 kg after rebuilding, wheras the milk composition and nitrogen efficiency in milk production remained almost unchanged for lactating cows. When the dry period was included, the nitrogen efficiency increased by 2 percentage units, from 25 to 27%. From 1999 to 2002, the yearly recruitment rate (percentage of heifer calvings) varied between 37 and 46% and the culling rate between 35 and 50%.

Animal cleanness on hind legs and udder improved considerably (Table 1), and the need for cleaning of stalls and grooming diminished substantially. At all recording occasions, the variation in dirtiness between cows was great. There was less variation between seasons, with a tendency of higher scores for hindfeet and udder during late winter than during the rest of the housing period. Cows that were kept in stalls with rubber-slat flooring before rebuilding were considerably cleaner than cows kept on ordinary flooring (5).

Table 1. Degree of dirtiness on different body areas before and after rebuilding (min.-max. of median scores for monthly recordings).

	Before	After
Hindfoot-hock[1]	8-9	3-4
Gaskin-thigh-rump[2]	2-3	1-2
Udder[3]	2-4	0-1

[1,2,3] Maximum sum of points attainable: 12, 6 and 9, respectively.

The incidence of clinical mastitis during the housing period dropped by 56%, from 0.69 cases/cow-yr before to 0.30 after rebuilding.
Milk somatic-cell counts remained low during the study; based on monthly test-day recordings, the average bulk milk-cell count was calculated to be 143,000 cells/ml both before and after rebuilding.
The prevalence of heel horn erosion, sole haemorrhage, sole ulcer and double sole at spring claw trimming dropped by 65-87% (Table 2). The total prevalence of any type of lesion varied between trimming occasions from 60 to 92% before and from 50 to 76% after rebuilding.

Table 2. Prevalence (percentage of cows diseased) of different hoof lesions at spring claw trimming before (one trimming) and after rebuilding (mean of two trimmings).

Lesion	Before	After
Dermatitis	12.3	20.5
Heel-horn erosion[1]	29.6	6.3
Heamorrhage[1,2]	38.3	13.4
Sole ulcer	6.2	0.8
White-line separation	16.0	5.5
Other lesions[3]	3.7	2.4
Any lesion	86.4	70.9

[1] Moderate or severe lesion.
[2] Heamorrhage in the sole or white line.
[3] Double sole, wharts, cracks or abnormal hoof shape.

In the spring before rebuilding, the prevalence of hoof lesions was generally lower in cows on rubber slats than on solid floor (5).
Before rebuilding, the prevalence of hock burns varied between 57 and 100%, and similar lesions on other body parts were rare. After rebuilding, however, the prevalence of lesions increased to 83% for the hocks, 2% for the knee area and 62% for the carpal joint. In most cases, lesions were superficial, small and without any appreciable complications. Moderate carpal hygroma was observed in some animals.
Reproductive performance remained excellent throughout the study, with 1.4-1.6 services/pregnancy, 81-87 d open, and a 11.7-12.0 mo calving interval.

Discussion

It is not possible to compare the cleanness of these animals with that of other dairy herds. With respect to clinical disease, official incidence estimates are missing, and some cases in the present study did not result in any veterinary treatment. Therefore, the incidences recorded in this study cannot be compared directly with figures from the official disease-recording system.

During the year before rebuilding, the incidences of clinical mastitis and acute hoof diseases were substantially increased, while the incidences of other clinical diseases were similar to other Swedish herds. After rebuilding, all common clinical diseases were in level with other Swedish herds. Cell counts may be compared with the arithmetic mean for all Swedish herds in the official milk-recording scheme during the same period, which was 185,000 to 212,000 cells/ml.
The prevalence of hoof lesions may be compared with the prevalence recorded (with the same scheme) by Manske *et al*. (7) in 101 Swedish dairy herds, showing that hoof health was relatively good before rebuilding (except for some white-line separations and sole haemorrhages) and good to excellent after rebuilding.

Conclusion

The results show that measures to reduce efficiently the ammonia emission from buildings for dairy cattle are compatible with improved animal health and production.

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CAN WE MINIMIZE NITROGEN EXCRETION IN DAIRY HERDS WHILE MAINTAINING PERFORMANCES?

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Introduction

In the last decades intensive animal production systems were encouraged. However, they generate considerable excess of N, the role of which being now well established in some environmental risks. The EC Directives make the management of animal excreta on farms more difficult and they will be reinforced in the future. In this context, reducing N excretion from the cows is one way among others to limit the impact of dairy herd to the N cycle on the farm. The paper directs attention to recent advances upon (i) the main factors affecting N excretion in the dairy cows, (ii) the role of protein feeding to promote balanced solutions between performances and N excretion and iii) the restitution of N on the grazing area.

Main factors affecting N excretion

Origin and composition of N excreted

In dairy cows, the quantities of excreted N (g/d) can be calculated by difference between N intake (NI) and N exported in milk (NM) as N retained is negligible. N excreted in faeces (NF) is mainly organic. NF only marginally varies with the composition of the diet and averaged 7.2 g/kg DM intake (DMI). On the contrary N excreted in urine (NU) is directly affected by the level and composition of ingested protein. Increased NU may originate from an excess of degradable N *vs.* rumen microbial requirements or from an excess or unbalanced amino acid supply *vs.* cow requirements. Both lead to the production of urea that is excreted in urine. Urea-N can easily be volatilised or leached. NU can be calculated as NI – NM – 7.2 DMI.

Quantification of N excretion per cow per year

Using the French feeding systems (INRA, 1988), Delaby et al (1995) have calculated the variations of annual N excretion. A dairy cow (7500 kg milk) fed with a well balanced maize silage based diet ingests 131 kg of N of which 50% come from the concentrate, secretes 40 kg of N in milk and excreted 42 kg in faeces and 49 kg in urine. N excreted equals 70% of NI, which is also equivalent to 12.1 kg per tonne of milk produced.

N excretion per tonne of milk produced decreased as the cow potential increases due to a dilution of maintenance requirement. With the same maize silage diet, excreted N decreases from 13.3 to 11.2 kg/t between 6000 and 9000 kg of milk. Consequences at the farm scale are difficult to extrapolate. Total N excretion per cow increases (from 80 to 101 kg), as does N excretion per ha of forage because extra amount of milk is mainly produced with concentrates. But in the same time the number of cows and the stocking rate will decrease to fulfil the quota.

N excretion varies with the forage system. Compared to the maize silage diet, a grass silage diet (15% CP) increased N excretion up to 153 kg from which 38 and 75 kg are excreted in faeces and urine. This high N excretion cannot be assimilated to a higher level of N losses at the farm scale because N exported per ha is higher for grass than for maize (Peyraud et al, 1995). Moreover the grass silage diet requires less N from purchased concentrate (49 vs. 61 kg). N excreted dramatically increased (from 14 to more than 20 kg per tonne of milk) with the level of intensification of grass production and this can lead to huge losses as stocking rate also increases.

N excretion depends of the feeding practice. Using security margins will increase N excreted. A 10% increase of the PDIE supply above needs will increase N excreted by 13 kg, whereas an 200 g/day excess of degradable N (PDIN > PDIE) will lead to an increase of 18 kg in N annually excreted (Peyraud et al, 1995).

Practical assessment of annual N excretion

The total amount of N annually excreted according to the major type of diets given to dairy cow (maize silage, conserved grass - 15% CP, pasture – 18% CP) and various durations over the year of the feeding sequences were calculated (Delaby et al, 1995, table 1). The calculations are based on a monthly basis and assume an optimised N supplementation.

Increasing maize silage decreases the total amount of N emitted indoors. Introduction of grazing increases the total amount of N excreted but reduces the amount of N to be collected indoors. The effect of the level of production may be integrated assuming a variation of 5% per 1000 kg of milk. When feeding is composed of mixed ration including grazing the distribution of the excreta between indoors and paddocks are fixed at 85/15 as long as the conserved forages represent less than 50% of the ration and at 65/35 beyond 50%.

Table 1: Effect of the forage system on annual excreted N for a cow producing 6000 kg milk

Restitution (kg N/year)		Indoors					Pasture
Maize silage (months*)	0	3	6	9	12		
Grazing (months*) 0	109	102	95	88	80	0	
3	87	80	73	65		29	
6	65	58	50			57	
9	43	35				86	

The sum of months with maize silage and months with grazing is make up 12 months by adding months with conserved grass

Protein supplementation to reduce N excretion while maintaining performances

Supply of degradable protein

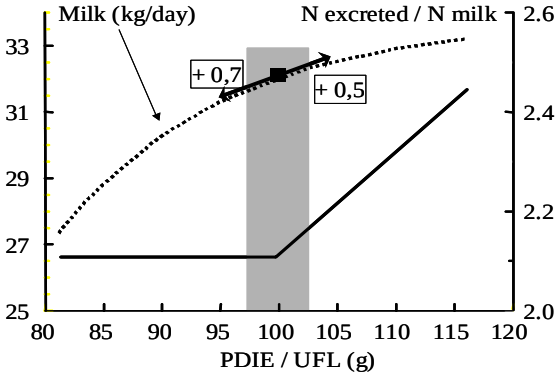
The degradable N supply is entirely recovered in urine and does not improve cow performances unless a large PDIN deficit occurs, which is rarely the case in usual dairy diets. In practice PDIN does not exceed PDIE supply to avoid unnecessary N loses and an 8% deficit in PDIN supply remains tolerable (Vérité et Peyraud, 1989) even for high producing dairy cows.

Supply of metabolisable protein (i.e. PDI supply)

Milk yield increases with the PDI supply as described in the PDI system (Vérité et Peyraud, 1989). To enlarge the response curves over a wider range of situations, 5 experiments were conducted in our station (Vérité et Delaby, 2000). Different protein to energy ratio varying from 85 to 115 g PDI/UFL were tested on a total of 250 dairy cows fed ad libitum with maize silage diets. The effects of PDI supply are very large as the responses between the extreme levels were 4.2 kg for milk yield, 1.4 g/kg for milk protein content and 1.6 kg/day for DM intake.

The response curves are curvilinear. Near the recommendations (100 g PDI/UFL), the marginal responses are 0.6 kg milk, 0.2 point of protein content and 0.25 kg DM intake and 7% for the global efficiency (kg milk/kg DM intake) for 5 g increase of PDIE/UFL. Above the recommendations these responses are very small. On the contrary feeding low PDI ration to reduce N excretion rapidly leads to large negative effects for all the parameters. The marginal responses are doubled for a 10% deficit.

Figure 1: Milk and N excretion responses to variation in PDIE/UFL ratio in the diet



N excretion is increased. NU increases linearly at a rate of 15 g/day for a 5 g increase of PDIE/UFL. However, the relative N losses (excreted N / milk N) stay at a minimum as long as the PDI/UFL is below 100 and increases rapidly for higher supply of PDI.

The level of PDI is therefore an important way to control performances and N excretion. The average value of 100 g PDI/UFL is really a threshold. Any reduction of PDI supply below this value rapidly reduces performances whereas increased supply above this value dramatically increased N excretion with only marginal responses in animal performances.

Restitution of N on grazing area

Effect of the level of N fertilisation

N fertilisation modifies both the CP content of grass, the herbage production and the number of grazing days per ha (GD). In a 5 years experiment Delaby and Peyraud (1998) have shown that decreasing N fertilisation level from 320 to 100 and 0 kg/ha provides a decrease in milk output per ha (16050, 12600 and 10700 kg) which is proportional to the decrease in GD (689, 550 and 456). But the N excretion per ha decreased more rapidly (368, 236, 174 kg N/ha), especially urinary N (276, 162, 112 kg/ha). Finally the relative N excretions were reduced from 22.9 to 18.7 and 16.3 kg N/ t milk. Excreta being for the most part emitted directly on the paddock, the risk of N leaching is sharply decreased by reducing N fertilisation rate. From a quantitative description of the fate of N excreted, Decau et al (1997) showed from these data that the amount of N, which will be able to leach at the end of the grazing season, decreases from 161 to 44 and 28 kg/ha.

Reducing stocking rate at a given level of fertilisation also allows to reduce urinary N excretion per ha but the amplitude of the variation for a variations of 100 GD is two time less than that previously observed (30 vs 60 kg N, Vérité et Delaby, 2000) because stocking rate do not modify CP content of grass.

Effect of feed supplementation

The use of cereal-based concentrates has almost no consequences on N excretion per cow and per ha so long it does not modify the number of grazing days. The use of a concentrate rich in protein increases NI per cow and per ha for a same number of grazing days. N excretion is then increased while the milk response remains very low unless the CP content of grass is below 14% (Delaby et al 1996). On a complete grazing season, Soegaard and Aaes (1996) reported a 145 kg/ha increase in N excretion when the CP content of concentrate increases from 14 to 32%.

Supplementation with maize silage is an efficient way to reduce N excretion per cow (see above) but the advantage is less obvious considering the N restitution per ha. In fact maize silage allow to increase the number of grazing days as cow eat less grass and it transfers N from maize area to grass area.

Conclusion

N excretion is variable but these variations are not directly linked to risk of environmental pollution especially between different forage systems. Protein nutrition (and utilisation of the PDI system) allow to control feed N efficiency. Reducing N fertilisation allow to limit the risks of N leaching while maintaining reasonable milk production per ha.

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APPLICATION OF NON-THERMAL PLASMA TECHNIQUES (NTP) TO REDUCE EMISSIONS FROM ANIMAL HUSBANDRY

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Introduction

In regions with high animal production density, it is a demand to develop efficient filters for the cleaning of exhaust air. Non-thermal plasma techniques (NTP) are used increasingly for various applications and provide an approach to destruct noxious manure gases, particulary ammonia, hydrogen sulfide, volatile organic compounds (VOCs) and biological particles (Rice R.G., 2003). Most of the work in this field focuses on the removal of greenhouse and toxic gases and odour by direct treatment in the plasma (Zhang R. et al, 1996; Wang Y. and Goodrich, R., 2003). However, it is a procedural problem to direct that great amounts of air flow through a reactor, that are accruing in pig or poultry production.

A plasma produces ozone and in addition to it a variety of chemical radicals with short lifetimes. It is to resolve, which contribution the products have to ammonia reduction.

In a current research project the application of a NTP-reactor for reducing emissions from an animal livestock is examined. The aim of this experiment was to draw conclusions about exhaust air cleaning with NTP by comparing the removal of ammonia in a flow-through and in a bypass configuration.

Materials and methods

The plasma equipment consists of a power supply and as plasma unit a dielectric barrier discharge (DBD) from UltraKat, Gaggenau (Germany). The volume of the reactor is 470 ml, with gap distance of 2mm. The power is continuously adjustable up to 2100 Watt. The plasma is generated by applying an alternating high voltage across the gap, leading to many small electrical breakthroughs (streamer) in the gap. The dielectric barrier between the electrodes prevent the formation of a conducting channel.

The experiment was carried out under laboratory conditions in two different strategies of the NTP application. With the help of a lateral channel sealer the air was pushed through the reactor with a flow rate of 60m³/h according to a space velocity of 6,0 m/s and a contact time of 16 ms.

In the first strategy, ammonia was added in front of the NTP reactor, in the second strategy, ambient air flows through the reactor and ammonia was mixed afterwards with air coming from the reactor. The experiment was carried out by using various ammonia concentrations (10, 50, 100 ppm) and electric power (0, 750, 1250 W). The concentration of NH_3, N_2O, NO, NO_2 and O_3 was determined by a FTIR-Spectrometer (ThermoNicolet).

Results

The temperature at the outlet of the reactor raised in the experiment up to 50°C when applying 750 W, and up to 70°C when applying 1250 W. Ammonia concentration was reduced by the flow-through treatment (Tab. 1) as well as by the bypass treatment (Tab 2), whereas the efficiency was higher in the flow-through configuration than in the bypass treatment. With increasing ammonia concentrations, the efficiency was decreasing by the flow-through treatment from 100% to 47% and from 80% to 24% in the bypass treatment with an applied power of 1250 W. Less power at the NTP reactor reduces the efficiency of ammonia reduction, too.

Tab. 1: Nitrogen exchange [ppm] by using flow-through treatment against initial ammonia concentration and power.

NH_3	Watt	NH_3	N_2O	NO_x	O_3
	0	11,5	0,8	0	0
10	750	0	3,0	1,9	2909
	1250	-	-	-	-
	0	52,7	0,8	0,5	2
50	750	28,3	3,8	2,3	2468
	1250	9,6	5,2	1,1	4329
	0	101,5	1,2	0,1	1
100	750	74,3	4,3	2,9	2603
	1250	53,8	6,2	2,1	4548

Tab. 2: Nitrogen exchange [ppm] by using bypass treatment against initial ammonia concentration and power.

NH_3	Watt	NH_3	N_2O	NO_x	O_3
	0	10,3	1,1	0,0	1
10	750	1,2	2,4	2,0	2632
	1250	2,1	3,3	1,8	5109
	0	55,6	1,0	0,0	3
50	750	43,3	2,5	2,6	2516
	1250	33,7	4,1	1,9	4800
	0	103,6	1,2	0,4	0
100	750	88,0	3,8	2,3	2974
	1250	79,0	5,4	2,0	5404

However, by using NTP other noxious gases can be generated. Laughing gas (N_2O) in this experiment increased up to 6,2 ppm and nitric oxides (NO_x) up to 2,9 ppm. To act as an indicator for ionized air, ozone originated in dependence of an applied electric power of 750 resp. 1250W up to 2537 resp. 4837 ppm O_3 that is an equivalent of 52 resp. 56 g(O_3)/kWh.

Discussion

As an indicator for ionised air ozone was the only species that was detectable by our measuring technique. The production of ozone is comparable with the devices that are used for ozone-generation with a DBE-reactor. We found a high correlation between ozone production and

ammonia reduction, in good agreement with the work of Ruan et al. (2004) and Wang & Goodrich (2003). However, for the reduction of ammonia it is not mandatory that the air is fed through the plasma reactor. The higher efficiency in the flow-through treatment could base on radicals decaying fastly after leaving the reactor, thus in the bypass-treatment a huge amount of theses radicals should be decayed, and ozone is assumed to be responsible for ammonia reduction.
The plasma induced chemical process of ammonia-reduction is characterised through a lot of complicated reactions and it's not possible to acquire all this reactions. In an experiment of several days we found deposits of Ammonium nitrate. This is an indication, that ozone oxidises ammonia in humid air to nitric acid (Rip G. Rice, 2003), but the proportion of this reaction is unknown and has to be verified in further investigations.

Conclusion

The use of the NTP-application for the treatment of exhaust air is an innovative approach, but requires the avoidance of the synthesis of noxious gases by varying the flow rate, amplitude, duty cycle or temperature. The air treatment by using a bypass configuration could be a solution to the great amounts of air flow in animal husbandry. Furthermore it is necessary to implement a catalytic converter to eliminate ozone. In accordance of the results in this investigation it is projected to apply the DBE-reactor in a pig fattening stable with 10 pig places. The exhaust air should be feed to a part of 500m³/h through the DBE-reactor, the other part of air-flow should be treated in a Bypass-prozess.

Acknowledgements

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GASEOUS EMISSIONS IN THE RAISING OF WEANED PIGS ON FULLY SLATTED FLOOR OR ON STRAW-BASED DEEP LITTER

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Introduction

Gaseous emissions from agriculture contribute to a number of environmental effects. Ammonia emissions are responsible of soil acidification and eutrophication. Carbon dioxide, methane and nitrous oxide are greenhouse gases taking part to the global problem of climate change. The global warming potential (GWP) is estimated to 21 for methane and 310 for nitrous oxide times the GWP of CO_2 (Billiard, 1998). Gaseous emissions from livestock houses are dependant from the housing and floor systems. Very few experiments have compared in standardized conditions gaseous emissions according to floor systems. The aim of the study was to compare emissions from a pig house with weaned pigs either on a fully slatted floor or on a deep litter.

Material and methods

Two identical rooms with an area of 30 m^2 and a volume of 103 m^3 were arranged to house simultaneously a group of 40 weaned pigs on a fully slatted floor in one and on a deep litter of straw in the other one. The slatted floor was in plastic panels with a void percentage of 37%; the floor area was 0.3 m^2/pig. The slurry pit was 50 cm deep. Before the arrival of the first animals, 600 l water were poured into the pit to have a 5 cm water layer in the bottom. Straw deep litter was realized with a 30 cm layer before the arrival of the animals. Thereafter supplementary quantities of straw were provided depending on the cleanliness of the litter. The available floor space per animal was 0.5 m^2. The total amount of straw used was 8 kg/pig.

Each room was ventilated with an exhaust fan. Fresh air entered through an opening which was connected to the service corridor of the building. The air temperatures of the two rooms and the corridor were measured automatically every hour. The ventilation rates were measured continuously and the hourly means were recorded with an Exavent apparatus (Fancom®).

In each room, two successive batches of 40 pigs were raised, without changing the litter or emptying the slurry pit between batches. The pigs were fed ad libitum, food containing 176 g crude protein per kilo. The quantities of food ingested and water consumed were determined per batch.

The concentrations of gases in the air in the two experimental rooms and the corridor supplying fresh air were measured with an apparatus from Innova Air Tech Instruments (1312 Photoacoustic Multi-gas Monitor) equipped for the measurement of NH_3, N_2O, CH_4, CO_2 and H_2O. For each batch, the concentrations were measured three times at about one week intervals and for 6 consecutive days respectively. The multi-gas monitor was programmed by conducting 2 measurements per hour at each sampling point. The emissions were calculated on an hourly basis utilizing the following formula : $E\ (mg \cdot h^{-1}) = D \times (C_i - C_e)$ with D, the hourly mass flow (kg air$\cdot h^{-1}$); C_i and C_e the hourly concentrations of gas in the air of the room or corridor (mg$\cdot kg^{-1}$ dry air). The mean emissions per pig per day were calculated for each series of measurements.

For each batch and each gas and for the combined data obtained with the two batches, the differences of the emissions with regard to the floor system were tested in the form of a mixed model for repeated measurements (SAS, proc MIXED).

Results

Climatic characteristics of the rooms

The average temperatures of the air were 26.4°C in the room with the slatted floor and 23.9°C in the room with the deep litter. The mean ventilation rates were 238 and 216 m^3/h for the two rooms respectively.

Performance of the pigs

The mean initial and final weights were respectively 7.2 ± 1.15 kg and 23.55 ± 3.3 kg. There was no significant difference between the daily weight gain of the pigs raised on deep litter (387 ± 65 g/day) or on slatted floor (379 ± 64 g/day). The food conversion ratio (kg/kg) were respectively 1.57 on deep litter and 1.74 on slatted floor.

Amounts and composition of manure

The amounts of slurry and of straw manure removed at the end of the experiment were respectively 37.0 kg per pig at 163 g dry matter (DM) per kg and 27.5 kg per pig at 326 g DM per kg. The slurry and deep litter nitrogen contents were respectively 64.5 g and 30.7 g per kg DM or 389 and 276 g per pig.

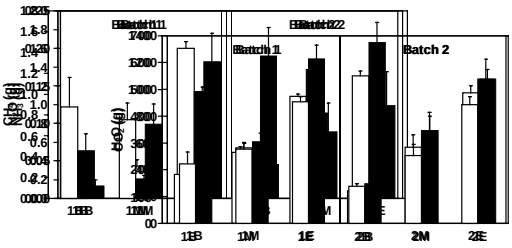
Gas emissions

Figure 1 shows the evolution of the emissions from the beginning to the end for each post-weaning period. Table 1 presents the mean emissions observed for each batch.

Over the 2 post-weaning periods altogether, pig raising on deep litter produced proportionately 100% more NH_3, 5% more CO_2, 16% more H_2O and 18% less methane than pig raising on slatted floor. Differences were however significant only for NH_3.

No N_2O was produced from the slurry and the emission from the deep litter was observed only during the stay of the second batch.

Figure 1. Gas emission (per pig per day) during the raising of 2 batches of weaned pigs on slatted floor (open bars) or on straw (closed bars). Three series of measurements per batches (mean ± s.d.) at the beginning (B), middle (M) and end (E) of the post-weaning periods.



Gas emissions increased regularly from the beginning to the end of each post-weaning period whatever the floor system.

Table 1. Gas emission (per pig per day) in the raising of two batches of weaned pigs on slatted floor or on straw.

		Slatted floor	Straw	s.e.	S
Batch 1	NH_3 (g)	0.35	0.80	0.18	
	N_2O (g)	0.00	0.00	-	
	CH_4 (g)	1.03	0.56	0.06	*
	CO_2 (g)	308	327	38.1	
	H_2O (g)	634	694	104	
Batch 2	NH_3 (g)	0.41	0.69	0.18	
	N_2O (g)	0.00	0.09	0.04	
	CH_4 (g)	0.79	0.95	0.09	
	CO_2 (g)	298	340	28.6	
	H_2O (g)	556	685	99.8	
Batch 1 and 2	NH_3 (g)	0.38	0.74	0.08	*
	N_2O (g)	0.00	0.05	0.02	
	CH_4 (g)	0.91	0.75	0.10	
	CO_2 (g)	303	334	19.7	
	H_2O (g)	595	689	48.1	

s.e. : mean standard error S : significance

Discussion

The emission of NH_3–N from piggeries with slatted floors is estimated to be 23% of excreted nitrogen (Guillou et al., 1993). In this experiment the NH_3–N emission from the slurry was only of 5% of wastes–N. The measurement period was however limited to 85 days and at the beginning of the experiment the slurry pit was clean with a 5 cm water layer in the bottom. In comparison, the NH_3–N emission from the deep litter was 17% of wastes–N. N_2O–N emission from the deep litter was 1% of wastes–N and emission of N_2 from the deep litter was estimated to be 17% of wastes–N. The cumulative N emission from these 3 gases was thus 35% of wastes–N. This value is lower than that observed in a previous experiment (59%) when five successive batches of weaned pigs were raised on the same litter without changing the litter between batches (Nicks et al., 2003). No N_2O production was observed from the slurry and according to the nitrogen balance these was no production of N_2. As a consequence the N content of the slurry was about 41% higher than that of the deep litter.

The levels of CH_4 emissions observed in this experiment suggest that the productions both in the slurry and in the deep litter were very low and that CH_4 in the 2 experimental rooms come essentially from the digestive tract of animals. In the same way, levels of CO_2 emissions indicate that the respiration of the animals was the principal source of CO_2 in the experimental rooms. So there was no significant difference in relation with the floor system.

In conclusion, the main differences concerning the environmental effects of rearing weaned pigs either on slatted floor or on deep litter are : a lower NH_3 emission when pigs are on slatted floor and a lower N content of the manure when they are kept on deep litter.

Acknowledgements

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MICROBIAL PHYTASE AS A MEANS TO REDUCE THE ENVIRONMENTAL IMPACT OF ZINC IN PIG FEEDING

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Introduction

Zinc oversupplied in pig feeding concentrates in manure and may cause environmental pollution (Jondreville et al., 2003). Lowering zinc dietary supply is undoubtedly an efficient way to prevent this adverse effect on environment. However, such an approach requires an accurate knowledge of zinc dietary requirement and of the factors by which it may be altered.

The NRC (1998) recommends a zinc supply of 80 mg / kg diet to weaned piglets. However, the zinc requirements of these animals have not been recently re-assessed.

In addition, although improved zinc availability by the addition of microbial phytase to piglet diets was previously reported (e.g. Lei et al., 1993), a zinc equivalency for phytase has not yet been established.

Therefore, four experiments were conducted in our laboratory in order to update the zinc dietary requirements of weaned piglets and to evaluate the sparing effect of phytase on the need for supplementation of diets with zinc as sulphate.

Material and Methods

In each experiment a basal diet was formulated to exceed all nutrient requirements for piglets weighing between 5 and 20 kg (INRA, 1989; NRC, 1998), except zinc. All of them were made of 79 to 90% of feedstuffs of plant origin. Their zinc, phytic P and Ca concentrations were 30 to 33 mg, 2.1 to 2.9 g and 8.3 to 9.6 g / kg, respectively (Table 1).

Table 1 : Characterisation of each basal diet without zinc and phytase added

Experiment	1	2	3	4
Plant feedstuffs (%)[1]	79	83	90	82
Dry matter (DM) (g / kg)[2]	905	896	880	903
P (g / kg)[2]	7.6	7.3	5.8	6.8
Phytic P (g / kg)[3]	2.5	2.9	2.5	2.1
Ca (g / kg)[2]	9.5	9.5	8.3	9.6
Phytase activity (U / kg)[2]	116	150	139	60
Zn (mg / kg)[2]	32	33	30	30
Cu (mg / kg)[2, 5]	26	8	6	11
Phytate:Zn[4]	27	31	29	25
(Ca x Phytate):Zn[4]	7.2	8.2	6.9	6.6

[1] Maize, soybean meal, isolated soy protein and heated wheat bran.
[2] Analysed according to the procedures described by Revy et al. (2004).
[3] Calculated from INRA-AFZ (2002).
[4] Molar ratio calculated accounting for a P content in a phytate molecule of 28.18% and a molecular weight of 660, 65.4, 40.08 for phytate, Zn and Ca, respectively. (Ca x Phytate):Zn is expressed as g / kg DM.
[5] Including 20, 5 and 5 mg / kg diet added as sulphate for experiments 1, 2 and 4, respectively.

The experimental diets were obtained by adding zinc as sulphate and/or microbial phytase (Natuphos ®, BASF AG, Ludwigshafen, Germany) to the basal diet. In experiment 3, dietary copper supply also varied (Table 2).

In each experiment, crossbred (Piétrain, Large White x Landrace) piglets, weaned at 28 d, were fed the basal diet without zinc nor microbial phytase added for a 7-day period. For the subsequent 19- to 21-day (experiments 1, 2 and 4) or a 30-day (experiment 3) period, animals were housed individually in stainless steel and plastic pens and fed one of the experimental diets. Six piglets were used per treatment. At the end of each experiment, the zinc status of piglets was evaluated through plasma alkaline phosphatase activity (APA), plasma zinc concentration and bone zinc concentration. The experimental and analytical procedures were detailed by Revy et al. (2004).

Table 2 : Experimental treatments

Exp.	Supplement [1]								
1	Zn	0	20	0	20				
	Phytase	0	0	1200	1200				
2	Zn	10	25	40	60	80	0	10	25	40
	Phytase	0	0	0	0	0	700	700	700	700
3	Cu	20	0	0	0				
	Zn	100	50	100	50				
	Phytase	0	0	0	850				
4	Zn	10	25	40	100	0	0	0	0
	Phytase	0	0	0	0	150	250	500	850

[1] Cu, mg / kg diet as $CuSO_4$; Zn, mg / kg diet as $ZnSO_4$; Microbial phytase, U / kg diet, as Natuphos ®

Statistical analysis of data was performed by means of the NLIN procedure of the SAS software (SAS Institute, Cary, NC, USA), using treatment means. The models included the experiment effect.

In accordance with the response of phosphorus availability to phytase supplementation (Kornegay, 2001), the equivalency equation of zinc added as sulphate for microbial phytase was supposed be of the following form: $a (1 - e^{- k Phyt})$, with Phyt = microbial phytase added (U / kg diet).

For plasma APA and zinc concentration the model was a linear plateau model of the following form: If $Zn + a (1 - e^{- k Phyt}) < Opt$, $Y = Max + b [Zn + a (1 - e^{- k Phyt}) - Opt]$; If $Zn + a (1 - e^{- k Phyt}) \geq Opt$, $Y = Max$.

For bone zinc concentration the model, which involved two linear splines with no plateau (Wedekind et al., 1994), was as follows: If $Zn + a (1 - e^{- k Phyt}) < Opt$, $Y = Max + b [Zn + a (1 - e^{- k Phyt}) - Opt]$; If $Zn + a (1 - e^{- k Phyt}) \geq Opt$, $Y = Max + c [Zn + a (1 - e^{- k Phyt}) - Opt]$,

with Zn = dietary zinc (mg / kg diet), Phyt = microbial phytase added (U / kg diet) and Y = response criteria.

Results

The parameters of the models are presented in Table 3 and the response of plasma zinc to dietary zinc at different levels of phytase is presented in Figure 1.

Without phytase added, the requirements were fulfilled when the supply of total zinc was 84, 75 and 87 mg / kg diet, accounting for plasma zinc, plasma APA and bone zinc as indicator of zinc status, respectively. All indicators

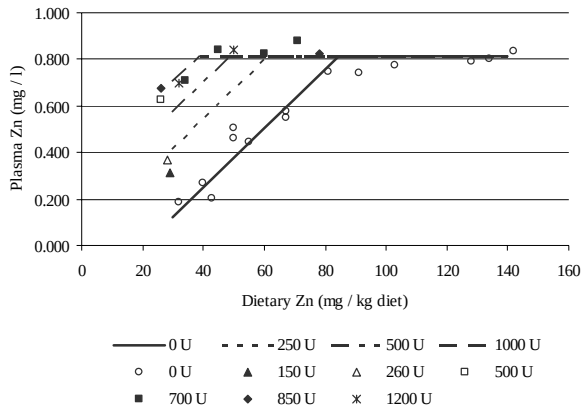
taken into account, the supply of zinc needed to meet the requirements was reduced down to 61, 49 and 42 mg / kg diet when the diets were supplemented with 250, 500 and 750 U of phytase, respectively. Two-hundred and fifty, 500 and 750 U of phytase were thus equivalent to 21, 33 and 40 mg of zinc as sulphate, respectively.

Table 3 : Prediction of indicators of zinc status according to dietary zinc (mg / kg diet) for different amounts of phytase added (U / kg diet) [1]

Phytase added	Breakpoint [2]	b	c	Max
		Plasma zinc (mg / l)		
0	84	0.0127		0.815
250	62	0.0127		0.815
500	49	0.0127		0.815
750	43	0.0127		0.815
		Plasma APA (U / l)		
0	75	4.52		237
250	58	4.52		237
500	47	4.52		237
750	41	4.52		237
		Bone zinc (mg / kg ash)		
0	87	2.57	0.635	226
250	65	2.57	0.635	226
500	51	2.57	0.635	226
750	44	2.57	0.635	226

[1] For plasma zinc and alkaline phosphatase activity (APA) calculated as follows: If Zn < breakpoint, Y = Max + b * (Zn - breakpoint), if Zn Ó breakpoint, Y = Max;
for bone zinc, calculated as follows: If Zn < breakpoint, Y = Max + b * (Zn - breakpoint), if Zn Óbreakpoint, Y = Max + c * (Zn - breakpoint).
[2] Breakpoint = Opt - a $(1 - e^{-k\,Phyt})$, with Opt = 84, 75 and 87; a = 49.8, 44.6 and 54.5 and k = 0.00247, 0.00191 and 0.00215 for plasma zinc, plasma APA and bone zinc, respectively.
Zn = dietary zinc (mg / kg diet) and Phyt = microbial phytase added (U / kg diet)

Figure 1 : Prediction of plasma zinc according to dietary zinc (mg / kg diet) for different amounts of phytase added (U / kg diet) [1]



[1] Parameters of the equations are presented in Table 3.

Discussion

The zinc requirements estimated from plasma and bone zinc are slightly higher than the 80 mg of zinc / kg diet recommended by the NRC (1998) for piglets between 10 and 20 kg. However, in accordance with the current results, Höhler and Pallauf (1994) also reported that a corn-soybean meal diet supplemented with zinc as sulphate to contain 80 mg of zinc / kg did not maximise plasma zinc concentration in weaned piglets.
The current zinc equivalencies for phytase are consistent with the 30 mg of zinc as sulphate for 1350 U of microbial phytase that can be derived from the plasma zinc data published by Lei et al. (1993). The equivalency values for the highest amounts of phytase added exceed the total zinc contained in the basal diet. This suggests that dietary zinc released by phytase is better absorbed than zinc added as sulphate in the basal diet and/or that microbial phytase prevents endogenous zinc to be complexed by phytates (Oberleas and Harland, 1996).
The body zinc retention by pigs being very low, zinc ingested is almost totally excreted (Jondreville et al., 2003). Thus, zinc excreted may be reduced by almost 30% by replacing 30 mg of zinc as sulphate by 500 U of phytase in a piglet diet formulated to contain 100 mg of zinc / kg.

Conclusion

From a practical point of view, the current results allow to update the requirements of zinc by weaned piglets. In addition, they indicate that microbial phytase provides an important means to reduce not only phosphorus but also zinc excretion by pigs.

Acknowledgements

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EFFECT OF A BIOSCRUBBER ON EMISSIONS OF BIOAEROSOLS FROM A DUCK FATTENING UNIT

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Introduction

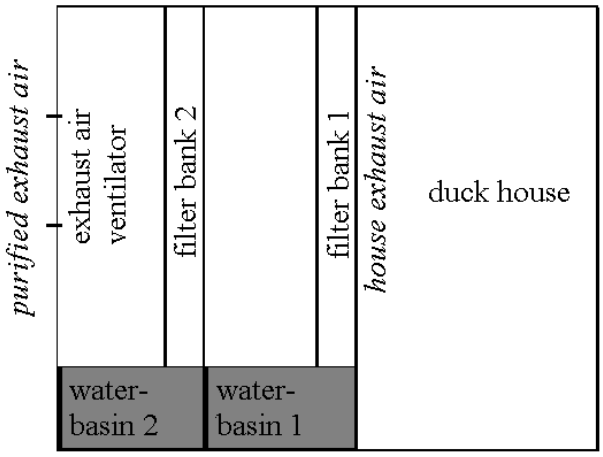
Bioscrubbers are used to reduce odour and noxious gas emissions from animal houses in poultry and pig production. However, the effect of bioscrubbers on emission of organic dust has not been well studied.
The aim of this work was to study the influence of a bioscrubber in a duck fattening unit on emission of airborne dust, bacteria, endotoxins and moulds.

Material and Methods

- principle of the bioscrubber

The determined exhaust air washer was a combined bioscrubber and chemowasher. The system consisted of two treatment processes. The air moved to two filter banks. Those filter banks were made of cellulose-combs. Through the filter banks water flows constantly from top to bottom. The water ran into a water basin and was circulated (figure 1).

figure 1: principle of the exhaust air washer



In this way dust and ammonia and odorous substances attached to dust, were washed out into the water basins. To get a higher reduction efficiency of ammonia acid was added to the washing fluid.

- investigated parameters

For our study we took air-samples from house exhaust air and from the purified exhaust air with filtration (PGP dust-sampling system; Ströhlein GmbH, Germany), impaction (Andersen-sampler; Andersen 1958) and impingement (AGI-30; Brachmann, et al. 1964). Further the hygienic quality of the washing fluid was investigated.

Following parameters were investigated:

- inspirable dust (PGP dust-sampling system)
- endotoxins in the inspirable dust (LAL-Test, QCL-1000, Cambrex)
- concentration of airborne aerobic bacteria (impingement, AGI-30; Standard I agar)
- concentration of airborne gram-negative aerobic bacteria (impaction, Andersen-sampler; MacConcey 3 agar; Zucker et al., 2000)
- concentration of airborne moulds (Andersen-sampler, DG-18-agar; Schütze, 2001)
- concentration of endotoxins in the washing fluid (LAL-Test, QCL-1000, Cambrex)
- concentration of aerobe gram-negative bacteria in the washing fluid (MacConcey 3 agar)
- species of gram-negative bacteria in air and washing fluid (api 20 NE and api 20 E; bio Mérieux).

Results

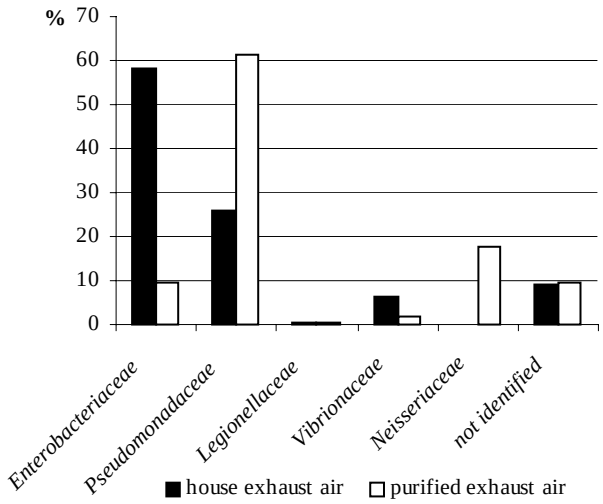
- reduction effects

The bioscrubber reduced the concentration of airborne dust, the concentration of endotoxins and the amount of total aerobic bacteria significant (Wilcoxon-test; SPSS).
In all cases (n=22) the emissions of dust, endotoxins and total aerobic bacteria were reduced. On average (median) the bioscrubber reduced the emission of dust by the factor 3.0 (min 1.0; max 33.5), the emission of endotoxins by the factor 12.5 (min 1.5; max 66.1) and the emission of total aerobic bacteria by the factor 6.1 (min 1.5; max 314.1).
There was no significant reduction of the emission of airborne gram-negative bacteria and airborne moulds by the washing process. The concentrations of airborne gram-negative aerobic bacteria were reduced in 14 of 22 cases and boosted in 7 cases. In one case the concentration didn't change. The concentrations of airborne moulds were reduced 16 times and raised in 6 times. In maximum the emission of airborne gram-negative bacteria increased by the factor 14.8. For airborne mould a maximal boost of the emission by the factor 3.8 was detected.

- Composition of airborne gram-negative bacterial flora in house and purified exhaust air

The species composition of the airborne gram-negative bacterial flora was different between house exhaust and purified exhaust air. In the house exhaust air we found mainly *Enterobacteriaceae* (58,3%) in the purified exhaust air mainly *Pseudomonadaceae* (61,4%) (figure 2).

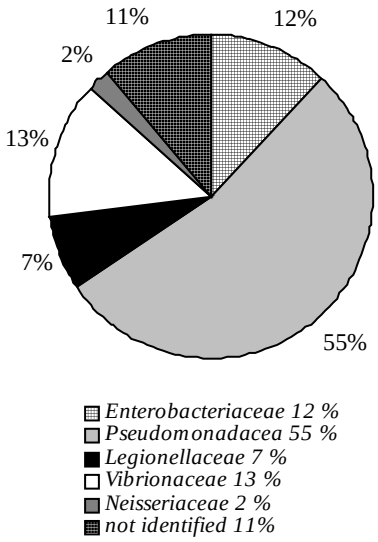
figure 2: composition of the bacterial flora in house and purified exhaust air



- Gram-negative bacterial flora in the washing fluid

In the washing fluid always high concentration of gram-negative bacteria were detected. *Pseudomonadaceae* were with 55% the predominating bacteria in the washing fluid, followed by *Vibrionaceae* (13%) and *Enterobacteriaceae* (11%) (figure 3).

figure 3: composition of bacterial flora in the washing fluid



Discussion and Conclusion

Our results indicate that the bioscrubber investigated in this study, which is mainly used to reduce odour and noxious gas emissions, has also the potential to reduce the emission of dust, total bacteria and endotoxins from animal houses. Further our results indicate that the washing fluid of the bioscrubber, which was recirculated, represented a secondary source for bioaerosol emissions.
Due to multiplying of different bacterial species (e.g. *Pseudomonas* spp.) in the washing fluid the composition of the bacterial flora in the purified exhaust air is different to the airborne bacteria flora in the duck house. Therefore techniques that are able to improve the hygienic quality of the washing fluid should be tested (e.g. UV-radiation, ozonation).

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STAPHYLOCOCCI AS AN INDICATOR FOR BACTERIAL EMISSIONS FROM A BROILER HOUSE

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Introduction

The air of animal houses contains bioaerosols like viruses bacteria, fungi, endotoxins and allergens that present a health risk for humans and animals [1]. Airborne pathogen bacteria, fungi that cause allergies and endotoxins were detected in poultry houses [2]. The spreading of bioaerosol emissions from poultry houses is less understood and therefore it is difficult to estimate the health risk on humans and animals caused by this emissions. A possibility to show the distribution of bioaerosols originating from the air of these livestock buildings is to use viable indicator bacteria. These bacteria need a sufficient survival time in the airborne state and should reach high emission rates because the detection of these organisms in longer distances downwind of the animal house depends on their source strength [3, 4]. Bacteria that possess these characteristics are staphylococci.
Staphylococci are commensales of the skin of poultry. Pathogenic and non pathogenic species were isolated from the skin and the nasal passages of the birds. Skin debris, broken feather barbules and particles from litter and faeces are dust compounds in poultry houses [2] to which the staphylococci can become attached. Staphylococci were the predominant airborne bacteria and the most present genus in airborne dust of broiler houses.
The objectives of this study were to quantify and to identify airborne staphylococci in the air of a Louisiana type broiler barn holding initially 40.000 broilers on litter and to estimate the travelling distance of these bacteria in the ambient air downwind the building.

Material and Methods

Sampling of bioaerosols

Bioaerosols were sampled by AGI-30 impingers in 50 ml 1:1 glycerol-phosphate buffer solutions placed at 1,5m height in the middle of the broiler house and at 1,5m, 4,0m and 10m heights at defined sampling places in the surroundings of the Louisiana barn. The sampling times were 30 min. in the barn and 90 min. in the field. The Impingers for the outdoor measurements were fixed in coloured [uv protection] white and insulated plastic holders at weather masts (Clark Masts Teksam NV, Belgium) to reflect sun radiation and prevent freezing in winter. The positions of the masts relative to the middle point of the barn were determined with a TC 110 Tachymeter (Leica Geosystems, Switzerland).

Meteorology

The wind speed and the wind direction were measured downwind (*lee* side) the barn with a UNIKLIMA 7 weather station and calculations of the weather data were made with the UK_TOSS-SOFTWARE (TOSS, Potsdam).

Cultivation of micro-organisms and identifying staphylococci to the genus level

Aliquots or diluted aliquots from the impinger solutions were plated on mannit-salt agar and on blood-agar basis (OXOID LTD, Basingstoke, Hampshire, England). After incubation the airborne colony forming units (cfu) per m^3 were calculated as described by Lin et al. [5].
Grown colonies of staphylococci on mannit-salt agar were identified to the genus level by morphology, motility, gram staining, catalase test, oxidase test and lysostaphin susceptibility.

PCR identification

A 16S-23S rDNA intergenic spacer PCR was used to identify *staphylococcus* species. A part of typical colony grown from staphylococci was transferred in a PCR reaction tube and incubated with 5 µl of a 100µg ml^{-1} lysostaphin solution for 15 min at 37°C. Then 5 µl of a GeneReleaser (BioVentures, Inc.) was added and a thermocycle programme followed by the manufacture's procedure was started.
A modified PCR amplification method based on a proposal of Mendoza et al. [6] was carried out.

Electrophoresis and imaging

PCR products were separated in 3 % agarose gels in TBE buffer. The gels were stained in TBE buffer with 0,5 µg ml^{-1} ethidium bromide and photographed with a BioDocAnalyze system (Biometra, Göttingen).

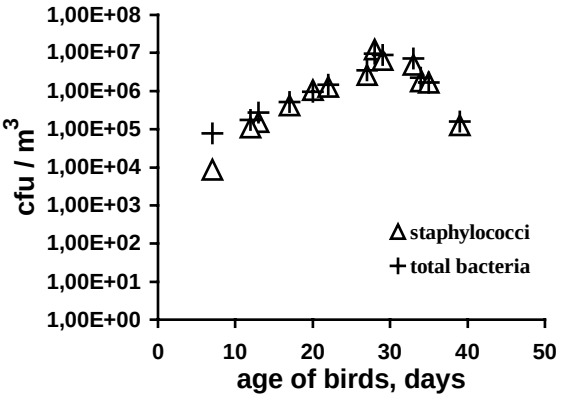
Identification of staphylococci species

Identification of the airborne staphylococci species was carried out as proposed by Mendoza et al. [6] and also with the ID 32 STAPH test (bioMérieux, Lyon).
Sometimes variations of the PCR fragments migration provide unclear results. In these cases we cleaned the PCR products with the MinElute PCR Purification Kit (QIAGEN, Hilden) and restricted the purified products with 10U DraI as described by the manufacturers protocol (Qbiogene, Heidelberg). For identification these patterns were compared to restricted PCR products of staphylococci strains from the DSZM (Deutsche Stammsammlung für Mikroorganismen und Zellkulturen, German type culture collection, Braunschweig).

Results

Figure 1 shows the results of the bacteria counts (total bacteria and staphylococci).

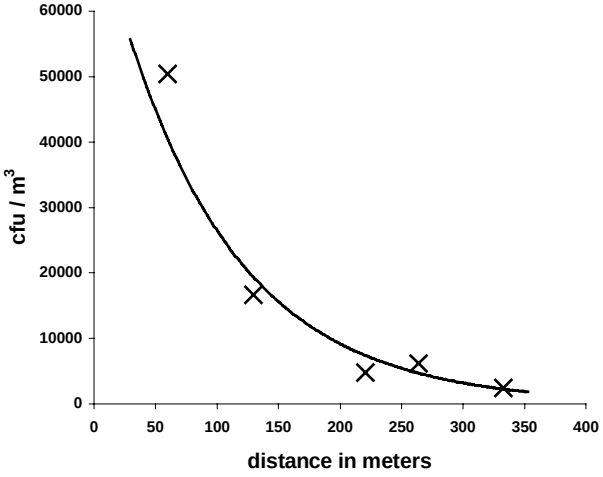
Figure 1 Numbers of airborne bacteria in a Louisiana stable holding initially 40.000 broilers on litter.



After one week the bacteria concentration is between 10,000 (staphylococci) and 100,000 (total bacteria). The counts rise to about 10 million after 30 days and drop to 100,000 again at day 40. This decrease is mainly due to the fact that at day 35 about 9000 of the biggest birds were taken to slaughter. The sometimes higher concentrations of staphylococci compared to the so called total bacteria count is caused by the different culture media which were used. It can happen that on blood agar base the colony counts vary considerably because of competitive growth or motile bacteria.

The staphylococci concentrations on the *lee* side showed a strong (r = 0,97) exponential decrease (Fig. 2). No staphylococci were found on the *luv* (upwind) side in the samples taken in parallel (detection limit 300 cfu of staphylococci per m^3).

Figure 2 Staphylococci on the *lee* side of a broiler house. Results from field measurements (May until September) at ages of birds older than 20 days. The sample points were positioned in the main wind direction and the wind speed was between 1.1 m/s and 4.1 m/s.



In addition to the overall quantification of staphylococci in the environment, the species of the genus *Staphylococcus* were also determined (Table 1).

Table 1 Predominant airborne *staphylococcus* species from samples in the house and from the parallel taken samples in the environment. At six different days 20 colonies from an outdoor sample and 20 colonies from an indoor sample were analysed.

Species found outdoor	Species found indoor
S. saprophyticus, S. cohnii, S. arlettae and *S. lentus*.	*S. saprophyticus, S. cohnii, S. arlettae* and *S. lentus*.

S. saprophyticus, S. cohnii and S. arlettae belong to the *S. saprophyticus* group whereas *S. lentus* is attributed to the *S. sciuri* group. *S. xylosus* was found in low concentrations and other species appeared occasionally only.

Discussion

The present study supports previous investigations in which the staphylococci were the predominant species in the air of broiler houses. The isolated and identified species in the indoor and the outdoor air were found on poultry skin and also in the litter. This indicates that skin particles and particles from the litter were the main source of airborne staphylococci. In this study the predominant species were coagulase negative. *Staphylococcus saprophyticus* is pathogen for humans and the other species may act as opportunistic pathogens in humans and animals. Additionally, these species can harbour a variety of resistant genes which could be transferred to pathogen bacteria like *Staphylococcus aureus*. A potential health risk from the identified species cannot be excluded for humans and animals.

The measured staphylococci concentration on the downwind side in relation to the distance of the animal house were higher as the concentration of indicator bacteria found in previous investigations. One important factor for detecting the dispersion of airborne bacteria from an emission source is the source strength [3, 4]. The source strength of the explored Louisiana barn was calculated based on the CO_2 balance method and it was about 1 x 10^9 staphylococci per second when the broilers were older than 20 days. This source strength for indicator bacteria was clearly higher as in studies, e.g. of Müller et al. [4]. Other factors like the sampling method, the meteorological conditions, the type of animal house and the topographic features have an effect on the dispersion of airborne bacteria [4].

The identification of indicator bacteria in more than 300 m distance downwind of the animal house suggests that other airborne bacteria including pathogens and dust bound viruses from the animal house can travel the same distances by air. More studies are necessary to verify the presented results.

Conclusion

Staphylococci seem to be a useful indicator bacteria to estimate the travelling distance of airborne bacteria from broiler houses. The results of this study can help to define safe distances between animal houses and between animal houses and residential areas.

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More specific literature with the authors on request.

INTERACTIONS BETWEEN BOVINE AND ROE DEER (*CAPREOLUS CAPREOLUS*) FOR *BABESIA SP.* INFECTIONS

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Introduction

Babesia divergens is the major agent of bovine babesiosis in France. This parasite expresses a lower specificity than other *Babesia* species; *B. divergens* can be cultured in human [1], sheep [2] or rat [3] red blood cells (RBC) and this protozoa can experimentally infect gerbil [4], rat [5] or sheep [2]; furthermore, *B. divergens* babesiosis is a zoonotic disease, notably in splenectomized human [6]. Field data about the potential hosts of *B. divergens* are very poor; but *Ixodes ricinus*, the vector of *B. divergens*, can bite different hosts, notably bovine or *Cervidae*.

In France, an increase of roe deer populations is observed and roe deers are more frequent in the fields where cattle are also present. Small forms of *Babesia* have been described in *Cervidae*, notably *B. capreoli* in roe deer in Europe [7]. They showed major serogical cross-reactions with *B. divergens* [8, 9].

The aims of this study were to confirm the seroprevalence of *Babesia* in roe deer in France using *B. divergens* antigens and to isolate and characterize roe deer *Babesia* for further investigations on interactions between bovine and roe deer for *Babesia* infections.

Material and Methods

A total of 355 samples were collected in roe deers killed by hunters in the 6 départments of Loire Atlantique, Mayenne, Maine et Loire, Sarthe, Vendée and Ille et Vilaine. Sera were extracted from the cardiac thrombus. Serological investigations were done using IFAT with *B. divergens* parasitized gerbil blood as antigen. The roe deer sera were diluted in phosphate buffered saline (PBS) pH 7.2 from 1:80 to 1:1280. Fluorescein isothiocyanate-labelled rabbit anti-goat immunoglobulins (SIGMA) diluted in PBS (1:100) were used to detect specific antibodies.

For blood parasite isolation, a total of 53 roe deer were investigated: 5 from the previous protocol, the others were trapped in the three wild fauna reserves of "Chizé" (Deux sèvres) (30 animals), "Trois Fontaines" (Meuse) (15 animals) and "La Haute Touche" (Indre et Loire) (3 animals). Blood samples were collected by jugular puncture with Citrate Dextrose Phosphate supplemented Venoject tubes.

In vitro cultures were performed as previously described [2, 10] with some modifications. Briefly, blood samples were centrifuged; plasma and buffy coat were discarded and the erythrocytes were washed in RPMI 1640 (Biowhitaker); cultures were initiated in 24 wells culture plates at 7.5 % haematocrit in RPMI 1640 supplemented with 20 % of fetal bovine serum; 50 µg/ml gentamicin and 2,5 µg/ml Amphoterincin B were added to avoid fungal, bacterial and *Trypanosoma* development. Cultures were performed in humidified 6 % CO_2 atmosphere at 37°C. The media were changed every two or three days during 3 weeks. For positive cultures, subcultures were done in autologous roe deer erythrocytes and in sheep erythrocytes with 10 % of previous culture. After 5 subcultures, cultures were performed in 25 cm^2 culture flasks, in autologous erythrocytes suspended at 2.5 % haematocrit in the same culture medium which were changed every two days. When parasitaemia reached at least 10 %, cultures were frozen in liquid nitrogen for further investigations.

For 7 *Babesia* isolates, gerbils (*Meriones unguiculatus*) were inoculated intraperitoneously with 2.10^6 parasitized roe deer erythrocytes.

Results

A total of 268 roe deers (268 / 355; 75 %) were positive in *B. divergens* IFAT. A total of 43 cultures (81 %) were positive for *Babesia sp.*; only 33 (62 %) subcultures could be performed in autologous RBC. For 32 of these isolates, a typical small form of *Babesia* was observed; morphological characteristics were very similar to those described for *B. capreoli* or *B. divergens*, with numerous parasites occupying the periphery or the margin of the erythrocyte and some tetrads (fig 1).

For these small form *Babesia*, subcultures in sheep red blood cells could not be performed; furthermore, inoculations to gerbils were unsuccessfull.

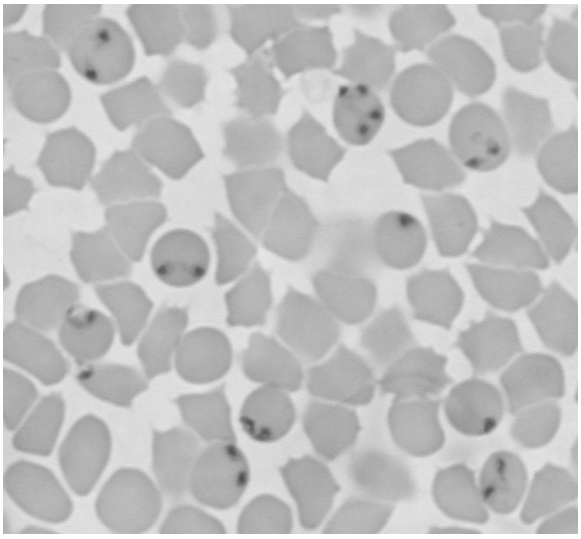

figure 1 : in vitro cultures of the small form of roe deer Babesia.

In one animal originating from "La Haute Touche", a large form of *Babesia* was observed with typical large geminated merozoites and numerous ovoid forms occupying the all diameter of the erythrocyte (fig 2). Subcultures in sheep erythrocytes were successful and this isolate could be continuously subcultured every 2 or 3 days. Inoculation to gerbil was unsuccessfull.

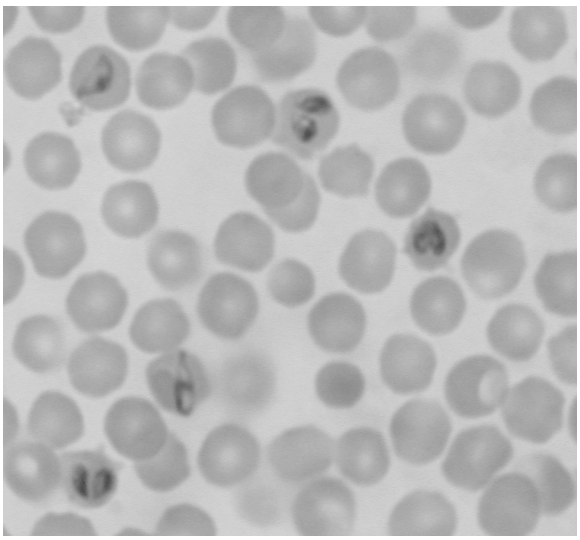

figure 2 : in vitro cultures of the large form of roe deer Babesia.

Discussion

As previously described [9], the serological prevalence against *B. divergens* was very high in investigated roe deer populations. Using in vitro isolation of the blood parasites, the prevalence of small forms of *Babesia* was similar to serological prevalence and 32 small forms isolates were produced.

B. divergens can be cultured in sheep red blood cells [2,10] and gerbils are experimental hosts [5]. In the contrary, the small *Babesia* isolates were unable to infect gerbils and were unable to grow in sheep red blood cells; furthermore, for 6 isolates, in vitro cultures were tested in human red blood cells as described for *B. divergens* [1] and were unsuccessfull. Because of these different biological properties, there is a high probability that the isolated small forms are *B. capreoli* and not *B. divergens*; the roe deers are probably not reservoir hosts for *B. divergens*. Further biological and molecular investigations should be done to finally identify the parasite.

A large form of *Babesia* was isolated in one case. Additional serological investigations were then performed using IFAT with sheep erythrocytes parasitized with this large form as antigen. About 20 % (70/355) of the roe deer investigated were positive. Further investigations are then needed about serological cross reactions between this *Babesia* isolate and *B. capreoli*. In roe deers, a arge form of *Babesia* is described in Siberia as *B. jakimovi* [11] but it has never been described in western Europe. Other large forms of Babesia are described in *Bovidae* in France, notably *B. motasi* in sheep and goat and *B. major* in bovine. The hypothesis of bovine or ovine origin of our isolate can be supported by the fact that it can be in vitro cultured in sheep red blood cells and also in bovine red blood cell (data not shown). Further studies are also needed to identify this parasite and finally evaluate the possible role of the roe deer as reservoir host for bovine *Babesia*.

The question of the interactions between roe deer and bovine for babesiosis should also be evaluated in the vector. For both *B. capreoli* and *B. divergens*, the vector is the three hosts tick, *Ixodes ricinus*. Infection with one of these two *Babesia* could modify the development in the tick and the transmission of the other one.

Acknowledgements

We particularly thanks Didier Van Laere for his help in the reserve of “Chizé”, Hubert Ferté for providing the blood samples from the reserve of “Trois Fontaines”, Xavier Legendre for his excellent assistance in the reserve of “La Haute Touche” and the hunters and “federation de Chasse” from Loire Atlantique, Mayenne, Maine et Loire, Sarthe, Vendée and Ille et Vilaine.

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Integration of animal farming systems in the environment

Posters

EFFECT OF *YUCCA SCHIDIGERA* EXTRACT, A FEED ADDITIVE, TO REDUCE AIR POLLUTANTS IN PIG FATTENING UNITS

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Introduction

Intensive pig breeding facilities are associated with high levels of air pollutants that may have adverse effects on both animal and human health. Hygienic standards of air quality in pig housing are determined by physicochemical microclimate parameters and air microorganism content. The emission of ammonia and malodor from stock breeding facilities that exert unfavorable effects both within and beyond the facilities are generated during the process of manure degradation and animal metabolism. Additives are defined as agents added to feed or manure in order to reduce the emission of ammonia and malodor, and characterized by specific properties to improve both microclimate and liquid manure quality. The effect of a commercial food additive based on the *Yucca schidigera* palm extract in reducing air pollutants in pig fattening units was investigated.

Material and Methods

The fattening units – the study was conducted at the Dubravica pig–breeding farm. During the 3 month study period there were about 450 fattening pigs. The fatteners were housed in boxes on a partially slatted floor, and feed and water *ad libitum*. The additive De-Odorase was mixed into the diet of the treated pigs in the amount of 112 g per 1.000 kg feed mix. The microclimate measurements were performed by use of standard methods and Testo instruments. Air samplers were taken with SAS 100^{TM}. Nutrition agar for mesophilic bacteria and Sabouraud maltosis agar for isolation of fungi were used.

Results

Table 1. Arithmetic mean of air pollutant levels in control and treated units

Parameters	Control unit	Treated unit	Reduction (%)
Ammonia (ppm)	14.5	10.2	29.7
Carbon dioxide (ppm)	3000	1500	50.0
Mesophilic bacteria (CFU/m^3)	4,80x10^4	3,20 x10^4	33.4
Fungi (CFU/m^3)	5,26 x10^4	3,72 x10^4	29.3

Table 2. Arithmetic mean of microclimate parameters in control and treated units

Parameters	Control unit	Treated unit
Temperature (°C)	16.9	17.2
Relative humidity (%)	77	73
Air velocity (m/s)	0.13	0.12

Discussion

The emission of ammonia associated with animal keeping and housing conditions originates from degradation of organic nitrogen compounds in feces and urea hydrolysis in urine. Fecal protein nitrogen mineralization occurs by the action of proteolytic bacteria and deaminases, whereas urinary urea undergoes hydrolysis by urease enzymes. The rate of conversion to gaseous ammonia and carbon dioxide depends on air temperature and pH. The process is retarded by low air temperature and low pH. As urea hydrolysis is a considerably faster process than nitrogen mineralization, urine is the major source of ammonia emission (Andersson, 1994). The microbiologic state of animal housing is reflected in the air microflora that mostly originates from animals (~80%), manure, and attending personnel (Methling *et al.*, 1981). Agents prepared from the *Yucca schidigera* palm extract have been used as additives to reduce adverse manure emissions, especially ammonia (McCrory and Hobbs, 2001). Studies performed to date report on different rates of ammonia reduction (Amon *et al.,* 1994). The agents appear to more efficiently reduce the level of ammonia emission in animal housing when used as feed additives, whereas by far greater amounts are required when added to manure. In the present study, the addition of De Odorase to feed resulted in 30% reduction of ammonia air concentration in a pig fattening unit, which is consistent with literature reports. Also, the concentration of other air pollutants such as carbon dioxide, bacteria and fungi was decreased.

Conclusion

The addition of De Odorase to animal feed resulted in about 30% reduction in the ammonia and carbon dioxide concentrations and air microorganism count in the pig fattening unit. Standards for assessment and evaluation of additive efficiency including environmental requirements should be adopted in the near future.

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HOW TO COMBINE PERFORMANCE AND ENVIRONMENTAL CARE? VEVOVITALL®

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Introduction

Ammonia (NH_3) is a well-known gas because of its odour and its toxicity. NH_3 can effect pig health and it has also been identified as one of the risk factors for chronic bronchitis, a work-related disease in pig farmers. The effect of NH_3 on animal performance is controversial, but most of the studies show a numerically negative influence with the typical NH_3 levels found in the commercial pig farms (10 to 25 ppm). NH_3 plays as well a role in the atmospheric pollution (acid rain) and in the eutrophication of water. Animal husbandry in Europe accounts for more than 80% of NH_3 emission and a specific EU Directive (2) requires a 13% reduction of NH_3 release into the environment by 2010 compared to 1990 levels. In pigs, 40 to 50% of the nitrogen intake is excreted via the urine and can turn into ammonia. A reduction in ammonia emission can be achieved by reducing the pH of the urine. Benzoic acid has been recently authorised by the European Union (5) for this purpose (VevoVitall®, DSM Nutritional Products). The Research Institute for Pigs Husbandry in Rosmalen performed studies, which have shown a significant reduction of the average pH of the urine and of the slurry as well as a reduction of ammonia emission (1% of benzoic acid in the diet lead to a 33% reduction of ammonia emission). In the same trials, there was a clear tendency for an improvement of weight gain and feed conversion ratio. A study was set up to better evaluate the influence of benzoic acid (VevoVitall®) on performances.

Material and Methods

The trial was conducted in the fattening house of Zootechnical Centre (K.U.Leuven R&D) and involved 93 animals, Piétrain * Hybrid, 11 weeks old at the start of the trial (average 24 kg). The experimental period was 118 days. The design of the experiment was a 2 treatments x 8 replications with 2 or 3 barrows and 2-3 sows per pen. The treatments were the following: a control with 0% benzoic acid and a 0,5% benzoic acid mixed in the feed. The feed was provided ad libitum in the form of meal. The following performance parameters were evaluated: feed intake, daily weight gain, feed conversion ratio.

Results

The results are presented in table 1. No particular health problems were observed during the trial. All improvements of technical performances have been noted during each phase of the trial. Even during the last phase (84 – 105 kg) one can see a positive trend on daily weight gain and feed conversion ratio.

Discussion

The improvement of performances are in agreement with the results found on fattening pigs (1, 4) as well as the improvement of performances observed by other authors in piglets (3).
An economic simulation is presented in table 2. Even when pig carcass prices are low, the use of VevoVitall® provides a benefit to the economic results.

Table 1: Final body weight, daily growth, daily feed intake and feed conversion ratio corrected for initial body weight.
LSMean +/- standard error. A different letter within a row indicates a statistical difference (P<0.05).

	Control	*VevoVitall®*
Body weight (kg)	103.8 +1.6 a	105.5 +1.6 a
Daily growth (g/d)	693 +14 a	731 +14 a
Daily feed intake (g/d)	1961 +39 a	1886 +38 a
Feed conversion ratio	2.82 +0.08 b	2.59 +0.08 a

Table 2: Economic simulation (in € / pig):

Carcass price / kg	*1,10 € / kg*		*1,25 € / kg*	
% VevoVitall®	0,0	0,5	0,0	0,5
Selling price / pig	89.40	90.35	101.59	102.67
Feed costs	46.82	46.04	46.82	46.04
Margin	42.58	44.31	54.77	56.63
Additional margin		+1.73		+1.86

Conclusion

The inclusion of VevoVitall® in growing-fattening pig diets is known to reduce the pH of the pig urine and the ammonia emission in pig houses. This study is showing that VevoVitall® is as well improving pig performances, leading to an economic benefit for the pig breeders.

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STUDY OF AN ELEPHANTGRASS PASTURE MIXED WITH CULTURES OF SUMMER AND WINTER SEASON: YEAR I

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Introduction

Systems more sustainable in production of milk and meat have in roughage as basis of animal nutrition. Within species studied is elephantgrass (*Pennisetum purpureum* Schum.) a perenne grass, with higher productivity and good adaptation at Brazilian climate conditions. His use is been intensificate under rotative grazing. This work had the objective study behavior and quality of elephantgrass pasture (EG) mixed with summer and winter cycle species during agricultural year of 2002.

Material and Methods

The trial was conducted in Santa Maria, RS, South region, Brazil. The EG cv. Merckeron Pinda was implanted in lines with 3mt of distance, in October, 2001. It was utilized organic manure, in form of swine waste (70 kg of N/ha) and bovine waste (30 kg of N/ha). The experimental area (0.33ha) was divided in two paddocks. During summer period, the pasture was composed by EG (in lines) and by species with spontaneous development (SSD), principally *Sida santaremnensis* and *Chloris gayana* (between lines). The time of utilization of pasture in summer period was January, 16th to April, 17th, 2002. The animals occupied the paddocks during one day and time of rest for paddocks varied of 33 to 45 days. Were utilized Holstein heifers with average weight of 160kg and they received 0.5% BW of concentrate (16% CP). During winter period, was utilized, between lines, black oat (*Avena strigosa* Scheb.) seeded at throw, in scaled form in April, 24th and May, 9th, 2002, in paddocks 1 and 2, respectively, using 110 kg of seeds/ha. Were made seven grazed during June, 12th, and October, 14th, 2002. In this periods, experimental animals utilized were Holstein lactation cows, with average weight of 530kg which received, later each milking, feeding supplementation (3.5kg of concentrate and 3.5kg of maize silage/animal/day). To determinate pasture quality were collected sample of grazing simulation after watching of feeding behavior of animals during 15min in start and finish of grazing. These samples were analyzed to determinate crude protein (CP), *in vitro* digestibility of dry matter (IVDDM) and neutral detergent fiber (NDF) level. The experimental design was randomized blocks with 6 treatments (grazing) and 2 repetitions (paddocks) for summer period and incomplete randomized blocks with 7 treatments (grazing) and 2 repetitions (paddocks) for winter period. The data were submitted at analysis of regression with support of SAS' statistical package (1996).

Results

In winter period, mean availability of pasture was 8257 kg DM/ha, where participation of EG was 32.5%. The medium level to CP, NDF and IVDDM of pasture were 11.52; 64.15 and 56.67%, respectively. Animal charge was 2344 kg BW/ha. In winter period, mean availability of pasture was 3278 kg DM/ha . The medium level of CP, NDF and IVDDM of pasture were 15.6; 58.6 and 63.92%, respectively. Animal charge was 690 kg BW/ha.

Discussion

The availability of EG observed in this trial (2688 kg DM/ha) is minor compared with data of Restle et al. (2002) which verified 2874 and 4126 kg of DM to January and April, respectively. A minor availability occurred due implantation and to be first year of utilization. In winter period, mean availability of EG (2501 kg de MS/ha) was minor at found by Botrel et al. (1994) where was observed value of 4599 kg DM/ha as mean of seven varieties of EG. Availability of EG during winter period is higher compared with summer period. This fact is explained by high presence of tiller (64.60%) and dead material (20.59%) in this period. In summer period, CP level of pasture was similar at data of Silva et al. (2002) that working with genotypes of EG under grazing, registered an average of 10.64%. In winter period , CP level was of 15.6% probably by presence of black oat. Frizzo et al. (2003) found values of 12.75% working with black oat and ryegrass. The values of NDF on two periods were minors at values obtained to Silva et al. (2002). Probably, the diversity of pasture had contributed to obtain of these values. The average IVDDM of pasture in summer period was superior found by Restle et al. (2002). In winter period, the pasture, constituted basically by black oat, presented a major level (63.92%) of digestibility.

Conclusion

The system of forage production composed by EG and SSD in Summer and EG and black oat in Winter demonstrate positive response as in complementation of forage mass as forage quality.

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BEHAVIOR OF LACTATION COWS ON ELEPHANTGRASS PASTURE MIXED WITH BLACK OAT, MANAGED UNDER AGROECOLOGICAL PRINCIPLES

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Introduction

In production systems of organic milk or agroecological with fundament in the free choice of animals and diversity of species in grassland establishment, animal behavior is an important instrument on evaluation of a pasture composed to perennial culture of summer cycle and other annual winter cycle specie.

Material and Methods

The trial was conducted in Santa Maria, RS, South region of Brazil, with objective to evaluate behavior of lactation cows under grazing. Was utilized an area with 0.33ha divided in two paddocks. The pasture was composed by elephantgrass (*Pennisetum purpureum*), established in lines and black oat (*Avena strigosa*), seeded between lines. Were utilized five lactation cows, of Holstein breed, with average weight of 530kg and average milk production of 14 liters/cow/day, which received them, later milking, feeding supplementation of 3.5kg of concentrate with 20% of crude protein and 3.6kg of maize silage/cow/day. Data collect, effectuated at each 10 minutes for two observers, was realized at 6 p.m. to 6 a.m. and 8 a.m. to 4 p.m.. Were made four evaluations (characterizing winter period) in June, 12th, July, 17th, August, 25th and September, 14th, 2002. The cycles of grazing were of 29 to 51 days and time of occupation of paddock was one day. Behavior parameters evaluated were: average time of grazing of elephantgrass (GEG), grazing of black oat (GBO), grazing of elephantgrass and black oat (GEGBO), rumination (R) and idleness (I). The experimental design used was randomized blocks with four treatments (grazing season) and five repetitions (cows). Data collected in each parameter were submitted at analysis of variance has been the differences between means compared by Tukey's Test, at 5% level of significance with support of SAS statistical package (1996).

Results

Average time of grazing on four evaluations was 7h 55min. It was verified that major time spent in grazing was during sunlight, with 59.22% of total time of grazing. The minor value to R occurred in 2nd evaluation. In others evaluations, varied of 8h 10min to 8h 37min, not occur significant difference among their (P>0.05). Average time of I on four evaluations was 4h. Data of behavior analyzed statistically found in Table 1. Data of botanical compounds of pasture are in Table 2.

Discussion

Average time of grazing is similar at value found to Phillips & Rind (2001) which working with Holstein cows grazing ryegrass (*Lolium perenne)* obtained 8h 9min, and Orr et al. (2001) observed time of grazing of 7h 42min. The major time of grazing occurred during sunlight. This behavior is waited during winter period where the temperatures guarantee a better thermic well-being. High values to GEG occurred in June to September, demonstrated that the animals feeding this culture although to be in senescence stage (June) and start of summer cycle (September), respectively. The major GEGBO occurred in last evaluation, when cows stayed short time in idleness, may be the needed to select diet, once time the black oat presented 62.92% of tiller and elephantgrass was composed basically by bud and dead material. Minor time of rumination and major time of idleness occurred in second evaluation, coinciding with major time of grazing in black oat, probably in function of better quality of pasture on period (Table 2).

Conclusion

It were verified differences in behavior parameters of cows influenced by changes of the pasture compounds.

Table 1. Behavior parameters of time of grazing in black oat (GBO), elephantgrass (GEG), elephantgrass and black oat (GEGBO), rumination (R) and idleness (I) on four evaluations.

Parameters	Treatments			
	June, 12th	July, 17th	August, 25th	September 14th
GBO	10.702^{b}	30.569^{a}	23.935^{a}	27.273^{a}
GEG	28.947^{a}	2.277^{b}	11.311^{b}	23.471^{a}
GEGBO	39.649^{b}	32.846^{c}	35.246^{bc}	50.744^{a}
R	43.158^{a}	34.309^{b}	41.639^{a}	40.826^{ab}
I	17.193^{b}	32.845^{a}	23.114^{b}	8.430^{c}

Means followed of distinct letter, in line, are different among their by Tukey' Test (P<0,05).

Table 2. Percentage of botanical compounds of elephantgrass (EG) and black oat (BO).

Botanical compounds (%)	June, 12th	July, 17th	August, 25th	September ,14th
LL (EG)	24.68	9.4	0.03	26.34
Tiller (EG)	66.32	67.04	72.63	56.78
D.M. (EG)	9.00	23.56	27.34	16.88
L.L (BO)	69.65	50.57	41.77	14.75
Tiller (BO)	25.00	38.11	37.12	62.92
D. M. (BO)	5.35	11.32	21.11	22.33

LL- leaf lamina ; D..M. – dead material

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HOW TO CONCILIATE BEEKEEPING AND AGRICULTURAL ENVIRONMENT ?

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Introduction

The domesticated bee (*Apis mellifera*) plays a main role in the biodiversity of plant life by its pollination. Moreover, it is endowed with a fabulous food hoarding instinct which is the reason why man has domesticated, bred and made the most of its produce: honey, pollen… The continuously changing agricultural landscape doesn't disturb the insect as long as it provides it enough good quality nectars. Therefore, beekeeping is able to adapt as long as beekeepers are …

Biology

A bee is a perenial social insect. The queen lives up to 5 years, the laying period varies according to the climate. In France, it starts end of Winter and ends in October-November. During the busy period of the swarms (Spring, Summer) the workers live up to 6 weeks (3 weeks working inside the beehive, then 3 weeks pollen gathering) whereas those born in Autumn will survive through the Winter with the queen, forming the winter group. The pollen gathering workers bring back the raw materials needed by the swarm: nectar, pollen, water, propolis (vegetal resin). Water and propolis are used straight away. But honey and pollen will be processed before storage. Honey is but the one ingredient bees need during the cold season: is will provide them with thermogenetic energy. Man makes the most of all raw materials (pollen, propolis) and processed material (honey, royal jelly) that bees produce. But only the surplus of honey can be taken away, not to endanger the life of the swarm at winter-time.

The bee and changing landscapes

The bee mainly keeps to pollen and nectar gathering for food purposes: it hasn't got any other choice ! Due to its adaptability, it has been possible to introduce it within a wide variety of climates, and has resisted the changing in habitats.

Since deforestation, the landscape is constantly changing with agriculture, cattle breeding, urbanization, industrialization… But these changes have no doubt been more important since the start of the 20th century. In the beginning, the French agricultural landscape is of wooded bocage: mixed farming and cattle breeding spread all over the country. Later mechanization and transport allow specialization in local or regional cultures. Regrouping more or less induces the loss of bocage landscape. One of the consequences being the death of hedge and enbankment plants, as well as changes in farming habits and unusual rotation of crops. So, sainfoin and sown-in meadows needed to feed draught horses disappear, whereas sweet corn, sunflower, peas, sorghum, rapeseed tend to develop. And most of them are of very little interest for beekeeping. If its survival hasn't been altered by the modification in its food supplies, its productivity has undergone great changes in both qualitative and quantitative terms.

Because of the new crop rotations, and particularly in large-scale farming zones, non-productive periods can be observed, for example between the sunflower and rapeseed flowering times. The beekeeper has had to evolve by adapting his apiarian techniques to any new setting, specially by moving the beehives to productive locations after a precise study of the local flora's potential.

The bee, a sentinel for the environment.

On the whole, beekeeping has succeeded in adapting to complex modifications through the past centuries. However, the bee – same as all other useful insects – turns out to be an easy target for pesticides. Since the large-scale use of insecticides in the fifties, beekeeping faces a great loss of swarms, due to (chemical) poisoning. A set of rules has been established to protect the bee: using conditions and official approval of pesticides. But it suffers insolvency. For example the official approval of fertilizers and herbicides doesn't take into account the larva-killing or sublethal effects on bees… Sublethal effects as a consequence of new systemic insecticides used in seed-coating. The launching of chemicals such as Imidaclopride (Gaucho®) or Fipronil (Régent TS®) on the market, to coat the seeds just before sowing was a very attractive technique meant for environmental protection. But they quickly proved to be dangerous,not only by Summer toxicity on sunflower/ sweetcorn, but also by higher Winter death rate due to toxic food consuming (sweetcorn and sunflower honey or pollen), and by their remanence in soil.

Conclusion

This domesticated animal has always exhibited «wild», and can be considered a true sentinel for the environment. Its survival in agricultural zones has recently become more than critical. In the long-lasting beekeeping setting –as well as for the long-lasting agriculture setting- solutions are to be found and fitted. These solutions depend on new cultural practice, new crop rotations, new rules about official approval of fertilizers and herbicides. The bee has been able to survive through centuries, and has adapted to various situations.It's now for her to find the right sort of food, as far as quality and quantity are concerned .

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POLISH ACCESSION TO THE EUROPEAN UNION AND ITS IMPACT ON HYGIENIC ISSUES IN ANIMAL PRODUCTION IN POLAND.

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Polish accession to the EU is connected with some issues in the animal hygiene as well from the scientific as from the practical point of view.

Systems of animal production in Poland are determined by the quality of soils and the size of the farms. In Poland most of the grounds (65,9%) are very good, good and average quality. And it is enough background to perform animal production. The majority of the total and commercial production belongs to pigs and dairy cattle (Ministry of Agriculture 2002). The main problem in introducing hygienic programs concerning animals' health protection is the structure of Polish, private farms. The average farm area is 9ha, compared to 19,4 ha in the UE. In Poland 86,9% of individual cattle farms keep from 1 to 9 cows per farm. The majority of Polish farms (72,4%) breed from 1 to 19 pigs per farm (CSO 2002). Breeding conditions in the majority of the small farms do not fully fulfill European requirements in the field of veterinary prevention and biosecurity. And the principal problem in prevention is identification and registration of animals (ARiMR 2004). The system of identification of cattle and pigs realized in the program IACS has already worked in Poland. Due to this activity it was possible to detect and explore the 16th case of BSE in Poland (Polak et al. 2003). According to the law regulations the system of identification of sheep, goats and deer kept in farming conditions should be completed in the nearest future.

To realize prevention and biosecurity activity it is necessary to have law regulations common with the EU. There are two, most important new Polish law regulations in this area:

1. Act from 11.03.2004 concerning protection of animals' health and combating infectious diseases of animals.
2. Law regulation concerning minimal conditions in keeping different species of farm animals (Ministry of Agriculture regulation from 2.09.2003).

European and Polish law regulations have common intentions and contain the same or similar thesis about prevention in animal production (Szymborski 2004).

According to these and other regulations the same hygienic standards should be obligatory in small and big farms. Right now it is impossible because of different technology in animal production in these two types of farms in Poland.

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IMPACT OF EU REGULATIONS ON PIG PRODUCTION IN FRANCE

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Introduction

French pig production and its industry have to face a raising number of regulation constraints, most of the time issued from EU directives or from imposed French procedures to reassure the consumer. The application of these measures induces extra costs at different levels of the industry. Some are taken in charge by the public authorities, the other ones by concerned firms.

Material and Methods

The impact of the most important measures (environment, welfare, traceability, sanitary security), expressed in cents of euros per kg deadweight (Table 1) has been estimated. Only definite extra costs corresponding to already set up rules are taken into account.

These additional costs include investment depreciations and interest charges on financial debts, and also running costs (operational charges, labour extra costs, additional inputs).

Environmental costs:

- building brought into compliance and pollution taxes: based on inquiries in public services.
- slurry treatment: based on an estimation from the Agricultural Census in 2000 of the quantities of nitrogen surplus in structural surplus zones and on an assessed unitary cost (1).

Welfare costs:

- In herds: keeping dry sows in groups takes place between 2003 and 2013, at which date its application will be compulsory. Global cost is based on a statistical survey on pig buildings in 2001, to estimate the number of concerned places, and on an assessed unitary cost (2).
- In transport: 50 000 exported breeders are concerned each year.

Traceability in pig industry

- Herds identification: all animals concerned, by tatoo or by microchip.
- Industrial food:
  - Security and traceability of raw materials: important consequences on supplying, manufacture, transport estimated with inquiries in feed firms.
  - Use of non GMO raw materials: a quarter of pig feed quantities is concerned (pigs belonging to quality signs).
- Slaughtering, meat processing: investment in computers and in labour is assessed, by inquiries.

Sanitary security:

- Growth promoters antibiotics ban: a partial ban was assessed (3) about 0,4 ct of euros/kg deadweight a total ban in 2006 would make it reach 2 ct.
- Bone meals and animal fats: since 2000, this ban has increased production cost of pig feed, an increase assessed through feed manufacturers.
- Elimination of slaughter by-products: this constraint borne by all slaughter firms is assessed by operator inquiries: small-sized companies and multi-species ones are the most penalized.

Results

Table 1- Annual extra cost induced by regulation in French pig industry in 2005.

In euros/100kg deadweight	2005
Environment (S and I) [(1)]	
· Buildings brought into compliance	0,9(S)+0,4(I)
· Nitrogen surplus treatment	0,6(S)+4,1(I)
· Pollution tax	0,09(I)
Welfare (I)	
· In herd	0,15-0,53
· In transport	0,007
Traceability (I)	
· Herds identification, control	0,7-3
· Animal food:	
- security-traceability of raw materials	0,3
- use of non GMO raw materials	-
· Slaughtering, meat proccessing	1,7
Sanitary security (I)	
· Growth promoters antibiotics ban	0,4
· Bone meals and animal fats in feed	0,22
· Elimination of slaughter by-products	1,8
Total	11-14

[(1)] costs borne by State (S) or by industry (I)

Discussion

The average total extra costs borne by the entire French pig industry is estimated but some of these costs will not be borne equally by all firms, according to their situation, to their size, their localization…

Conclusion

The whole regulation constraints applied to French pig production have an important impact on production cost, between 11 and 14 cents of euros per kg deadweight; the impact is likely to be reinforced in the years to come. Marketing or regulation solutions have to be found, so that the production will not be penalised by its outside competitors who do not apply the same rules.

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Animal Health

ROLE OF OIE IN WORLD ANIMAL HEALTH MAINTENANCE

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Introduction

Preventing the spread of animal diseases and zoonoses through international movements is one of the important objectives of the World Organisation for Animal Health (OIE). This is accomplished by establishing international standards and guidelines aimed at preventing the importation of pathogens dangerous for animals and humans while avoiding unjustified sanitary barriers and through surveillance, notification and control of diseases.

The OIE was founded in 1924, before the creation of the United Nations. Initially 28 countries united with a mandate to share information on animal disease outbreaks to allow the Member Countries to take the appropriate control methods to protect themselves and to prevent further spread of the disease. There are now 167 OIE Member Countries. Providing a mechanism for prompt reporting of disease outbreaks/occurrences is still one of the primary roles of the OIE.

The OIE objectives and activities for the prevention and control of infectious animal diseases and zoonoses are focused on the following areas.

Transparency in animal disease status worldwide

Each OIE Member Country is committed to report to the information department on its health status regarding significant animal diseases and diseases transmissible to humans. The OIE then disseminates the information to all Member Countries to enable them to take appropriate action and to protect themselves.

Collection, analysis and dissemination of veterinary information

Using OIE network of internationally recognised scientists, Collaborating Centres and Reference Laboratories, the OIE collects, analyses and publishes the latest scientific information on significant animal diseases, including those transmissible to humans, especially regarding control and prevention methods

Strengthening of international coordination and cooperation in the control of animal diseases

Improving the legal framework and resources of National Veterinary Services

The OIE provides technical expertise to Member Countries requesting assistance with animal disease control and eradication programmes, particularly in developing countries. These activities are performed in coordination with and in support to other Regional and International Organisations and with donor countries and agencies responsible for supporting and funding the control of infectious animal diseases and zoonoses. Under OIE-World Bank Official Agreement, surveillance of animal disease is recognised as an international Public Good.

The OIE is strongly committed to convincing national policy makers and international donors that the cost of strengthening Veterinary Services so that they can provide better surveillance, early warning systems and management of epizootics, including zoonoses, is negligible compared with the economic loss resulting from the accidental or intentional introduction of infectious animal diseases and zoonoses

Sanitary protection of world trade in animals and animal products while avoiding unjustified sanitary barriers. Guarantee of the safety of food of animal origin and promotion of animal welfare through a science-based approach

The OIE develops standards for use by the Member Countries to protect themselves against disease incursions as a result of trade in animals and animal products, while avoiding unjustified sanitary barriers. These standards are developed by experts from Member Countries and from the OIE's network of 170 Collaborating Centres and Reference Laboratories and in collaboration with FAO and IAEA/FAO Joint Division experts.

In 1995 the standards developed by the OIE were recognised by the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) of the World Trade Organization (WTO). In order to harmonise SPS measures and remove unjustifiable sanitary or health restrictions to international trade, the Agreement states that governments should use these international standards, guidelines and recommendations. The goal of the Agreement is to minimise the risk of importation of pathogens and to remove unjustifiable sanitary or health restrictions to international trade. The Agreement states that it is the sovereign right of a country to provide an appropriate level of animal and public health protection at its borders. However, this sovereign right is not to be misused for protectionist purposes: an importing country could only apply sanitary measures to imports if a similar level of protection is applied to all imports and internally by the importing country. Member Countries can introduce standards providing a higher level of protection than that provided by the OIE standards if there is a scientific justification, but these standards must be based on a science-based risk analysis.

ORGANISATION OF THE OIE

International Committee

The International Committee is the highest authority of the OIE. It comprises all the Delegates nominated by the governments of 167 Member Countries (as of May 2004) and meets once a year during the General Session in Paris in May.

Voting by the Delegates within the International Committee respects the democratic principle of one country, one vote.

The principal functions of the International Committee are:

to adopt international standards in the field of animal health and zoonoses;

to adopt standards and resolutions for the control of the major animal diseases;
to elect members of the OIE's statutory bodies (President and Vice-President of the Committee, Members of the Administrative Commission, Regional Commissions and Specialist Commissions);
to elect the Director General of the OIE;
to examine and approve the annual OIE activity report, programme of activities, financial report and budget presented by the Director General.

During the General Session, changes affecting the distribution of the major animal diseases throughout the world are closely monitored.

Administrative Commission

The work of the International Committee is prepared by the Administrative Commission.
The Administrative Commission, consisting of the President of the International Committee, the Vice-President, the Past President and six elected Delegates, represents the Committee in the interval between the General Sessions. Members are elected on a geographical basis.
The Commission meets twice a year to examine, in consultation with the Director General, technical and administrative matters, in particular the programme of activities and financial documents to be submitted to the International Committee for approval.

Regional Commissions

The five Regional Commissions study specific problems affecting the Veterinary Services and organise cooperation within each of the Regions:

Africa
Americas
Asia, Far East and Oceania
Europe
Middle East

Each Commission holds a meeting every two years in one of the countries of the region to study technical items and regional cooperation on animal disease control.
The Regional Commissions also meet during the General Session of the International Committee. They report to the Committee on their activities and submit recommendations for final endorsement before implementation by the Director General.

The Director General and the Central Bureau

The Central Bureau, located in Paris, is managed by the Director General of the OIE. He is elected by the International Committee. The Central Bureau implements the strategies determined by the International Committee and coordinates the corresponding activities in the fields of information, international cooperation and scientific dissemination.
The Central Bureau also provides the secretariat for the annual General Session of the Committee, the various meetings of the Commissions and technical meetings held at the OIE. It also contributes to the secretariat for Regional and Specialised Conferences.
With the help of voluntary contributions from some of the Member Countries, the Central Bureau provides the impetus for activities such as organising regional training seminars and coordinating control programmes.
The Central Bureau has become an international resource centre at the service of animal health (including zoonoses) officials worldwide.

TOWARDS GREATER TRANSPARENCY IN THE ANIMAL HEALTH SITUATION WORLDWIDE

The OIE is the worldwide observatory for animal health. Its key mission is to keep national Veterinary Services and International Organisations informed of the appearance and course of epizootics in any country in the world that represent a threat to animal or public health (zoonoses). The system is based on official animal disease information that the Veterinary Authorities of OIE Member Countries have an obligation to report to the OIE. The use of standard reporting forms ensures that the system is fed with the required data in a standardised format. The strength of the OIE Animal Disease Information System is its 'legal' basis defined in Chapters 1.1.2 and 1.1.3 of the OIE *Terrestrial Code* and in Chapters 1.1.3 and 1.2.1 of the OIE *Aquatic Code*.
The OIE Animal Health Information System has the following components:

The International Early Warning System, which consists of an alert procedure to warn of exceptional epidemiological events (natural or intentional) occurring in Member Countries. Information is aimed at decision-makers and other stakeholders to enable them to take the necessary preventive measures. Under this system, the occurrence of a disease or any exceptional epidemiological event, including zoonoses, must be reported as soon as possible to the OIE Headquarters, which then redistributes the information through a variety of channels. Follow-up reports are provided weekly to allow end-users to follow the epidemiological situation as it develops.
The International Monitoring System, with procedures for gathering monthly and annual animal health data from around the world. Periodical information is collected for all OIE-listed diseases having the potential for rapid spread, adverse economic impact or having a zoonotic potential, while annual information is collected for 130 listed infectious animal diseases and zoonoses.
To improve the transparency of animal health information, OIE is developing a verification procedure for non-official information from various sources on the existence of diseases outbreaks that have not yet been officially notified to the OIE. These processes use different sources of information such as such as diagnostic results from OIE Reference Laboratories, scientific papers, field projects, newspapers, internet, Global Public Health Intelligence (GPHIN), ProMed, etc.
In order to improve the control of highly contagious diseases, OIE and FAO have recently developed a new initiative, called the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs). The concept of this initiative is

based on a regional and international approach to animal diseases. GF-TADs will improve disease information and epidemiological intelligence. The Global Early Warning System (GLEWS) and response is an integral aspect of the GF-TADs programme. The GLEWS is defined as an instrument to be developed by FAO/OIE/WHO for the international community and stakeholders alike to assist in predicting and preventing livestock animal disease threats through epidemiological analysis and the integration of additional factors that might have an impact on the occurrence and spread of such diseases (e.g. economic factors, civil unrest, climatic changes, etc.). The most important action is to share information on animal health/zoonoses in humans among the three organisations. Results of disease information tracking systems are shared among the three organisations in order to search for additional information for verification purposes. OIE through its verification system would verify information with the Delegate of the Member Country. This will significantly improve the quality of official information.

While every effort is made to improve the OIE Animal Health Information System, the major difficulty encountered, as with any international activity, is the quality of the information received, especially information from countries where the resources available for Veterinary Services are inadequate lack of trained veterinarians/epidemiologists, poor equipment and laboratory facilities, poor involvement of stakeholders in national surveillance systems, absence of disease control programmes, etc). In such countries potentially dangerous situations might go unnoticed or not be dealt with quickly, thereby increasing the risk of the disease spreading to other countries.

The OIE has a limited source of emergency funds for use in rapidly assisting Member Countries faced with exceptional epidemiological situations. Typically, these funds are used to send experts from OIE Reference Laboratories or Collaborating Centres immediately to assess the epidemiological situation in the field and prepare the actions of national authorities and other international organisations.

- Member Countries receive alert messages on disease outbreaks or suspicion thereof via fax or e-mail.
- The OIE annual compilation entitled *World Animal Health*, provides a wide variety of information on the animal health situation worldwide and reports on the disease control methods Member Countries apply.

A selection of all this information is integrated into *Handistatus* – a regularly updated computerised database available on the OIE Web site (www.oie.int).

Scientific information is disseminated through other publications, including the OIE *Scientific and Technical Review*, which contains research articles and guidelines of the very highest standard for animal disease control.

By collecting, processing and disseminating data on animal diseases throughout the world, the OIE endeavour to ensure transparency in the animal health situation worldwide for the benefit of its Member Countries.

TOWARDS IMPROVED HEALTH SAFEGUARDS IN INTERNATIONAL TRADE

The smooth flow of animals and animal products requires:

- the development and adoption by the international community of animal health regulations aimed at avoiding the risk of importing and spreading diseases and pathogens transmissible to animals and humans;
- the harmonisation and greater transparency of sanitary regulations applicable to trade in animals and their products so as to avoid unjustified sanitary barriers.

The WTO Agreement on the Application of Sanitary and Phytosanitary Measures advocates the use of standards developed under the auspices of the Office International des Epizooties.

Various normative works, approved by the OIE International Committee, are designed to promote the harmonisation of regulations applicable to trade and animal disease control:

- The *Terrestrial Code*, for mammals, birds and bees, developed by the Terrestrial Animal Health Standards Commission, and the *Aquatic Code*, developed by the Aquatic Animal Health Standard Commission. They are updated annually and are available both as an electronic version on the OIE Web site and in a printed version. The Commissions are elected by the General Assembly of the Member Countries of the OIE.
- The *Terrestrial* and *Aquatic Codes* also have guidelines for disease reporting. These Standards state that Member Countries should proceed according to chapters 1.1.2 and 1.1.3 of *the Terrestrial Code* and chapters 1.1.3 and 1.2.1 of the *Aquatic Code* to notify disease occurrence. This information is then forwarded immediately to other Member Countries.
- The OIE now takes a proactive approach to disease reporting and will also report information on confirmed positive results provided by OIE Reference Laboratories or from unofficial sources, such as scientific publications, ProMed and lay publications after the information has been verified by the Member Country.

The *Terrestrial Manual*, developed by the Biological Standards Commission, and the *Aquatic Manual*, developed by the Aquatic Animal Health Standard Commission, presents standard methods for diagnostic tests and vaccine production to be applied notably in the context of international trade and national animal disease control programmes. Both texts constitute the reference standards for the international harmonisation of the diagnosis of animal diseases and vaccine control; they also contain specific chapters on sampling methods, packaging and transport of samples, quality management and biosecurity of veterinary laboratories, tests for sterility and freedom from contaminants, human safety in the veterinary microbiology laboratory, veterinary vaccine production, disinfection and inactivation procedures and laboratory methodologies for bacterial antimicrobial susceptibility testing.

- The OIE *Quality Standard and Guidelines for Veterinary Laboratories: Infectious diseases.*

This OIE publication describes the standards for the management, biosecurity and technical requirements for laboratories conducting tests for infectious diseases as well as specific details with respect to test method validation, reference reagents and laboratory proficiency testing.

Lastly, the OIE, through the work of the Scientific Commission for Animal Diseases develops and updates lists of countries recognised as being free from some serious diseases, most notably foot-and-mouth disease, bovine spongiform encephalopathy, rinderpest and contagious bovine pleuropneumonia. These lists make a substantial contribution to the health security of international movements.

TOWARDS OBJECTIVE AND IMPARTIAL EXPERTISE IN ANIMAL HEALTH

The International Agreement of 25 January 1924 establishing the OIE made it responsible for promoting and coordinating research on the surveillance and control of animal diseases throughout the world.

This objective has been attained by the creation of a worldwide animal health network, involving the setting up of Specialist Commissions and Working Groups, the designation of Collaborating Centres and Reference Laboratories, the organisation of meetings of experts and the continuing publication of scientific articles.

Specialist Commissions

The Specialist Commissions study problems of animal disease surveillance and control and questions relating to the harmonisation of international regulations.

The Terrestrial Animal Health Standard Commission contributes to the development, in collaboration with other Specialist Commissions, of the generic and specific chapters in the *Terrestrial Code*, promote the adoption by the International Committee of animal health (including zoonoses), animal welfare and animal production food safety standards, guidelines and recommendations concerning the trade or international movement of mammals, birds and bees and their products, and harmonised disease control regulations.

The Scientific Commission for Animal Diseases contributes to the development of better strategies and methods for animal disease surveillance and control. The Commission convenes groups of specialists of the highest standard, particularly in the event of an animal health emergency or to verify or evaluate the status of Member Countries in terms of specific animal diseases.

The Biological Standards Commission harmonises methods for the diagnosis of animal diseases and the control of biological products, especially vaccines used for veterinary purposes. The Commission coordinates a programme to develop standard reagents aimed at standardising diagnosis.

The Aquatic Animal Health Standard Commission collects all available information on disease control methods for fish, molluscs and crustaceans. The Commission harmonises rules governing trade in aquaculture products as well as diagnostic methods. It also organises scientific meetings on these topics.

All the standards proposed by the various specialist Commissions need to be approved by the International Committee before publication. All the standards, recommendations and guidelines of the OIE relating to animal health, zoonoses and international trade in animals and animal products are recognised by the WTO.

OIE Reference Laboratories and Collaborating Centres

These OIE Reference Laboratories and Collaborating Centres, of which there are 170, covering 92 diseases and topics and located in 31 different countries, provide OIE Member Countries with support and scientific advice on all matters relating to the surveillance and control of animal diseases. This support can take many forms: such as the provision of experts (150 world renowned scientists), preparation and supply of diagnostic kits or standard reagents, seminars, courses, and organisation of scientific meetings.

Working Groups

Three OIE Working Groups are currently active:

Wildlife Diseases
Animal Welfare
Animal Production Food Safety

These Working Groups meet to review progress made in their subject field and to ensure that the information is made available rapidly to all OIE Member Countries. They also contribute to the organisation of scientific meetings, seminars, workshops and training courses.

The OIE Working Group on Wildlife Disease urges Member Countries to recognise the importance of wild animals as potential reservoirs (and even as targets of deliberately introduced biological agents) when planning responses to outbreaks of disease, exotic or otherwise.

The WGWD has determined that relatively few countries have developed plans for responding to any disease incursions that may affect wild animals. In order to assist OIE Member Countries that may wish to undertake such planning, the WGWD will, in the course of the next 3 years, review preparedness and response plans that may have already been prepared. The Group will identify from these plans the major components and information requirements essential to this planning. The outcome of the investigation will be reported to the OIE International Committee in 2005.

The OIE Working group on Animal Welfare developed a detailed work programme.

The International Committee had decided that the OIE would give priority to the welfare of animals used in agriculture and aquaculture and that, within that Group, the topics of transportation, humane slaughter and killing for disease control purposes would be addressed first, following by housing and management.

The OIE organised the first International Conference on animal welfare in February 2004.

The OIE Working Group on Animal Production Food Safety, established between the OIE and high level representatives of the *Codex Alimentarius* Commission, is responsible for hazards for consumers likely to occur during animal production (on the farm). This Working

Group also covers intentional actions likely to be done on the farm.

During the 72nd OIE General Session, Member Countries recognised that zoonotic diseases are emerging and re-emerging with great frequency, and indicated their overwhelming support for a greater OIE role in confronting the challenges of such zoonoses. They also recognised the need for coordination of activities among animal and public health officials and organisations and vertically through national, state, and local groups. For this purpose Resolution No. XXIX was adopted during the 72nd General Session with a clear indication for including this activity in the fourth OIE strategic plan (2005–2010) and to create an Ad hoc Group on Emerging Diseases with member from the Working Groups on Wildlife Diseases and Animal Production Food Safety, the Ad hoc Group on Epidemiology and other relevant bodies or experts, in particular OIE Reference Laboratories.

DEFINITIONS OF HEALTH AND DISEASE IN TEXTBOOKS OF VETERINARY MEDICINE

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Introduction

The concept of health, as well as the concept of disease, must be regarded as essential to veterinary medicine. Nevertheless, it appears to be extremely rare that broader discussions about these basic concepts occur within the veterinary society. The increasing diagnostic possibilities to identify diseases make it crucial to define disease and health, as this basic distinction gives the very fundament of disease classification.

The naive definition of health in veterinary medicine seems to be that health is no more than the very absence of disease, which can be considered as a dichotomous definition. I suppose that this position often is the case for basic assumptions in e.g. epidemiology, where the disease frequency commonly is calculated based on that disease is binary, which means that either the animal has the disease or it does not have the disease.

The epidemiological methods used to investigate the excellence of a disease test, e.g. the sensitivity and the specificity of a serological test, is built on the understated assumptions of well-defined concepts of disease and health. However, it seems to be rare that any evaluation of diagnostic methods is based on any scrutiny or precise definitions. For example in a well known textbook on veterinary epidemiology quite extended parts of the book emphasize the theoretical problems associated with diagnostic tests (Martin et al., 1987). But the concept of health and disease is just briefly reviewed and the authors refers to that productivity is commonly used as a surrogate definition of health in veterinary medicine.

Pathology has always been an essential part of veterinary medicine. Naturally it is self-evident that the pathologist should be able to determine health from disease in order to put a accurate diagnosis, although it seems rare to find any explicit definition of these central concepts or even to find the subject being investigated further.

No matter in what area the veterinarian is active, the daily work involves health and disease in animals, directly or indirectly. Therefore, I found it natural to examine how different concepts of disease is used within veterinary medicine, and to examine textbooks in veterinary medicine, e.g. veterinary pathology, internal medicine, epidemiology.

The aim of the present study is to examine how the concepts of “health” and “disease” are defined in veterinary textbooks in pathology, epidemiology, internal medicine and other areas.

Material and Method

Within the scope of this study I examined the veterinary textbooks at the libraries of the Swedish University of Agricultural Sciences (SLU) during the spring of 2003. The literature examined, compiled veterinary textbooks in several disciplines, such as pathology, internal medicine, bacteriology and immunology.

My approach in examining the literature was to perform a scanning of the content, indexes and introductory chapters of every volume. The scanning was done to identify any definition or discussion about health and disease. Only textbook with explicit definitions of health and disease were selected for further examination. Thus, this study do not investigate implicit definitions of health and disease, as the identification of such definitions would demand far more advanced analyses, as well as, I would run increased risk of getting arbitrary definitions.

Descriptive results

About 80 out of the 500 relevant books I found within veterinary medicine were written for non-veterinarians, such as veterinary nurses, farmers or the common man. Thirty-nine of the 500 books (8%) comprised any explicit form of, more or less developed, definition of health and/or disease. Twenty-two books out of these 39 were written for veterinarians or veterinary students, five were veterinary dictionaries, two were handbooks for veterinary nurses and ten were written for farmers or animal owners.

Twenty-five volumes were written in English, four were in German, five were in Swedish, two were in Norwegian, two were in Danish and one volume were written in French.

Four out of 39 volumes were available in several editions. These different editions were counted as single handbooks, and special attention was paid to any added or extended health or disease definition. Different editions revealed an extended or modified definition of health (Blood & Henderson, 1974; Blood, et al., 1979; Blood & Radostits, 1989; Radostits, et al., 1994; Radostits, et al., 2000). Other handbooks had unchanged text sections about health/disease throughout several editions, e.g. a German textbook for veterinary nurses (Geyer & Grabner, 1983; 1988; 1991).

I found that more textbooks written for non veterinarian contained definitions of health or disease, compared textbooks written for veterinarians (15% versus 7 %). The three textbooks about alternative veterinary medicine with explicit health definition were written for laymen and covered homeopathic treatment of animals (Brock & Nielsen, 1986; Day, 1995; Thoresen, 1997). These books gave not only a definition of health from point of view of alternative veterinary medicine, but also a definition of what the authors saw as conventional medicine, which they rejected. However, there were no references to other publications in these books.

With few exceptions the books only had one single definition of health or disease. The exception was, apart from the books alternative medicine, a dictionary of

veterinary medicine where alternative definitions of health were given (Blood & Studdert, 1988; Blood & Studdert, 1999).

Discussion

General impressions

I found that few textbooks had any health definition at all. In general, common textbooks of pathology were lacking any suggestion of how to define health and disease. Typically, textbooks of pathology and internal medicine comprised chapters about each system of organs, i.e. one chapter about diseases of the integument, one about disease of the respiratory system, one about disease of the digestive system and so on. These textbooks contained of contributions from several authors, responsible for text sections of the diseases of an organ system or a group of diseases. Then the book chapters were put together by the editors (see for example Thomson, 1988 or Ettinger & Feldman, 2000). This type of books rarely contained any introductory part covering the very concept of disease.

Categories of health definitions

A subdivision of health definitions is in itself arbitrary. However, such a division may increase the possibility to comprehend heterogeneous items of more or less developed definitions of health and disease. My subdivision should be regarded as a tool to bring order in this study, rather than to establish a definition of schools of the subject once for all.

I found different approaches to the concept of health and disease in the literature reviewed. My suggestion is to split it into the following five categories:

1. Health as normality
2. Health as biological function
3. Health as homeostasis
4. Health as physical and psychological health
5. Health as productivity including reproduction

1 Health as normality

It is relatively common to look at 'health' and 'disease', as normal or abnormal. However, these definitions are usually very limited in their extension and sometime only fragments. An example of normality definition comes from a manual for animal health auxiliary personnel written by FAO (1983), where a healthy animal is described to have a normal appearance and behaviour. The animal should have normal features, including normal body position and movements. This definition is actually not a definition of the concept of health as such, but an operational definition of clinical health, assuming normality.

John Websters (1987) has a similar approach to health in his disease definition which relates disease to a normal state, which is not further defined.

The best way to recognise signs of ill-health in a cow is to stand and stare at healthy cows 'long and long', for signs of ill health always appear as departure from normality in posture, movement, alertness, appetite , etc., and the first signs may be very very subtle. (Webster, 1987)

Furthermore, Webster puts our attention to the importance of getting clinical experience of the normal health animal in order to understand the normal variation of healthy animals.

In a German veterinary dictionary (Wiesner & Ribbeck, 1983) 'disease' is defined as disturbance of the normal function of the body.

Krankheit: Störung der normalen Funktion des Körpers oder seiner Organe und Organsysteme als Ergebnis verschied, exogener Faktoren (Exposition, Umwelt) im Zusammenhang mit einer sich zeitweilig ändernden Anfälligkeit (Disposition) sowie der Reaktionseigentümlichkeit des Organismus. (Wiesner & Ribbeck, 1983)

Arnall och Keymer (1975) define health as a soundness of the body and an absence of disease, which assume that there is a normality of health. They refer to definitions of health without giving a clear opinion of their own.

Everyone understands the general meaning of health, but it is not easy to define the state precisely. Dictionaries give varying definitions: "a soundness of body" or "a normal condition of body with all its parts functioning well", whilst one authority merely states that it is "an absence of disease". (Arnall & Keymer, 1975)

Baker and Greer (1980) gives a semantic analysis of the disease concept before they propose a normality-based health concept.

Disease may be correctly defined as "not at ease" because the prefix "dis" denotes reversal or separation from the root "ease". Animal ill health is synonymous with the word "disease". They both describe a condition that results from any structural defect or functional impairment of the animal body. Some diseases are not easily detected until they are in the terminal stages; however, most diseases are manifested by signs of disturbances called symptoms. The herdsman should be fully aware of the appearance, movement, and daily habits of healthy animals so that he can detect any abnormal behaviour early. (Baker & Greer, 1980)

In the previous definitions there are clearly stated attempts to base the health concept on normality, but elements of biological function is also introduced as relevant (Baker & Greer, 1980; Hoopes och Thwaits, 1997). The passage by Baker and Greer (1980) about *"structural defect or functional impairment of the animal body"* leads us to the next school of definition; Health as biological function.

2 Health as biological function

Slauson and Cooper (1990) propose in their textbook of comparative pathology a definition of disease, where the disease can be seen as a manifestation of malfunctioning physiology.

Disease is a manifestation of physiology gone wrong, and it ultimately reflects some structural or functional alteration in the cells of which all living things are made. (Slauson & Cooper, 1990)

Gillespie and Timoney (1981) define disease as disturbances of proper performance of body functions.

Disease may be defined as an alteration of the state of the body, or of some of its organs, which interrupts or disrupts the proper performance of the bodily functions. Functional disturbance soon is manifested by physical signs which usually can be detected by others.
Diseases may be of external or of internal origin. (Gillespie & Timoney, 1981)

Norman F. Cheville (1988) proposes that veterinary pathology is abnormal biology in a wide sense:

Pathology, in the broadest sense, is abnormal biology. --- Pathology is essentially the search for and the study of lesions, the abnormal structural and functional changes that occur in the body. (Cheville, 1988)

The idea of biological functioning is related to the idea of homeostasis, which could be said to make functioning more precise.

3 Health as homeostasis

Health defined as homeostasis is an old idea. The concept of homeostasis relates to the maintenance of a delicate balance within the organism. This is a common way of looking at health. Here is one example:

Krankheit entsteht, wenn das normale Zusammenspiel der Körperfunktionen gestört ist. Ihre Entstehung ist abhängig von der Gesamtverfassung des Organismus (Konstitution), von einer Veranlagung und Krankheitsbereitschaft (Disposition) sowie von den auf den Körper einwirkenden äußeren und inneren Krankheitsursachen, z.B. Infektionserreger, Umwelteinflüsse, Giftstoffe, Stoffwechselveränderungen. (Geyer &Grabner, 1983, 1988, 1991)

The idea of health as homeostasis is commonly used in veterinary homeopathy. In the three homeopathy books I found the discussion about health was quite extended and the homeostasis idea was usually contrasted to conventional medicine. The homeostasis idea was commonly expressed as being holistic, which can be illustrated by Christopher Day (1995), who writes about homeopathic veterinary medicine and conventional medicine.

In holistic terms, the word " health " implies the concept of a mind an body together in harmony with the environment. When the organism, comprising the mind and the body, is out of the harmony within itself or with its environment, then we have the state of disease (literally dis-ease).
Modern conventional medicine tends to view disease as a set of signs and symptoms, recognisable combinations of which are called 'disease'. Each of these given a name and is assumed to have an identity of its own. In holistic medicine we view disease differently. We see the signs or symptoms simply as a result of, and expression of the body's reaction to, the disease forces which impinge upon it, threatening to disturb its internal equilibrium. Like all systems in equilibrium, the body – a very sensitive and active equilibrium system- reacts to disturbing forces in an attempt to retain or regain balance. (Day, 1995)

Homeopathic health definitions are often stated to be 'holistic' and they are mainly based on the idea that health depending on homeostasis. However, as health definition of conventional veterinary textbooks also can be based on the 'homeostasis idea', it can not be proposed that 'homeostatic thinking' logically leads to homeopathic medicine. One example of this is that Lagerlöf, Hallgren and Ekesbo (1968) define health as a state where all organs are in a delicate balance with each other and with the surrounding world. This definition has been further refined by Ekesbo (2002). These authors clearly belong to conventional veterinary medicine and not homeopathy.

4 Health as physical and psychological health

It is common in the debate about animal welfare to propose a wide definition of health which also includes psychological aspects of health (Broom, 1996; Fraser et al., 1997). However, it seems to be uncommon to include welfare and well-being in health definitions of veterinary textbooks. But there are some authors who propose psychological aspects to be included in health definition.

Martin, Meek och Willeberg (1987), make thorough descriptions of theoretically problems related to diagnostic tests , but they give a brief definition of health. They actually gives references to the human health definition stated by WHO, but they also think that productivity is a substantial part of health of farm animals.

Although health in humans has been defined as a state of complete physical, mental, and spiritual well being, in veterinary medicine, productivity is often used as a surrogate measure of health. (Martin et al., 1987)

Blood and Studdert (1988, 1999) gives in a veterinary dictionary expression for similar thoughts.

***health** a state of physical and psychological well-being and of productivity including reproduction.*(Blood & Studdert, 1988; 1999)

The definition is very short but in my opinion it mixes two different approaches, which could be regarded as contradictory. At first there may not be any conflict

between well-being and productivity, but how do we make priority between these two aspects. Are both parts necessary to fulfil in order to get stay health?

This dual health definition leads us to the last category of health definitions, namely;

Health as productivity including reproduction

The previous definitions could easily be universal, i.e. the definitions could be applied to all animals including humans. But, it would probably be hard to used a health definition that says that 'Health equalises productivity including reproduction, as a general health definition for humans or other non-producing animals, e.g. pets.

The idea that health in animals solely is the same as productivity is quite rare in the literature, but it is proposed by C. S. G. Grunsell in Black's veterinary dictionary (West, 1995):

--- *(health) is now more accurately regarded as a state of maximum economic production".* (C. S. G. Grunsell in West, 1995)

However, quite frequently elements of reproduction and productivity are incorporate in health definitions proposed for farm animals. For example Aspinall (1976) makes the following statement:

It is extremely difficult to give a definition of health, but in practical terms a healthy animal grows, reproduces, and behaves in a manner which has come to be regarded as normal for its species and type. (Aspinall, 1976)

In Blood and Studdert "Comprehensive Veterinary Dictionary" (Baillière Tindall (1988) respectively W. B. Saunders (1999)), propose several definitions of disease and health.

Disease *traditionally defined as a finite abnormality of structure or function with an identifiable pathological or clinicopathological basis, and with a recognizable syndrome of clinical signs. Its cause is more often than not unknown.*
This definition has long been widened to embrace subclinical diseases in which there is no tangible clinical syndrome but which are identifiable by chemical haematological biophysical microbiological or immunological means. Nowadays it is becoming so that the definition is used even more widely still to include failure to produce at expected level of nutritional in the presence of normal levels of nutritional supply and environmental quality. It is to be expected that the detection of residues of disqualifying chemical in foods of animal origin will also come to be included within the scope of disease.

health *a state of physical and psychological well-being and of productivity including reproduction.*
healthy *1. a state of being in good health. 2. pertaining to, characterized by , or promoting good health.*
(Blood & Studdert, 1988; 1999, texts are identically in both editions)

David Sainsbury (1986) propose a slightly modified definition based on productivity, where he uses the concept of positive health.

Good health is the birthright of every animal that w rear, whether intensively or otherwise. --- I believe it is not sufficiently understood by those interested in animal welfare that good health may be the most vital factor of all. --- An animal which is medicated to control disease is not as truly healthy as one which is maintaining health by living in a totally favourable environment. No one has found the perfect word to describe this state, but the term 'positive health' has been used and is perhaps a logical expression. Essentially, this means the provision of a complete die, an environment that is optimal for the animal's physiological needs, comfortable to the animal's senses, in which the animal is secure and free from fear, and with no undue challenge by pathogenic micro-organisms or predators. (Sainsbury, 1986)

General discussion of concepts

It is rare that veterinary textbooks give explicit definitions of health and disease. The main reason for that may be that the general purpose of the textbooks is not to investigate what is healthy or what is diseased in principle, but to describe diseases and their causes.

It is not uncommon that textbooks use different aspects of health definition (e.g. Wiesner & Ribbeck, 1983). This makes it harder to actually know what the main health idea is in a textbook.

When an explicit definition of health is presented in a textbook it is almost always given without references to other health definitions. I assume that the reason for this is that the authors are focused on the general topic of the book and not philosophy. It is extremely rare that the general debate of welfare in animals is referred to in veterinary textbooks (e.g. Broom, 1996; Duncan, 1996).

The authors seem to write in isolation from other authors' definition of health and disease. However, there is a remarkable exception and that is the school of homeopathy. All three books about veterinary homeopathy found are referring to what the authors find as 'bad' health definitions, i.e. the mechanistic approach in conventional medicine (Brock & Nielsen, 1986, Day, 1995, Thoresen, 1997).

Homeopathic health definitions are stated to be 'holistic' and are mainly based on the idea that health depending on homeostasis. However, as health definition of conventional veterinary textbooks also can be based on the homeostasis idea, it can not be proposed that 'homeostasis thinking' logically leads to homeopathic medicine. This observation may illustrate that the basic theory behind a health definition do not have inevitable consequences for the veterinary practice.

Conclusions

The concepts 'health' and 'disease' are rarely defined in veterinary textbooks. The explicit definitions of health can be categorized into five different categories:

• Health as normality, i.e. to define health in relation to what is regarded as normal. Normality could be regarded as biostatistical (quantitative) or as more qualitative.

• Health as biological function, where the deviation or disturbance of the biological processes is regarded as disease

• Health as homeostasis, where homeostasis is an old idea, which relates health to the maintenance of a delicate balance within the organism.

• Health as physical and psychological health, where the health is defined as physical and psychological health, often related to terms like well-being or even harmony.

• Health as productivity including reproduction, which is common in veterinary medicine for farm animals. This definition refer that the concept of "health" in (livestock) animals should integrate, or even be replaced by, productivity and reproduction.

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REPRODUCTIVE BIOTECHNOLGIES AND RISKS OF DISEASE SPREADING

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SUMMARY

The present paper deals with the risk analysis of the three first generations of Reproductive Biotechnologies: Artificial Insemination (AI), in vivo collected and in vitro produced Embryo Transfer (ET). Those technologies are used at large and worldwide. There are theoretical risks to associate pathogens with gametes and embryos to be moved hence contaminating the recipients. For each of those technologies, as they were becoming available and used on the field, the veterinary community has been able to perform numerous investigations allowing to generate guidelines and recommendations based on sound science. The Intergovernmental Agency, the Office International des Epizooties (OIE) has assessed those elements and has approved those recommendations as now published in the Terrestrial Animal Health Code. For artificial insemination, the basic rules rely on the presence of semen donors in pathogen free studs under the official veterinary supervision. For transfers of both in vivo collected and in vitro produced embryos, and in addition to the strict application of the guidelines such as those published in the International Embryo Transfer Society (IETS) Manual, the basic concept of biosecurity relies on the officially approved embryo collection or production and transfer teams. Many decades have proven that when such guidelines and recommendations are rigorously followed, those transfers can be achieved with a maximum level of security.

INTRODUCTION

There are classically four generations of Reproductive Biotechnologies (RB) that have built up progressively (Thibier, 1990). The first introduced after the last world war is that of Artificial Insemination (AI) and the last, transgenesis appeared with the first breakthrough reported by Palmiter et al., (1982) in producing transgenic mice. In between, were those of "classical" Embryo Transfer (E T), involving in vivo embryo collection and transfer (second generation) and those of in vitro embryo transfer and nuclear transfer (cloning). Currently, on the field, it is a fact that only Artificial Insemination, in vivo collected embryos and in vitro produced embryos transfers are used with quite a different range in numbers. Insemination by artificial means has been developed in many species of mammals, birds and insects. A recent worldwide survey by Thibier and Wagner (2000, 2001) has shown that, in the bovine where it is the most widely applied, more than 100 million females are inseminated each year. This corresponds to approximately one sixth of the total population. It is now over a quarter of century since the second generation of RB became operational in the field. Due to the relatively high cost of obtaining offspring (around 7OO €, for cattle in the European Union), its global uptake has been restricted mainly to cattle. As shown by the annual survey of the International Embryo Transfer Society (IETS) Data Retrieval Committee, around 500,000 bovine embryos are transferred annually across the world (Thibier, 2003). The transfer of in vitro produced embryos became operative for special purposes in cattle and, to a lesser extent, in other species some ten years ago. More than 80,000 bovine in vitro produced embryos were transferred worldwide in 2002 (Thibier, 2003).

Since the beginning of implementation on the field of reproductive biotechnologies, starting with Artificial Insemination, in the late forties, the veterinary community has taken the greatest care to ensure that no pathogen transmission would be associated with semen. The same held true with the more recent reproductive biotechnologies.

The risk analysis, as called nowadays, includes first an assessment of the risks that some pathogens could be associated with the gametes or embryos that are collected or produced and further transferred. A considerable number of investigations have been made and have resulted into some recommendations based on sound science that were then taken and approved by the Office International des Epizooties (OIE) and further incorporated in the relevant OIE Appendices. We will here report in the first section of this presentation some highlights of this assessment. Risk management is the second step of the risk analysis and involves a clear, well-defined code of practice ensuring that the recommendations are followed all along the line of the process so as to guarantee transfer of pathogen free gametes or embryos. This will be here reported in the second section of this paper.

1. THE RISK ASSESSMENT.

Genital shedding of pathogens can result from primary infection of the genitalia or from a more generalized infection. It is hence quite logical to assume that there are risks of contamination associated with collecting gametes or embryos and transferring them in any recipient without appropriate control measures.

1.1. Semen sterility is virtually unachievable, so the first question is how to control the population of so-called "non-specific microorganisms in semen efficiently and the second question is how to prevent any association of specific pathogens.

Numerous studies have identified specific putative agents associated with semen that are able to contaminate inseminated cows (see reviews by Thibier, 1998 and Thibier and Guérin, 2000). Are so concerned the 15 major diseases listed by OIE in List A (table 1). They are all of viral origin with the exception of contagious bovine pleuropneumonia, which is caused by a mycoplasma. For almost all these diseases during their chronic phase, the pathogenic agent has been reported to be present in semen. Semen transmission has been well established and documented for several such agents such as in particular Foot and Mouth Disease and blue tongue in ruminants. Almost 80 diseases are listed by the OIE in List B and nine of them can affect multiple species. There are other

important diseases not listed which must be also considered such as BVD for example. Of the viral diseases in List B, those that have been investigated the most are enzootic bovine leucosis (EBL), the herpes virus disease so-called, infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/PV). The EBL virus is exclusively associated with blood cell, so contamination of semen from infected animals is only possible if blood cells are included. IBR/IPV has been the subject of considerable investigation. As with most of herpes viruses, bovine herpes virus (BHV1), the pathogen of this disease, has phases of excretion and phases of latency. Intermittent excretion of BHV1 in semen has been shown to occur in bulls (Guérin, 1989) and reactivation of latent infection without warning or noticeable signs is a great hazard. The BVD virus has been identified in semen and the major risk arises from the so-called " persistently infected" animals which continuously shed the virus. Bulls with persistent infection, as well as those with the acute form of BVD, may shed the virus in semen and thus transmit the disease (Kirkland, et al., 1997). Numerous bacterial agents can also be associated with semen and among those a special care should be addressed to brucella and mycobacterium tuberculosis. With regard to cattle, it should be mentioned that prions do not pose a risk of transmission through semen contamination (Wrathall et al., 2000, 2001).

1.2. With regard to in vivo collected embryos, quite a number of pathogens have been investigated in their interaction not only with bovine but also with ovine/caprine and swine embryos, using different approaches: in vitro contamination of embryos, in vivo collected embryos from affected donors and search for the association of the pathogens to the embryo either in vitro or from recipients receiving possible infected embryos. Table 2 summarizes some of the data collated in the IETS Manual (1998). These results show that, provided that defined sanitary practices (see below) are followed during the process, the risks of transmission of a given disease from the donor to the recipient via an embryo is minimum.

1.3.As far as the in vitro produced embryos are concerned, the sanitary risks associated with the donor females have recently been reviewed by Guérin et al., (2000). The first point of interaction is the oocyte itself and its follicular environment, (surrounding cells of the oocytes and the follicular fluid). Contamination of such cells by two types of viruses, BVDV and IBR/IPVV (those two viruses are those that are the most commonly studied) has been reported by several investigators in different parts of the world (see the referred above review). Those viruses appeared to adhere to the oocyte zona pellucida and hence are "external" to the oocyte. It would be interesting to investigate further if a given pathogen could be found "inside" an oocyte collected from the ovary with then the possibility of interacting with the genome directly. Of course if that was the case, it could also theoretically occur in the natural process, except that here with the in vitro procedure, one collects oocytes that otherwise might never have spontaneously ovulated. To date and to our knowledge, no infectious agent has been retrieved inside the oocyte with the notable exception of a report by Bielanski (1994) on Camplylobacter fetus that suggested an intracellular contamination of that bacterium. Of course the conditions of this observation were totally experimental and it is hence difficult to assume that it mimics exactly the normal situation. This however should be more thoroughly investigated and could be a theoretical or potential challenge as no field evidence has arisen of infected progeny.

The zona pellucida of in vitro produced embryo seems to interact with pathogens differently than from in vivo embryos. One further evidence of this was reported by Marquant-LeGuienne et al., (1998) investigating quite an important pathogen in ruminants and swine, the FMD virus. These authors in vitro contaminated in vitro produced embryos and showed that the 10 washings of the embryo recommended were unable to remove the association of the FMD virus (type O) from the embryo as opposed to what was reported for in vivo derived embryos (Singh et al., 1986 and see review in the IETS Manual). It remains to demonstrate if the other types of FMD virus behave in a similar manner and if enzymes such as trypsin would be effective for each of the types of the virus in dissociating them from the in vitro produced embryo zona pellucida. Various bacteria have also been investigated and a recent report on another type of pathogen, Tritrichomonas foetus, has recently shown that this parasite experimentally associated with in vitro produced bovine embryos was not further detected in embryonic cells of ZP intact embryos or hatched embryos after culture hence rendering the potential risk of transmission unlikely (Bielanski et al., 2004).

1.4. The possible sequence of hazards. As proposed by Thibier and Guérin (2000) for the in vitro production of embryos, the sequence of hazards in terms of infectious agents includes (1) those related to the female donor and the mode of collection (abattoir collection or ovum pick up), (2) the maturation process, (3) the fertilization (introduction of semen), (4) the co-culture in vitro development, (5) the cryopreservation before (6) the last step, thawing and transfer. The sequence is similar for artificial insemination and for transfer of in vivo collected embryos with their relevant "routes".

For semen donors, the environment in which the animal is located has the utmost importance in terms of likelihood to be in contact with a given pathogen. The same holds true for embryo donors. For the latter, Stringfellow and Givens (2000) nicely summarized from an epidemiological point of view, the risks at stake. "If a pathogen was to be transmitted by transfer of in vivo derived bovine embryos, an uninterrupted sequence of events would have to occur". This sequence includes (1) the exposure to pathogen, (2) the continued association of pathogen with the embryos, (3) the maintenance of infectivity of pathogen throughout embryo manipulation and processing and finally (4) delivery of an infective dose of pathogen to a susceptible recipient. Several factors well identified in the review of Stringfellow and Givens (related to the own properties of the embryo such as those of the zona pellucida or to the handling and

procedures used: washing, antibiotics etc.) give some explanation to those observations.
This can be extended to all reproductive technologies and greatest care is always to be taken to ensure that there is no addition of pathogens or contaminants during the whole process.
A special notice should be given to the risks associated with materials of animal origin. Any biological product of this kind used for recovery of gametes, sperm and oocytes or embryos, dilution, in vitro maturation of oocytes, washing and storage is potentially a source of contamination. This is of particular relevance with regard to the Transmissible Spongiform Encephalopathies (TSE) as discussed at large by Wrathall, 2000.
The putative contamination of semen or embryos while stored in liquid nitrogen (LN) tanks for example, as an additional source of contamination, has received recent attention. Bielanski et al., (2003) have demonstrated the occurrence of microflora in LN tanks such as Stenotrophomonas maltophilia that was able in experimental contact with semen to decrease the motility of semen. These authors have indicated that direct contact of contaminated LN with embryos may lead to their association with viral agent. However, they have also shown that all sealed samples of embryos stored in contaminated LN tanks tested negative for the presence of bacteria or viruses.

For in vivo-collected embryos, and with the proviso that all guidelines published by IETS and OIE after official approval, are rigorously followed, the IETS relevant committee, namely the Health and Safety Advisory Committee (HASAC) has categorized diseases according to the risks assessment analysis, into four categories. The category one is "that for which sufficient data are available to determine the risks to be negligible provided that the embryos are properly handled between collection and transfer". As seen in the table 3, there are only seven diseases listed in this category and it is unlikely, unfortunately, that this number will increase in the near future due to the insufficiency of research in this area.

2. THE RISK MANAGEMENT.

If for the risk assessment section there are similarities and dissimilarities between semen and embryos, clearly for the risk management, the procedures to be followed and accordingly the official recommendations are radically distinct.

2.1. For semen, the basic epidemiological rule is the following: for a given pathogen, the semen may be guaranteed to be free of a given pathogen if the semen donor is free from it **and** if the donor is one of a group of individuals that are free from it. This approach requires a very strict and well-monitored system of control of the male studs. Specifically, the semen should be collected (i) in an approved semen collection center (SCC), (ii) in a hygienic manner by technically trained and experienced people and (iii) under a rigorous program controlling the health status of the sires. The quarantine station in which the bulls are to stay prior to their entry to the SCC is important. The station management should ensure that only individuals free of specific diseases enter the SCC. If this quarantine station is the primary line of defense, the second refers to the adequate design and management of the SCC. Its general organization should be officially approved by the veterinary authorities according to the recommended guidelines and the center should adhere to a biosecurity program under a quality assurance system. Guidelines are all based on recommendations laid down in the OIE Terrestrial Animal Health Code Appendices: 3.2.1, 3.2.2 and 3.2.3. (OIE Code, 2003). One of the prime examples of the application of these measures is the EU Council Directive on semen, referred to as "Directive 88/407 for the bovine species.
The processing laboratory must also be monitored; only authorized personnel should be allowed to enter. The basic organizing idea in such a laboratory is the FORWARD rule: once the semen has been collected, it should move forward from one place to another with no return or crossing. Health surveillance and testing is also to occur according to the recommendations provided in the OIE Code. The principles are first, to control and monitor individuals prior to their entry into the quarantine station and further prior to entry in the SCC. Health considerations of the area, herd of origin and each individual animal must be considered. Second, regulation examination and testing of the males in the center must be performed. Three major types of monitoring are required at regular intervals (once or twice a year): (i) thorough clinical examination of all individuals, (ii) detailed andrological examination of the collected semen and (iii) complete testing for various diseases recognized as the major sources of risk, such as tuberculosis, brucellosis, IBR/IPV, BVD, campylobacteriosis, tritrichomoniasis etc…

2.2. For embryos collected in vivo, the practical procedural guidelines for collecting and handling the in vivo derived embryos are described in details in the IETS Manual. It should be considered as a code of good practice and could be included in a quality assurance system wherever possible. The first step of course refers to the thorough clinical examination of the donor animal and its environment (lack of infectious contagious disease in the area or in the herd). For the handling of embryos, the basic recommendations are as follows. The first stage is to ensure an appropriate washing, 10 times consecutively with a new pipette each time, with immersion of the embryo(s) in each wash for duration of 1 min. with light agitation and with at least a dilution factor of 1/100 between each washing. There are now means to do this in a convenient manner and consuming little time. The embryo should be very carefully inspected under magnification (X 50) and should only been processed if the embryo has an intact zona pellucida and no adherent debris because such cells could serve as a source of contamination and allow for carry over the pathogen. The treatment of embryos with the enzyme trypsin is often recommended when dealing with "sticky" pathogens such as the herpes virus BHV1. This was shown not to be always necessary (Thibier and Nibart, 1987) but is nevertheless a good procedure and often required for exported embryos. The way trypsin is to be handled is also relevant since as a protein enzyme, it is

quite sensitive to the environment. It should also be mentioned that such a treatment is not by any mean, a panacea. Even if used, it should not be considered as replacing the need for sanitary precautions with the environment of the embryos.
The media may also be of some concern as discussed above. Its nature and origin should hence be selected with great care. The addition of antibiotics is also of some value if used appropriately. The quality control of the whole process is now necessary for a given team (see below) and regular testing in the media collected and stored for assay should be a standard procedure. This could involve search for a putative contamination by various viruses that might originate from the collected donor of from some serum used in the media, and the status for pathogenic and also for saprophytic microflora. This should contribute in the mid-term to establish and verify the effectiveness of the quality assured production process procedure.
These procedural considerations are part of the OIE recommendations (Terrestrial Animal Health Code: Appendix 3.3.1.) that specifically refer to the guidelines published in the IETS Manual. They are also most of the time included in the regulations for moving embryos from one farm to another. In doing so, it is right to state that embryo transfer contributes to improving the animal health status of a given population in controlling very strictly such movements of germplasm between herds. The basic concept of those regulations relies on that of the official approval of embryo transfer teams. This was a very important step in the scope of the veterinary regulations that generally rely on the animals, its confinement and its products. Here the safety of the industry fully relies on the ethical and technical excellence of the man/woman in charge, head of the embryo transfer team. The criteria conventionally used by the veterinary authorities to give their official approval relies on four major points:

(1) the supervision of the team by one veterinarian, with often the requirement for adequate training in terms of hygienic procedures for the personnel involved,

(2) the necessary equipment to proceed adequately to the different steps of the procedure,

(3) the commitment of the head of the team to strictly follow the procedural guidelines as stated in the IETS Manual,

and (4) be regularly submitted to official tests of flush fluids, washing fluids and degenerated embryos in terms of possible viral or bacterial contamination.
These teams are under the overall supervision of the official veterinary authority and are regularly inspected.

2.2. For the in vitro produced embryos, a set of recommendations to control risks associated with such embryos have been elaborated within the IETS and been published in the relevant chapter of the IETS Procedures Manual. Here too, they should be considered by all practitioners as a mandatory code of good practice. The first step to survey is the health status of the area, the herd of origin when relevant and the donor herself making sure that no infectious, contagious disease are present at the time of collecting the oocytes. A special note is to be given when dealing with animal from a given species or breeds threatened by extinction. It may well be for reasons of biodiversity or germplasm conservation that the general conditions required are not met. There could consequently be some exceptions, because of the considerable power of this technique for quality control (see below) and this, incidentally, constitutes one comparative advantage to this technique. When ovaries are collected from the slaughter house, it is of the greatest importance to trace back the herd situation of those females and check for example that they do not come from any depopulated herd for health reasons. The premises and working areas should be so designed that individual specialized units are set aside for particular tasks with restricted access. Wherever possible, a laminar flow chamber should be in place with close attention to cleaning and disinfecting procedures as rightly stated by Guérin et al., (2000).
The handling of embryos during the various steps should always be conducted with great care and under highest hygienic conditions. The quality of the media and of the co-culture cells system when relevant is one of the most critical point of the procedure. All biological products should be strictly controlled and guaranteed free from microorganisms (virus, bacteria or fungi). Sera containing antibodies against agents of particular concern should be avoided. It is also strongly advised to have knowledge and confirmation of the inactivation procedures from the manufacturers when relevant.
Adding antibiotics to the media is also always of good practice as it contributes to remove permanent or opportunistic pathogenic agents or saprophytic microorganisms inadvertently introduced at the collection point or at the time of fertilization from semen that can never be sterile (Guérin et al., 2000). Finally, the recommended washing procedure such as that described above for the in vivo derived embryos contributes to further reduce the likelihood of associating pathogens with the embryos so produced and released from the lab for transfer. The interest of adding trypsin is still a matter of debate as insufficient studies have been yet performed to assess the advantage of such a procedure with no detrimental effect. One of the major comparative advantages of this technique is that the production system provides control points and sufficient time to allow for each batch of embryo produced to be monitored and assessed to relative to their sanitary status. In addition, the many different media used provides an excellent source of sampling as it has been shown that the media, as a mediate environment of the embryos, serves as a good indicator of the pathogens to which they could have been exposed during the process (Thibier et Guérin, 1993). The quality control is here of particular relevance.
As for in vivo derived embryos, based on full consideration of relevant scientific peer reviewed papers, an Appendix (Appendix 3.3.2.) in the OIE International Animal Health Code for in vitro produced embryos has been elaborated and approved, with subsequent adoption of national regulatory frameworks such as the European Union Directive (89/556 modified 93/52 and 94/113).
The official guarantee of safety in terms of animal diseases relies on two factors: first only an officially approved team is allowed to process such embryos and

second such teams are under the control of the veterinary authorities of a given country (Thibier, 1993).
As for in vivo derived embryos there are four major conditions to be met for the teams to be officially approved:

(1) the supervision of the team by a well trained veterinarian in terms of hygienic and sanitary procedure,
(2) the capacity of the team to work in satisfactory conditions with particular attention to the premises, arrangement of the lab and equipment
(3) the commitment of the team to strictly follow the procedural guidelines as referred to in the IETS Manual,
(4) the regular submission of the team to the inspection of the veterinarian authorities and to sanitary controls of the degenerated or non- fertilized embryos, maturation and culture fluids stored for this purpose.

CONCLUSION

In conclusion, when moving gametes or embryos, there are definitely risks at stake of associating pathogens with them. However, as those Reproductive Biotechnologies developed, the veterinary community has devoted a considerable number of investigations both in vivo and in vitro to make clear assessments of those risks. This has led to recommendations and guidelines validated by the relevant intergovernmental Agency, as far as the control of infectious diseases are concerned, namely the Office International des Epizooties. There is still room for further research for some agents, particularly for exotic diseases and this should certainly be encouraged. However, the system in place worldwide has proven to be effective with considerable numbers of such transfers. It is based on science and integrity in the collection and processing procedures. It is hoped that when the new Reproductive Biotechnologies such as nuclear transfer or transgenesis when appropriate will be implemented, the same approach will be taken by both scientist and regulatory agencies.

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Table 1. List A diseases in mammals susceptible to be transmitted through AI (from Thibier and Guérin, 2000).

Disease or pathogenic agent	Bovine	Ovine/ Caprine	Porcine	Equine
Foot and Mouth Disease	P; Tr	P;Tr	P; (Tr)	
Vesicular stomatitis	(P); (T)			
Swine vesicular disease			P; Tr	
Rinderpest	P; (Tr)	P; (Tr)	(P)	
Peste des petits ruminants		P; (Tr)		
Contagious bovine pleuropneumonia		(P); (Tr)		
Lumpy skin disease				
Rift Valley Fever	P; (Tr)			
Blue Tongue	(P)			
Sheep pox and goat pox	P; Tr	P; T		
African Horse sickness		P; (Tr)r		
African swine fever				(P)
Classical swine fever			P; Tr	
			P; Tr	

P: presence demonstrated; Tr: transmission demonstrated; () highly probable

Table 2. Summarized results of studies of pathogens- intact zona pellucida embryos interaction (derived from the IETS Manual, 3rd ed., 1998)

Types of pathogens	No. of embryos exposed (*)	Assay of embryos
In vitro contamination and assay of bovine embryos (**)		
Viruses	12 - 169	0
Other viruses (***)	29 - 144	36 to 100 % positive
Bacteria	38 - 96	0 –26 % positive
Mycoplasmas	20 - 111	30 – 100 positive
Assay of embryos from zona pellucida intact bovine embryos from infected or seropositive donors		
Virus	2 – 372 (****)	Negative
Brucella	309	Negative
Chlamydia	5	Negative

(*) range of number of embryos per pathogen studied
(**) high concentration exposure mimicking a “worse case scenario”.
(***) BHV-1, BHV –4, VSV.
(****) FMD virus-infected donors.

Table 3. List of IETS/OIE diseases in category 1[1].

DISEASE	SPECIES	NOTE
Foot and Mouth Disease	Cattle	
Enzootic Bovine Leucosis	Cattle	
Bluetongue	Cattle	
Brucella abortus	Cattle	
Infectious Bovine Rhinotracheitis	Cattle	Trypsin treatment required
Pseudorabies	Swine	Trypsin treatment required
Bovine Spongiform Encephalopathies	Cattle	

[3] according to the conclusions of the Research sub-Committee of the IETS HASAC (OIE, Terrestrial Animal Health Code, Appendix 3.3.5,2003).

[1] Special categorization for in vivo derived embryo-pathogen interaction

Tools and strategies for fighting diseases

Oral Communications

ACUTE PHASE VARIABLES TO ASSESS HEALTH IN VARIOUS SPECIES

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Introduction

During tissue damage, infection and inflammation, pro-inflammatory cytokines are liberated. This first reaction of the body to immunological stress is the innate, non-specific, response preceding specific immune reactions. Within a few hours after infection the pattern of protein synthesis by the liver is drastically altered (1). Some proteins show an increase in concentration, the positive reactants (such as C-reactive protein (CRP)), whereas others decrease, the negative ones (such as albumin, retinol binding protein (RBP) and alpha-a1 lipoprotein). Individual variability in reactivity occurs and depending on energy balance, the negative blood variables may be more indicative of a changed metabolism than the positives. The positive APPs are regarded as having general functions in opsonisation and trapping of micro-organisms and their products, in activating complement, in binding cellular remnants like nuclear fractions, in neutralising enzymes, scavenging free haemoglobin and radicals, and in modulating the host's immune response. Measurement of positive acute phase variables in combination with negative ones and calculation of an index results in a rather sensitive method to analyse the nutritional and inflammatory state of an individual.

The index has been used as prognostic inflammatory and nutritional index (PINI) for human patients (2,3) and as acute phase index (API) for cattle (4). When well chosen it becomes a *nutritional and acute phase indicator* (NAPI). Such index enhances sensitivity and specificity in comparison to single APPs remarkably detect non-healthy subjects in populations of normal animals, as was shown for cattle and finishing pigs at slaughter (4-6) and was favoured by findings in experimental pigs with *Streptococcus suis* infection (5).

Results from measurements in cattle and pig will be shown as model for other species including man. Furthermore preliminary results from milk analyses are presented. Milk is described to contain a specific isotype of SAA, measurable in mastitis cows at an earlier moment compared to the elevation of SAA found in blood (7). Isoelectric focus (IEF) was used for a time curve of samples from cows intra-mammarily infected with *E. coli* 0:157 (30 cfu in one quarter) for detecting SAA isoforms in blood and milk. With these findings an easy and reliable to determine, early determent of mastitis might be found.

Materials and Methods

The index was calculated as a combination of positive and negative reacting proteins. The format used is stated below:

$$(N)API = \frac{\text{value of a rapid positive APP} \times \text{value of a slow positive APP}}{\text{value of a rapid negative APP} \times \text{value of a slow negative APP}}$$

The data used were obtained from previous performed well-described experiments (4-6). In those studies several blood values were determined. These values are calculated here.

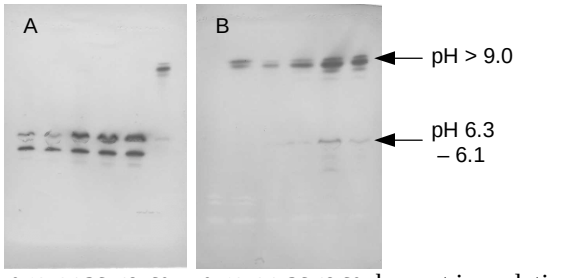
Isoelectric focus (IEF) and Western blot (WB) techniques were performed routinely. For detecting SAA isoforms on the WB after IEF, the biotinylated anti human antibody from a commercial kit (TP 802, Tridelta, Ireland) in 1:100 dilution was used, followed by Streptavidin-Horse Radish Peroxidase, 1:4000. (DAKO, Denmark), and visualization by incubating for 10 to 15 minutes with a TBS solution containing 0.5 mg/ml 3,3'-diaminobenzidin (Sigma-Aldrich, USA) and 0.02% of 30% hydrogen peroxide (Merck, Germany).

Results

When compared 21 healthy control cows with 233 clinical patients the API of the normal cows revealed a value of 0.001 whereas the non-healthy group had a API value of 2.9. The API calculated for these cows contained haptoglobin and SAA as positive reacting proteins and albumin and alpha-2-macroglobulin as negative ones.

In the case of pigs experimentally infected with Streptococcus suis the measurements before time point of infections were used as control. The API used contained CRP and Haptoglobin as positive reacting proteins and Albumin and Vitamin A (reflecting RBP) as negative one. The API value at the time points before infecting was 0.005, whereas this value increased up to 9.1 at day 3 post-infection.

Figure 1. Western blot of IEF of time series of plasma (A) and milk (B) samples after inoculation with E.coli 0:157 (30 cfu in one quarter). The 60 hours sample from the milk was run on both gels to allow comparison between them.



Discussion

The findings presented indicate the calculated index to be more powerful in discriminating between normal and diseased animals.

Furthermore the results indicate milk SAA to be a marker for infectious mammary disorders. The milk isoform has a different pI, compared to the plasma isoform and it was measurable at an earlier time point compared to the blood plasma SAA.

Ackowledgement

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IMPLEMENTATION OF THE ACUTE PHASE PROTEIN HAPTOGLOBIN IN ENCOMPASSING PREVENTIVE HEALTH PROGRAMS IN PIG PRODUCTION

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Introduction

Traditional quality control - performed at the end of the production process - is no longer the means to economically ensure food safety and quality.

Important aspects of the extended comprehension of quality in meat production are, for instance, simultaneous ideas about the type of production, housing of animals or environmentally friendly production. Therefore it is essential in agriculture and in the food industry, as it is the case in other industrial sectors, to implement new strategies aimed at process improvement. Thus an efficient quality and health management becomes more and more essential in animal production (1).

As sensitive markers for disturbances of the homeostasis, acute phase proteins are discussed as potential parameters to assess animals' health and welfare (2). Haptoglobin (Hp) belongs to the positive acute phase proteins in pigs (3) indicating inflammatory or infectious lesions by increasing serum concentrations (4). It is a very sensitive parameter that can support the evaluation of the general health status of pigs within the scope of veterinary herd-health-analysis or comprehensive preventive health programs (2, 5).

The purpose of three studies was to prove how to implement the screening parameter Hp in preventive health management systems, i.e. determination of sampling time in the production stage as well as the evaluation of benefits.

Material and Methods

The first study aimed at the assessment of new control strategies including the screening parameter Hp in different piglet rearing systems.

Seven piglet breeding farms, 15 piglet rearing farms and two fattener farms were available. According to the customer-supplier-contacts the piglet rearing farms were divided into five categories:

Category I: own breeding
Category II: one supplier
Category III: up to 40 suppliers
Category IV: some suppliers and
Category V: work distributed pig production.

In every farm a weak-point-analysis was performed using check lists which constituted as main criteria housing, building, production process, germ contamination, parasites, sty climate, fodder/water and individual animal health. In the categories I and II blood sampling was performed a few days before moving the piglets to rearing, 3 weeks after housing and a few days before moving the animals to the fattener. The categories III to V realized blood sampling directly after receiving and a few days before moving the animals to the fattener. During the whole production period diseases and rearing performance were recorded by the farmers.

In a second study Hp was tested as a marker of less hygienic conditions (evaluated by check lists) in two breeder-fattening farms (A, B). Clinical examinations and blood samples of indicator groups of 16 pigs each were performed.

The third study examined Hp as a tool to monitor pig health status at slaughter. Blood and muscle samples from diaphragmatic pillar (d.p.) and m. brachiocephalicus (m.b.) were collected from 330 slaughter pigs. Meat juice was obtained after freezing and thawing the muscle samples and post mortem examination including bacterial analysis as well as the determination of salmonella-antibodies in meat juice were performed.

In all studies Hp determination in serum respectively meat juice was performed according to the method of HISS and co-authors (6).

Results

In study one it turned out that piglets from one origin had significant lower Hp concentrations at the beginning of the rearing period than animals received from different origins. The retrospective view showed that animals causing medical treatment costs over € 1,50 had higher Hp levels at the time of coming into the rearing than animals which caused lower costs. The prospective view about the expected growth performance in the fattening period showed that piglets in the rearing with daily weight gain over 350g had lower Hp concentrations at the end of the rearing period. Obviously a close connection between the hygienic status of the rearing farm in the receiving inspection, the in-process inspection and the final inspection and the Hp serum concentration did exist. Out of these results a test strategy for Hp-Screening was developed (figure 1).

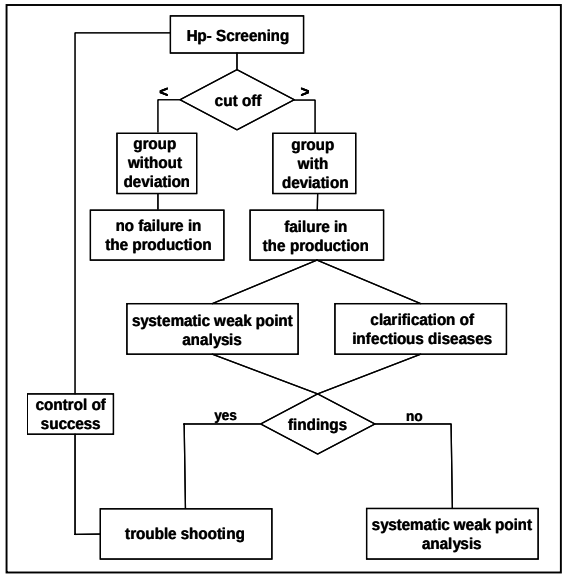

Figure 1: Test strategy for Hp-Screening

The results of the second study proved this connection. In contraste to farm A, farm B only acquired half of the maximally available points concerning relevant hygiene

factors and was therefore rated „unsufficient“. The pigs of farm B showed more often clinical symptoms than those of farm A which was reflected by comparing the time course of growth of the two fattening groups. The performance of pigs in farm A followed the normally observed physiological course of growth while the growth of pigs in farm B was depressed (figure 2). It was conspicuous that there were also statistically significant differences in Hp concentration between animals showing no observable clinical deficits from farm B in comparison to pigs from farm A.

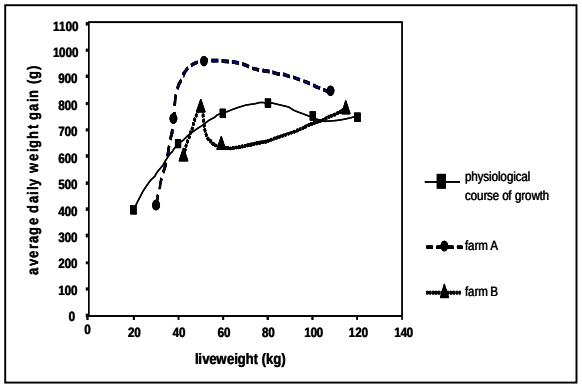

Figure 2: Physiological cost of growth and average daily weight gain of pigs in farm A and B

In the third study Hp concentrations in blood could be correlated significantly ($p<=0.001$) with those in d.p. juice ($r=0.7$) and m.b. juice ($r=0.8$). Significant ($p=0.046$) higher Hp levels in pigs tested positive for salmonella-antibodies could be found in blood (figure 3). Furthermore higher Hp levels appeared in meat juice of salmonella positive samples but could not be proved statistically.

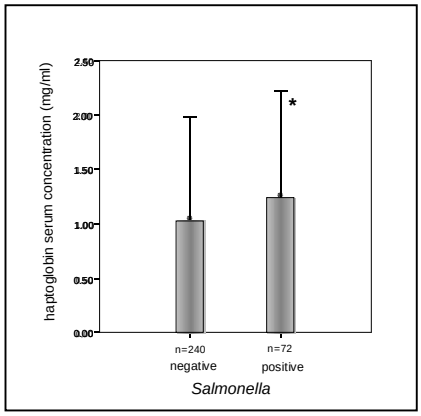

Figure 3: Hp serum concentration of Salmonella positive and negative slaughter pigs

Discussion

The significant higher Hp levels of pigs from category III to V could be the result of the crowding-effect during the transport of the pigs. Different studies proved the relationships between the daily weight gain and the Hp concentration (7, 8). Our own studies prove this relationship. Furthermore Pedersen and Dahl (9) as well as Ice and co-authors (10) found out that different environmental and management factors affect animals' health and its performance.

Also this close connection between the hygienic status of the farms and the Hp values of the pigs could be observed in our field studies.

Conclusion

A test strategy combining the measurement of Hp, check lists and ranking system in the preventive health management is suggested. Favourable points in time for the receiving inspection are three days before moving the animals to the breeder or directly at the receiving time, for the in-process inspection three weeks after receipt and for the final inspection three days before moving to the fattener. Hp was demonstrated to be a useful tool to assess animal health in all production stages and to identify animals living in less hygienic environment. Furthermore, Hp quantification in meat juice might be a useful parameter to assess meat quality in terms of animal health at slaughter.

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AUTOMATIC MEASUREMENT SYSTEM FOR COW LEG DISORDER DETERMINATION

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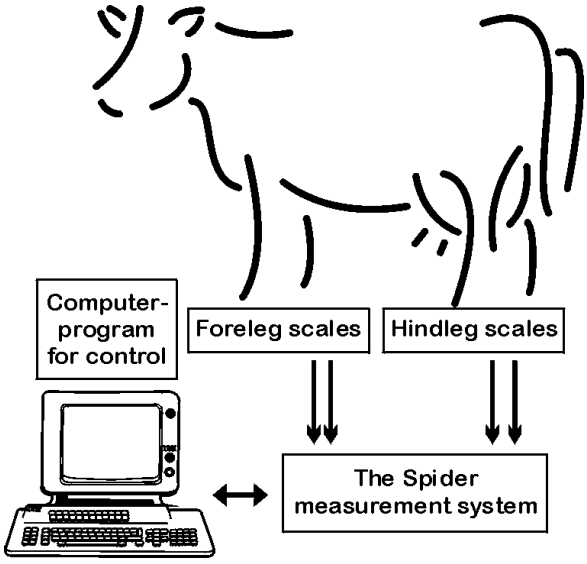
Introduction

Limb disorders cause serious welfare, health and economic problems at the loose housing cattle keeping (Klaas et al, 2003; Juarez et al, 2003). In bigger herds it is very complicated to track the early stages of lameness and other disorders. Therefore, the automation of relevant procedures is needed. Walkthrough scales for lameness detection are under development at present (Rajkondawar et al, 2002). The automatic measurement of static load of each leg is quite a suitable means for this purpose as well. It could be carried out when the cow is in some of the self-service units (concentrate feeder, milking robot etc). The objective of this study is to estimate the leg load distribution of cows.

Material and Methods

The Interrobo co-operation project between the University of Helsinki and the Estonian Agricultural University has developed a four-scale measurement system at a milking robot (AMS) in Suitia experimental cowshed in Finland (Figure 1).

Figure 1. Measurement systems for the investigation of the leg load distribution of cows



Four strain gauge scales were installed taking into account the point of support of each leg. Prior to this study it was ascertained that the application of scales with the area of 30×40 cm would guarantee that all four legs are situated on the scales with 95% probability (Hämälainen, 2003). The Spider measurement system (HBM, Germany) consisting of an amplifier, an analogue-digital converter and a controller were connected to the scales. Special software was created to ensure the data flow from the Spider to a PC and to control the measurement cycles. The load measurement had an accuracy of ± 1.0 kg and the measurement frequency was 10 Hz. When a cow entered the AMS, leg load measurements started automatically. Measurements were stopped and written into hard disc also automatically after the cow left the AMS. The data files were later downloaded from the PC via a network of the Helsinki University. Leg load index (LLI) that indicates the partial load of a leg in relation to the body weight was created for each leg:

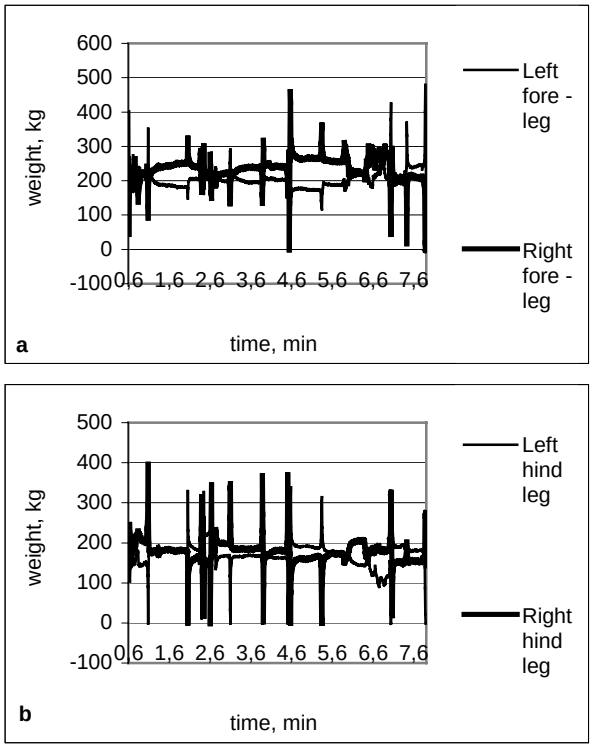
$$LLI_n = L_n / W,$$

whereas L_n is the leg load (n=1-4), and $W = \sum L_n$ is the body weight. These indexes were used to evaluate the leg load distribution during the study. For the experiments, 42 dairy cows were chosen as focal animals who regularly visited the AMS. Altogether 1 055 AMS visits were analysed with the EXCEL software package.

Results and discussion

An example of leg load changes during the milking is presented on figure 2. Typically the forelegs' load is higher (54%) than for hind legs (46%). The peaks on the graphs indicate kicks .

Figure 2. Forelegs (a) and hind legs (b) load during the milking



The results of the study showed that there are static and dynamic differences in LLIs between different legs and cows (Figures 3-7). Cows A and B on figure 3 have normal pattern of LLIs, cow C has a considerably smaller left hind leg LLI, and cow D has a much smaller right foreleg LLI. The results of the whole study showed that the mean values of the hind legs load were unevenly distributed: the LLI of the left hind leg was 0.206 and for the right hind leg 0.253 (Figure 4). This was obviously caused by the cows' behaviour in connection to the AMS.

Figure 3. LLIs of four cows

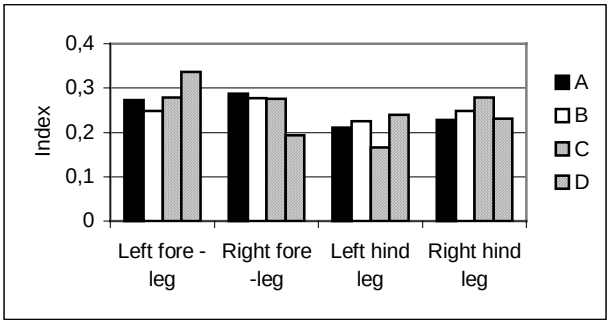
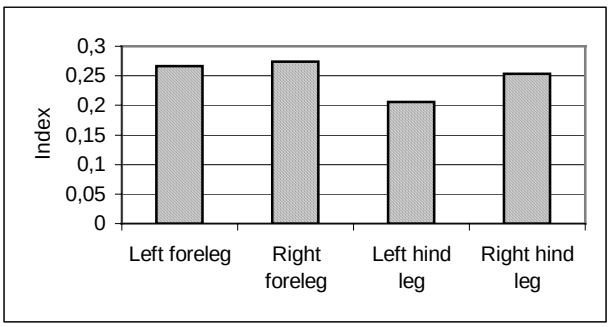
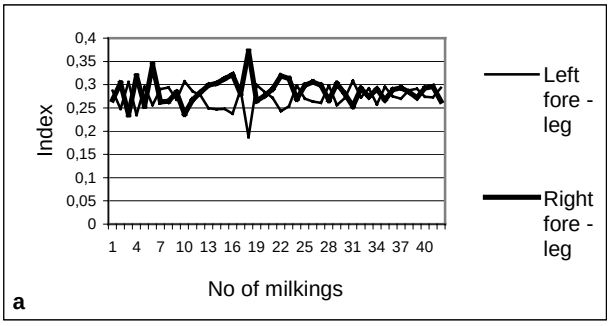


Figure 4. Mean LLIs during the study



LLIs of different cows had quite a specific dynamic pattern during the study. The appearance and duration of the differences in LLIs between the neighbouring legs can be used as parameters for the evaluation of abnormalities (Figures 5-7).

Figure 5. The dynamics of forelegs (a) and hind legs (b) LLIs of cow A



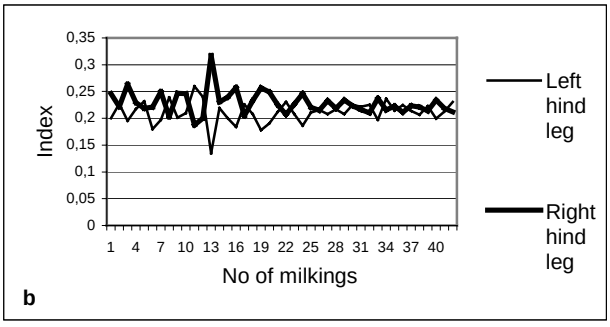


Figure 6. The dynamics of hind legs LLIs of cowC

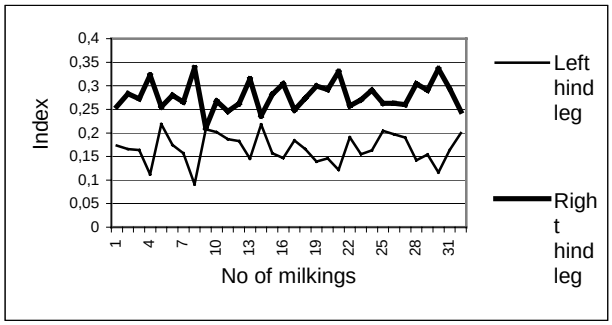
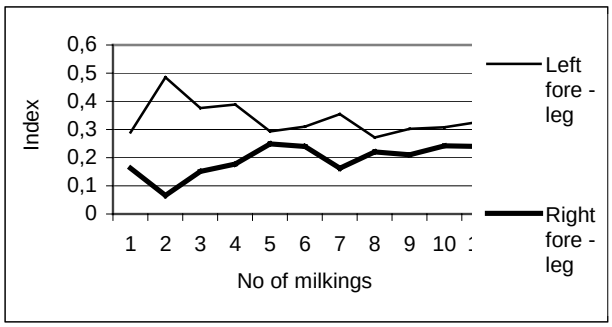


Figure 7. The dynamics of forelegs LLIs of cow D



Conclusion

The static distribution of leg load indexes (LLIs) and their dynamics in time can provide a basis for the detection of leg disorders. To a certain extent the cow's behaviour influences the leg load distribution. An automatic system for the estimation of LLIs can be used to monitor the health status of animals.

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USE OF POOLED SAMPLES FOR A COLLECTIVE BVDV-INFECTION CONTROL SCHEME IN BRITTANY (WESTERN FRANCE)

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Introduction

In Brittany (western France), 98% of farmers are affiliated to bovine health organisations (Groupements de Défense Sanitaire - GDS -).
A control scheme against bovine viral diarrhoea virus (BVDV) infection has been implemented by the GDS since 1986 ; until 1996, its main aim was to detect and slaughter PI animals in herds with clinical signs (mucosal disease, runting desease, abortions …). During this period, the herd annual incidence rate was 3 %, but many recontaminations were observed (corresponding to 10 % of the total incidence). Therefore, it was decided by farmers to implement a collective BVDV-infection control scheme, aiming at controlling the risk of new infections in all herds. In a preliminary step, specific studies were carried out to assess the prevalence and dynamics of BVDV infection in Brittany (1996 – 2000). Since 2000 all the dairy herds have been included in this new collective BVDV control scheme.

Material and methods

Basically, the control scheme is organized in three steps.

The first step consists in the determination of the BVDV-infection status of each herd. It is based on levels of BVDV-antibodies measured in bulk tank, using a blocking P80 ELISA test. Results are expressed in percentage inhibition (found to be correlated to the within-herd prevalence of antibody-positive cows) and split in three classes (Table 1).

Percentage of inhibition	class	Prevalence of antibody positive cow (mean)
≤ 35 %	0	0 – 10 % (5 %)
35 < < 60%	1	10 % - 30 % (22 %)
≥ 60 %	2	> 30 % (66 %)

Table (1) : relationship between percentage inhibition of bulk tank milk and prevalence of antibody-positive cows(1)

The herd-status is based on results of three consecutive testings four months apart. Based on Table 1, the 27 (2^3) possible combinations are gathered in 5 different statuses (Table 2).

status	definition	
A	presumed non infected	000, 001, 010, 100
B	not recently or lowly infected	011, 101, 110, 111, 121, 210, 211, 012, 112, 021
C	recently infected (?)	102, 002
D	heavily infected	222, 221, 212, 122, 022
E	undetermined	220, 120, 200, 202, 020, 201

Table (2) : Definition of the 5 herd-statuses

The second step involves only herds having a B, C, D or E status. It aims at detecting PI among dairy cows. In these herds, a bulk milk sample of the first lactating cows is tested using the blocking P80 ELISA test ; in the case of a positive result, a BVD RT.PCR technique is carried out on the whole bulk tank milk. In the case of a positive PCR test, all dairy cows are serologically tested then virologically for the negative ones.

The third step involves all herds having a D status and those of B, C or E status experiencing a first lactating-cows positive test using ELISA. Five pregnant heifers and five young heifers (older than 6 months) are serologically tested using the blocking P80 ELISA test. When more than 2 heifers are found positive, the whole group comprising the positive-tested heifers is serologically tested, then seronegative animals are virologically tested. PI are slaughtered within one month following detection. This procedure is implemented six and twelve months later when young calves are older than 6 months. Investigations are stopped when three consecutive heifers groups are seronegative.

Results

Herds statuses

	Feb. 2001	Feb. 2004
A	40 %	42 %
B	20 %	22 %
C	1 %	1 %
D	37 %	33 %
E	2 %	2 %

Table (3) : distribution of herds according BVDV-infection statuses in february 2001 and february 2004

The proportions of herds in the different statuses were almost steady from February 2001 to February 2004 (Table 3).

Complementary investigations

Status	First lactation bulk milk	PCR result in bulk tank milk
B and E	+ 38 %	+ 1 %
		- 99 %
	- 62 %	Not concerned
C	+ 50 %	+ 0 %
		-
	- 50 %	Not concerned
D	+ 65 %	10 %
		-
	- 30 %	Not concerned

Table (4) : results of RT PCR applied to bulk milk tank according herd-status and bulk milk of first lactating cows

Among B and D statuses, respectively 23 and 38 % of pregnant heifers groups have at least one antibody positive heifer. In total, less than 10 % of the herds hold at least one PI animal ; this proportion is higher in D + herds status (D with a seropositive test on first lactation cows) than in the other cases, as shown in table (5).

Status	Status with first lactation cows	Percentage of herds with at least a PI animal	
		Within status	On whole herds
A	-	-	0 %
B & E	**B ⊕ E ⊕**	17 %	1 %
	B - E -	0.5 %	0,1 %
C	**C ⊕**	50 %	0,5 %
	C -	5 %	0,05 %
D	**D ⊕**	30 %	7 %
	D -	2 %	0,15 %
TOTAL			10 %

Table (5) : Proportion of herds with PI animals

Discussion

A BVDV control scheme may be very costly. Investigations based on (i) individual serological tests and (ii) virological tests in antibody-negative animals cost about 10 € for each animal.

Farmers wish efficient and not expensive control schemes. Our method, based on use of pool samples in successive linked steps (focussing on target animals, e.g. primiparous and heifers) is much cheaper : about 1,5 € per animal. The method allows to give priorities and to adjust the means, expecially the human ones.

An action only in D + herds (20 % of the herds) should allow to detect at least 70 % of herds with PI animals.

Relevant indicators are necessary to assess the efficiency of our scheme. Analysis of transition probabilities between statuses from February 2001 to February 2004 shows that the survival rate in A status for a herd located in an area (geographical department) applying the collective scheme is higher (83%) than for A-status herds in other areas (69%).

Conclusion

BVDV control scheme needs new tools and methods : screening tools, sampling, risk assessment are necessary for an economic approach of animal health management. These methods must be associated with patience and risk acceptation. Brucellosis and tuberculosis control required more than 20 years !

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TOWARDS A TOOL FOR RETROSPECTIVE ASSESSMENT OF EXPOSURE OF NON-WEANED CALVES TO THE BOVINE RESPIRATORY SYNCYTIAL VIRUS (BRSV)

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Introduction

Respiratory disorders are usually considered as a major health issue in cattle, particularly in young calves [1, 8, 13]. Among the great number of viruses and bacteria potentially involved, the Bovine Respiratory Syncytial Virus (BRSV) appears to be the most common and significant aetiological agent of respiratory disorders [8, 9, 13]. Oubreaks of BRSV infection mostly occur in non-weaned calves less than three months of age [14]. Currently, the most widespread method of indirect detection of BRSV infection relies on detection of total immunoglobulins G (IgG) specific of BRSV by Enzyme Linked Immunosorbent Assay (ELISA). Evidence of an active immune response to BRSV is a specific rise in titer of total IgG from at least 400% between two blood samples three weeks apart [5]. However, taking into account the very strong seroprevalence of specific BRSV antibodies in cows (between 70% and 95%) the frequency of calves with BRSV specific maternally-derived antibody is very high [4, 9]. Maternally-derived antibody suppresses antigen-specific serum antibody response during the first months of life. So, reliance on paired-serological testing to identify BRSV in calves less than 3 months of age is not recommended [15]. The passively transferred antibodies are mainly of the IgG1 isotype, whatever the etiologic agent concerned [4, 6]. Amount of IgG2 is very low in the colostrum [2, 7, 10]. Moreover, if IgG2 seem to appear only 3 weeks after the infection, they remain detectable during more than 80 days [7, 11, 15]. However, the only study dealing with IgG2 specific of BRSV in calves less than 3 months and in spontaneous BRSV natural infections was carried out on only five calves [11]. This study aimed at describing, under field conditions, the level of total IgG and IgG2 specific of BRSV obtained from a large sample of sera from young calves before and after colostrum intake and from their dams.

Materials and methods

Blood samples were collected from young calves of commercial herds located in the south of the area Pays de la Loire by the veterinarians of 3 clinics located near the National Veterinary School of Nantes. On the occasion of a calving or a caesarean, 5 ml of blood was collected into sterile vacutainers by jugular venipuncture of the newborn calf before colostrum intake. Five ml of blood was collected into sterile vacutainers by caudal venipuncture of their dams. Between 3 and 6 days after birth, 5 ml of blood was again collected into sterile vacutainers by jugular venipuncture on the same calves. These calves should have taken an adequate amount of colostrum under supervision of the farmer. They also should have been healthy from birth to the day of this second sampling. Lastly, these calves should not have been vaccinated. Sera were separated by centrifugation and stored at –20°C until required for testing. All the sera were analysed blindly the same day. Finally, 100 paired sera of calves and the 100 sera of their dams were available for serological testing.

Detection of TGIg and G2Ig was made using commercial ELISA tests (LSI RSV Bovine Serum IgG and LSI RSV Bovine Serum IgG2). The cut-off value proposed by the manufacturer is S/P = 0.2 (for the two kits).

Results

The distribution of the S/P ratio for newborn calves before and after colostrum intake are shown in figure 1. Among the 100 newborn calves, before the intake of colostrum, 98 had a S/P (sample/probe) ratio of G2Ig < 0.04 and 99 had a S/P ratio < 0.08. After the intake of colostrum, the same 99 calves still had a G2Ig S/P ratio < 0.08, but only 6 of these 100 calves had a TGIg S/P ratio < 0.08. Among the 100 dams, 94 had a G2Ig S/P ratio > 0.08 and 97 had a TGIg S/P ratio > 0.08.

Discussion

This study allowed to confirm (i) the absence of detection of Ig G total and Ig G2 specific of BRSV on newborn calves before colostrum intake, (ii) the transmission of total IgG specific of BRSV by colostrum intake of their dams which have high sera level of total IgG and IgG2 specific of BRSV and (iii) lastly, under field conditions on a large sample of newborn calves, the quasi-absence of IgG2 specific of BRSV after colostrum intake. Based on the cut-off values provided for adult cows by the manufacturer the seroprevalence obtained on the dams, 90% of IgG2 and 95% of total IgG specific to BRSV, in Western France, seems to be similar to the high seroprevalences found in adult cattle in other European countries like the United Kingdom [9] and Denmark [11]. According to the syndesmochorial placentation of cattle, the calves were born almost agammaglobulinemic [10]. This step of checking allowed us to estimate the ability of the two ELISA tests used (total IgG and IgG2) to classify all the newborn calves sampled before colostrum intake as non infected. The results provided by the tests fit well with the agammaglobulinemia of newborn calves before colostrum intake. After colostrum intake, the S/P ratio of total IgG and IgG2 specific of BRSV increase (fig 2). Nevertheless when S/P ratio of IgG2 or total IgG specific of BRSV of the dams are high, S/P ratio of IgG2 remain low for their calves. As discussed by Uttenthal et al.[11], the presence of cattle with IgG1 but no IgG2 probably represent newly infected animals or animals with residues of maternally derived antibodies whereas cattle with IgG2 but no IgG1 probably represent convalescent animals. So detection of IgG2 could be used on non-weaned calves in endemic areas of BRSV infection. Concerning the newborn calf having a S/P ratio of IgG2 specific of BRSV of 0.1245, this value remains much higher than the values obtained on other calves. This calf should be considered as an outlier. This high value can be easily explained by a lack of specificity of ELISA tests [15]. Moreover, this calf had the highest S/P ratio of total IgG

specific of BRSV, so a non-lethal infection during the gestation, sometimes suspected but never evidenced, cannot be thrown out [7].

Conclusion

The obtained antibodies profiles are consistent with previous experimental studies [2, 10, 14]. Then, the potential interest of this technique based on the detection of IgG2 specific of BRSV, as a method for the retrospective detection of the circulation of the BRSV for non weaned calves, comes from the quasi-absence of this isotype in the colostrum which would make it possible to detect only a post-infectious immune response of the calves, with only one blood sample contrary to the usual methods. From our results it is possible to propose that a cut-off value adaptated to non-weaned calves can be lower than the cut-off value of 0.2 provided (for adult cows) by the manufacturer. Indeed, values around 0.08 comply with two classical methods of cut-off value determination. The first one consists in taking the highest value of a negative population. So, in this situation to propose cut-off values around 0.08. And the second method, consists in taking the average value increase of 2 standard-deviations. So, in this second situation to propose the cut-off value of 0.0732. Then, cut-off values around 0.08 seem to be relevant [3].

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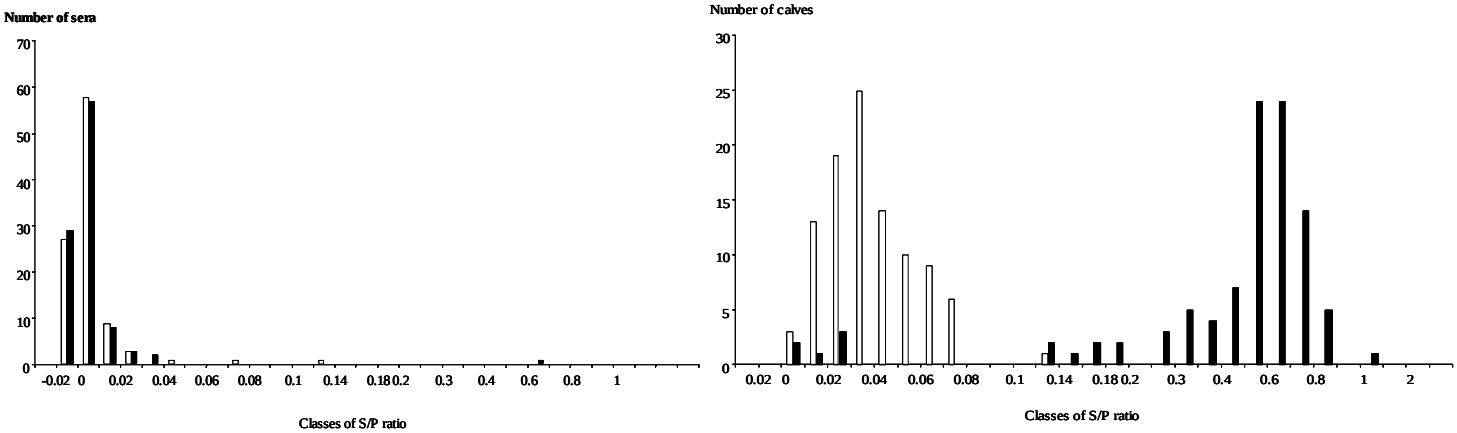

Fig. 1: Number of sera of the newborn calves before(left hand)and afte (right hand) colostrum intake according to classes of S/P ratios obtained with the IgG2 test (□) and the total IgG test (■) specific of BRSV.

RISK OF A *BRUCELLA* TRANSMISSION BY PORCINE EMBRYOS: AN *IN VITRO* STUDY

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Introduction

The cryoconservation of pig embryos with intact zona pellucida would be a major advantage to control and then insure minimal risk of disease transmission during embryo transfer of genetic material. Moreover, international rules on embryo transport outside of the country origin require that only embryos with an intact zona pellucida may be exported [9]. A vitrification technique is currently being studied in several species to resolve the problems of cryopreservation. Recently, a new technique has been developed to increase the cooling and warming rates of vitrification named Open Pulled Straw (OPS) technology [11]. The faster cooling rate seems to be one of the key solutions to protect porcine embryos from chilling injuries and to obtain piglet births after vitrification of unhatched blastocysts [2,3]. The OPS method allows the conservation of genetic resources and opens possibilities for porcine embryo transfer industry. In particular, exchange of genetic material between countries may then be simplified.

However, pathogens could be cotransferred with embryos. Transmission by embryo transfer of viruses to swine has been studied [10], and transmission of bacteria has been few investigated [7]. This study reports the evaluation of risks of transferring *Brucella* species via porcine embryos. The choice of *Brucella* genus relies on the re-emergence of brucellosis in pigs due to *Brucella suis* biovar 2 responsible for abortion, orchitis and sterility in swine, since 1993, in France and on the enzootic presence of this biovar in other European countries [5].

Material and Methods

1- Embryo production and collection

Superovulated Large White hyperprolific gilts (n=20) were used as embryo donors. Gilts were artificially inseminated 12 and 24 h after initial detection of oestrus using fresh semen. On days 5.5 or 6 of the oestrous cycle (Day 0= onset of oestrus) they were slaughtered after electronarcosis, and their reproductive tracts were immediately removed. Embryos were recovered by flushing the uterine horns with phosphate buffer at 39°C containing 2% New Born Calf Serum. Embryo development stages were evaluated under a stereomicroscope, with 20x magnification. Only unhatched blastocysts (n=394) were selected and assigned for this study.

2- Embryos washings before contamination with *Brucella* species

Before contamination with *Brucella*, blastocysts were washed 10 times according to the recommendations of the International Embryo Transfer Society (IETS) for bovine embryos transfer. However, antibiotics have been used only in the 5 first washings-steps to avoid action on the further infection with *Brucella* strains.

3- Contamination with four *Brucella* strains

Blastocyst stage embryos from Large White hyperprolific gilts were selected (from 10 to 35 embryos per gilt). They were submitted *in vitro*, in 6-wells microplates (Costar, Corning, USA) at the rate of 1 to 8 embryos per well to a massive infection (10^6 cfu/mL) with one of the four *Brucella* strains:

- *Brucella abortus* biovar 1 reference strain 544, isolated from bovine,
- *Brucella suis* biovar 2, and *Brucella melitensis* biovar 3, two wild strains isolated from swine, and
- *Brucella ovis*, a wild strain isolated from ram.

The *Brucella* strains and the embryos were incubated in the M199 culture media at 39°C with 5% CO_2. *Brucella* multiplication was measured after 17h of incubation, by culture of the M199 incubation media on trypticase soy agar yeast medium (TSA-YE) or TSA-YE added with 5 % horse serum for *B. ovis* (TSA-YES), after dilution in PBS.

One hundred and seventy six embryos from ten sows were used in each trial.

Trial 1. After 17h of incubation with *Brucella*, embryos were washed 10 times with Dulbecco's PBS, without antibiotics.

Trial 2. The second trial is incubated like the 1st one, except that washings have been performed with Dulbecco's PBS added with penicillin (100IU/mL) and streptomycin (100µg/mL), as preconised by the IETS for bovine embryos transfer.

In both trials, viability of embryos after washings was controlled by observation under a stereomicroscope.

4- Washing bath controls

Washing baths number 1, 5, and 10 were cultured on TSA-YE or TSA-YES, to look for the presence of *Brucella*. Moreover, all washed embryos themselves were mashed after the 10th washing and cultured also to look for the presence of *Brucella*.

Results

The control of *Brucella* culture from the M199 media showed that *Brucella* were still alive and were recovered on both TSA-YE or TSA-YES plates, after 17h of incubation in M199 media (data not shown). Enumeration showed that *Brucella* had survived or proliferated depending on the assays.

Stereomicroscopic observation of embryos after 17 h of incubation with *Brucella*, did not show any alteration.

In the 1st trial using Dulbecco's PBS without antibiotics (Table 1A), *Brucella* strains were always massively recovered in the first washing samples. Only 10 to 30 % of the 5th washing samples were positive and in addition the cultures were then less intense. *Brucella* were never recovered in the last washing. Moreover, *Brucella* were detected from 10 to 30% of the mashed embryos, according to the strains (the density of the culture was similar to that observed in the 5th washings).

Table 1. *Brucella* recovered in washing baths or embryos after 17h of incubation at 39°C, expressed in percentage of positive culture for each strain and each trial (the number of embryos tested are given between brackets).

A. *Without antibiotics in the 10 washings*

Washing bath	*B. abortus* biov.1 (45)	*B. melitensis* biov. 3 (45)	*B. suis* biov. 2 (44)	*B.ovis* (42)
1	100	100	100	100
5	30	10	10	0
10	0	0	0	0
Embryo	30	20	10	10

B. *With antibiotics in the 10 washings*

Washingbath	*B. abortus* biov.1 (44)	*B. melitensis* biov. 3 (43)	*B. suis* biov. 2 (44)	*B. ovis* (45)
1	100	100	0	40
5	10	10	0	0
10	0	0	0	0
Embryo	0	0	0	0

In the 2nd trial (Table 1B) using antibiotics in the washing buffer, *B. suis* was never recovered whatever the washing step. Unlike *B. suis,* other strains were recovered in the 1st washing and only in 10 % of the 5th washing for *B. melitensis* and *B. abortus.* No *Brucella* culture was positive for the 10th washing. Moreover, all the washed embryos were free of *Brucella*.

Discussion

Most of the studies concerning the sanitary risks of *Brucella* transmission by embryo transfer previously published were done on cows and showed that *in vivo,* artificially or naturally infected cows [1,4,8] did not seem to retain *Brucella* in their uterus. Transfer of cryopreserved cow embryos is now well documented whereas pig embryo transfers are more recent because of the sensitivity of these embryos to damage caused by cryopreservation. This problem being now solved by the development of the OPS method, we tried to document the critical point of the potential cotransfer of bacteria, and particularly *Brucella*, with embryos.

The inoculum of *Brucella* strains used in this study is extremely high. It was chosen to apply drastic conditions being probably over what is expected in practice. However the growth of embryos is not disturbed after 17 hours of incubation with *Brucella.* Ten percent of them were hatched blastocysts at the end of culture. Results obtained could be a good indication that if *in vitro,* no *Brucella* are recovered, *in vivo, Brucella* would also probably be removed. These results evidenced the need of antibiotics. We have demonstrated that without antibiotics in the washings, if no *Brucella* were recovered from the 10th washing, embryos were still contaminated (from 10 to 30 %). On the opposite, the trial using antibiotics as recommended by the IETS, showed no *Brucella* neither in the last washing nor on the embryos. Moreover, no *B. suis* were recovered whatever the washing sample cultured.

Even if our *Brucella* inoculum is higher, our results are consistent with those obtained by Mallek *et al.* [6], on the effects of *in vitro* contamination by *Brucella abortus* (10^1 to 10^5 cfu/mL) on both mice and cows embryos who have demonstrated that ten washings were sufficient to eliminate *Brucella* from the transfer medium. However they did not use antibiotics in their media. Similar results were obtained with porcine embryos *in vitro* infected by various pathogens such as *Pasteurella multocida* or *Streptococcus suis* by Smits *et al.* [7], integrating a cocktail of penicillin and streptomycin in the washing bath, that corroborate ours.

Conclusion

These results emphasise the need of antibiotics in the buffer used to wash embryos before performing vitrification and transfer. Considering that the level of contamination with *Brucella* used in these assays is probably higher than what is expected in practice, we could conclude on the real effectiveness of the washings. According to this study, the application of washing procedures with antibiotics is necessary and sufficient to allow porcine embryos transfers without risk of transmission of *Brucella*.

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ONLY PHASE I Q FEVER VACCINE PROTECTS PREGNANT GOATS AGAINST CHALLENGE WITH COXIELLA BURNETII

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Introduction

Q fever, a zoonosis caused by the obligate intracellular bacteria *Coxiella burnetii* is endemic throughout the world and infects arthropods, birds, pets, domestic and wild mammals and humans. The disease is known since the 1930th and has been reported worldwide except in Antarctic region and perhaps in New Zealand where its presence is not really confirmed (5). In livestock, *C. burnetii* is associated with reproductive disorders: abortion, stillbirth, and delivery of weak and unviable newborns, placentitis, endometritis and infertility (7). Such reproductive failures are accompanied with shedding of great number of *Coxiella* into birth products, urine, faeces and milk of infected animals. In human, the acute disease currently appears like a flu-like, usually self-limiting illness accompanied by myalgia and severe headache. Complications may occur such as pneumonia or hepatitis. Endocarditis in patients suffering from valvulopathy and premature delivery or abortion in pregnant women, are the main severe manifestations of the chronic evolution of the disease (8).

Q fever is essentially an airborne disease. The main route of *C. burnetii* infection is by inhalation of contaminated aerosols or dusts containing the microorganism shed from infected animals. Transmission of *C. burnetii* among domestic ruminants is mostly associated with abortion and among them sheep flocks. The source of human infection is often unidentified, although sheep and goats are more frequently involved in the disease cycle than other animal species. As *C. burnetii* is very stable in environment, resisting to elevated temperature, desiccation, osmotic shock, ultra violet light and disinfectants, direct contact with the aborted female is not required. This environmental resistance allows *C. burnetii* to be transported by wind far away from its original source leading to the appearance of Q fever cases in urban areas, where an important percentage of patients fails to report direct contact with animals (10). More wild and domestic birds, which are able to transmitted Q fever via their feces or their ectoparasites, can also be responsible of human cases in urban areas or apparently without animal contact. Oral transmission, by ingestion of contaminated raw milk or dairy products in particular goat dairy products could lead to seroconversion and in few cases to Q fever. Ticks are also considered to be a major reservoir in several countries.

Several actions could be proposed to prevent and reduce the animal and environmental contamination:

1) antibiotic treatment to reduce the number of abortions and the quantity of *C. burnetii* shed at parturition,

2) the destruction of placentas and fetuses in order to prevent there ingestion by domestic or wild carnivores which could disseminate the disease and

3) the treatment of the manures which could also spread the disease faraway, and be spread in fields when the wind blows,.

However, the only way to really prevent the disease in ruminants is to vaccinate uninfected flocks close to infected one, with an efficient vaccine preventing abortion and shedding of the bacteria. Several vaccines have been developed for this purpose. However *C. burnetii* presents phase variation, which is similar to smooth-rough variation in the lipopolysaccharide (LPS) of enterobacteria (2). Phase I that corresponds to smooth LPS is infectious for animals and humans contrary to phase II that is obtained after several passages in chicken embryos or cells culture.

Phase I vaccines are difficult and hazardous to obtain but are described as the only efficient vaccines (9). So in this study the efficacy of 2 commercial vaccines compounded of inactivated *C. burnetii* reference strain Nine Mile, one phase I vaccine (Coxevac, CEVA Santé Animale France) and one phase II vaccine (Chlamyvax-FQ, Merial France) were assessed in goats by comparing the 2 vaccinated groups with a control one for the kidding performances and the shedding of *C. burnetii* in placenta, vaginal mucus, faeces and milk.

Material and Methods

Two months before mating, according to the manufacturers' instructions, 17 goats were subcutaneously vaccinated with the phase I vaccine (group Ph I) and 16 goats with the phase II vaccine (group Ph II).

At 84 days of gestation, the goats from these 2 groups as 14 unvaccinated control goats (group NV) were subcutaneously challenged with 10^4 *Coxiella burnetii* strain CbC1 which was isolated from an aborted goat. The animals were kept in separate pens in a level 3 biosecurity building until about 6 weeks after delivery. The animals were observed daily for clinical signs. At the end of the study, the goats and their kids were necropsied for further of *C. burnetii* researches in different organs (spleen, liver, lungs, and in addition for goats, uterus and mammary lymph nodes.

For detection of specific antibodies directed to *C. burnetii* by ELISA, (CHEKIT-Q-Fever enzyme immuno-assay kit; Bommeli diagnostics, Switzerland), blood samples were collected at the time of vaccination and then twice a month during all the experiment.

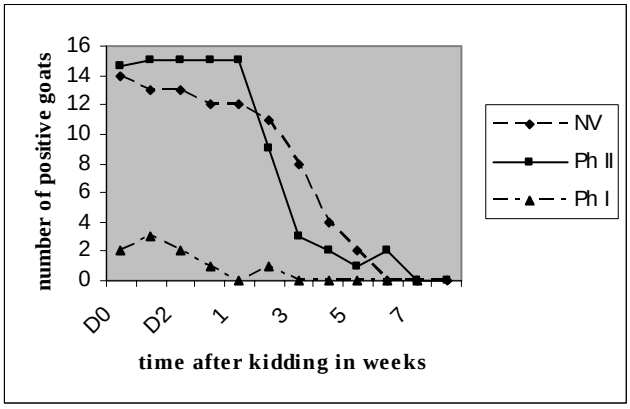
The bacterial shedding was checked by Trans-PCR (3), on placental cotyledons, vaginal mucus, fecal samples and milk. For this purpose, fecal samples were collected as previously described (1) 17 days after *C. burnetii* inoculation and then twice a month until the end of the experiment. Placental cotyledons were collected at parturition. Vaginal swabs were sampled at parturition, on the 3 subsequent days and then every week. Milk samples were taken at the parturition day and daily for 3 days after, and then once a week.

Results

The phase I vaccine prevented abortions as only 1/17 goat aborted in group Ph I whereas 13/15 and 9/12 aborted in groups Ph II and NV respectively. The average length of gestation (days ± SE) is normal for the group Ph I

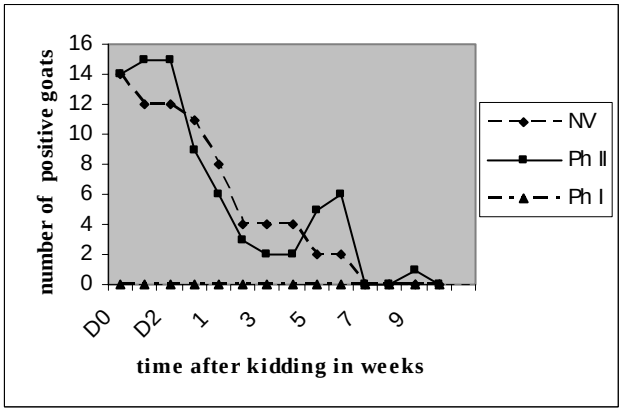
(153±3) but was too short (134±15 and 141±8 respectively) for the groups Ph II and NV. The kidding performances of the group Ph I, 22/26 (85%) live kids, were similar to the one observed in does of original flock when only 7/23 (30%) kids survived in group NV and 9/18 (33%) in group Ph II.

Fig1 Shedding of C burnetti in vaginal mucus



C burnetii was detected in vaginal mucus (Fig 1), faeces or milk samples (Fig 2) of all the goats of group Ph II and NV while none of the milk samples was positive in group Ph I, only 7/17 goats had a transient bacterial shedding in vaginal mucus (1.5 days in average in comparison to 16 days and 22 for groups Ph II and NV respectively) and 12/17 in faeces (10 days in average in comparison to 28 days and 27 for groups Ph II and NV respectively).

Fig2 Shedding of C burnetti in milk



Antibodies after challenge increased following almost the same pattern in the phase II vaccinated and the unvaccinated animals whereas, their increasing was quickly stabilized in the phase I vaccinated goats.

Discussion

The efficacy of vaccines against Q fever has never been tested in experimentally infected goats. The used dose of *C burnetii* CbC1 strain has been established in a previous experimental infection (1). It induced the abortion of about 80 % of the non immune pregnant goats, which is sometimes but extremely hardly ever observed in field conditions. Indeed, often in ruminants' herds, few females abort while the others are asymptomatic but shed the bacteria during several months (4). However in some caprine flocks more than 30% and even 90% of the pregnant female abort the reason of this difference of gravity of the disease is always unknown, nevertheless the phase I vaccine is able to protect the pregnant goats even against a very high challenge.

Conclusion

In our experimental conditions, which were very severe, only Coxevac vaccine was efficient and dramatically reduced abortion and excretion of bacteria in the milk, vaginal mucus and faeces, reducing environmental contamination and thus the risk of transmission to humans. In contrast, Chlamyvax FQ did not modify the course of the disease. So phase 1 vaccine must be used to control the disease. The large use of such a vaccine in cattle in Slovakia in the 70-ties and 80-ties has significantly reduced the occurrence of Q fever in this country (6, 11).

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DECREASED COLONIZATION WITH A HIGHLY VIRULENT *S*. TYPHIMURIUM DT104 AFTER VACCINATION WITH AN INVASIVE ATTENUATED *S*. TYPHIMURIUM-MUTANT.

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Introduction

Salmonellosis is one of the most important enteric infections in man as well as in livestock. Various *Salmonella* serotypes can cause self limiting gastroenteritis and systemic diseases. Besides *S*. Enteritidis, *S*. Typhimurium is the most frequent cause of food poisoning. Although poultry and pigs usually do not develop clinical salmonellosis, they become carriers and shedders (via meat and faeces) resulting in a substantial disease causing potential for humans (2).

Increasing interest can be observed in the use of vaccines to reduce the level of *Salmonella*-infections in livestock. Thereby, among the available approaches for triggering an efficient mucosal immunity, the use of live attenuated *Salmonella* strains is the best studied strategy (3). Several attenuation strategies have been employed to render salmonellae avirulent. Those include the generation of temperature-sensitive mutants, mutants deficient in the biosynthesis of aromatic amino acids (e.g. *aroA*, *aroC*, and *aroD* mutants) or purines (e.g. *purA* and *purE* mutants), mutants altered in the utilization or synthesis of carbohydrates (e.g. *galE* mutants), mutants altered in production of adenylate cyclase (*cya*) or of the cyclic AMP receptor protein (*crp*), or mutants with an affected global regulatory system (*phoP*). Additionally, different metabolic drift mutations of salmonellae were analyzed for their potency in immunization studies using experimental animals as well as livestock.

One of these metabolic drift mutants of *S*. Typhimurium, S. Tm. Nal2/Rif9/Rtt (*gyrA-cpxA-rpoB*), has been successfully used for immunoprophylaxis of latent *Salmonella* infections in chicken. Here, the prevention and reduction of *Salmonella* transmission between poultry and man is the main goal of vaccination strategies. The application of this vaccine leads to reduction of colonization by wild strains as well as to a reduced shedding period in infected laying hens.

The aim of this study was to characterize the efficacy and the humoral immune responses of a *S*. Typhimurium *gyrA-cpxA-rpoB*-mutant-based vaccination in pigs. Therefore, an oral infection model that results in a clinical infection and subsequently persistent shedding of *S*. Typhimurium in piglets was used (1). Here we demonstrate, that similar to the situation in chickens after homologous challenge infection, oral live vaccination of pigs revealed a significant reduction of the colonization of tissues and inner organs combined with a shortened shedding period as well as a prevention of clinical signs of salmonellosis (4).

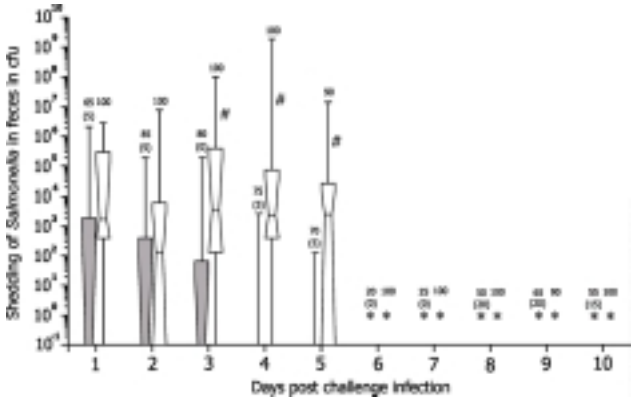
Material and Methods

Twenty 4-week-old male hybrid SPF piglets were immunized orally with single inoculation of total $5x10^8$ bacteria. A control group was treated with a placebo. The animals were challenged orally with a highly virulent S. Typhimurium DT104 strain three weeks post immunization. All pigs were controlled daily for clinical symptoms of salmonellosis. Additionally, fecal samples were investigated daily for salmonellae. At day ten post vaccination, all pigs were euthanized. For *Salmonella* sp. examination, tissue samples (n=13) were collected aseptically and quantitatively and qualitatively examined. In addition, the systemic antibody response was investigated by ELISA.

Results

The clinical investigations revealed that the vaccination protected against symptoms of salmonellosis. While all placebo animals revealed moderate to severe clinical symptoms, the majority of vaccinated pigs did not develop disease. For instance, only 25 % of the immunized pigs showed slightly increased rectal temperatures (mean 39.5 °C), but 40 % of the placebo group had elevated rectal temperatures (40.0 – 41.3° C, mean 39.9 °C). All animals (100 %) of the non-immunized group showed slight to moderate disturbed demeanour, while this was observed only in 10 % of the immunized pigs. A total of 60 % of the pigs in the placebo group, but only 5 % in the immunized group showed anorexia and diarrhoea.

Fig. 1: Quantitative isolation of Salmonella in faeces from immunized (grey notch boxes) and placebo-treated (white notch boxes) pigs. The percentages of animals tested bacteriologically positive for S. Typhimurium DT104 using pre-enrichment procedures (qualitatively detection) is depicted separately in numbers. The percentage of animals tested positive for immunization strain S. tm. Nal2/Rif9/Rtt is shown in parentheses. (#), indicates significant differences ($P<0.05$) between immunized pigs and those of the placebo-treated group.

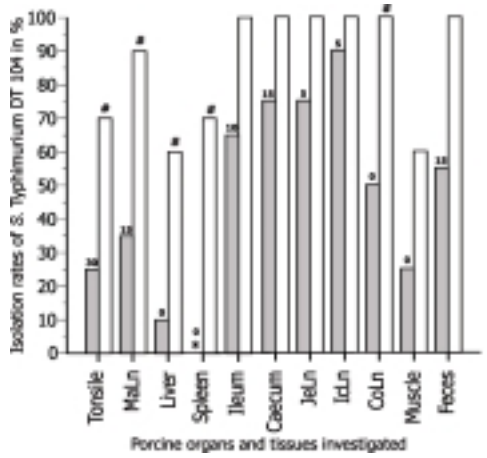


Furthermore, the bacteriological investigation showed also marked effects of vaccination.

Significant differences could be observed between the immunized group and the placebo group at shedding of the challenge strain (Figure 1). Beginning at day 3 post infection the group of immunized pigs shed *S*. Typhimurium DT104 to a significant lesser extent ($P<0.05$) than non-immunized pigs. Additionally, it could be demonstrated that vaccine strain invaded the gut and

gut-associated lymph nodes. Furthermore, vaccinated pigs showed a significantly decreased rate of colonization (42.5 % versus 87.5 % at the placebos) of the inner organs (Figure 2). Particularly in organs, which were used for human consumption (muscle, liver and spleen), the rate of colonizing was significantly increased. The challenge strain could not be detected in liver, spleen, and muscle.

Fig. 2: Rate of colonization of different tissues and inner organs by S. Typhimurium DT104 in immunized (grey boxes) and placebo-treated pigs (white boxes). MaLn = mandibular lymph node, JeLn = jejunal lymph node, IcLn = ileocolic lymph node, CoLn = colic lymph node. The percentage of animals tested positive for immunization strain S. Tm. Nal2/Rif9/Rtt is given separately in numbers. Asterisks (*) indicate, that S. Typhimurium DT104 could not be cultured from the samples. (#), indicates significant differences (P<0.05) between immunized pigs and those of the placebo-treated group.



Significant differences could be observed between the immunized group and the placebo group (Figure 3) at shedding of the challenge strain. Beginning at day 3 post infection the group of immunized pigs shed *S.* Typhimurium DT104 to a significant lesser extent (P<0.05) than non-immunized pigs. Additionally, it could be demonstrated that vaccine strain invaded the gut and gut-associated lymph nodes. Furthermore, vaccinated pigs showed a significantly decreased rate of colonization (42.5 % versus 87.5 % at the placebos) of the inner organs. Particularly in organs, which were used for human consumption (muscle, liver and spleen), the rate of colonizing was significantly increased. The challenge strain could not be detected in liver, spleen, and muscle.

Serological investigation shows that in comparison to non-immunized pigs, immunized animals revealed a markedly higher IgA antibody activity in the serum.

Additionally, a significant discrimination (P<0.05) between vaccinated and non vaccinated pigs was observed when IgM was measured, but, here the non-immunized animals had an elevated humoral antibody response.

Discussion

As in poultry, the aim of vaccination in pigs is the protection of the human consumer from a *Salmonella*-infection by infected or contaminated meat (2,3,6,8,10). Therefore, vaccination of pigs against *Salmonella* ssp. should decrease the number of viable salmonellae in both meat (muscle, liver, spleen) and faeces. This was achieved by vaccination as presented here. Vaccinated animals shed substantially smaller amounts of the challenge strain and for a shorter period of time. Therefore, it can safely be assumed that vaccination of pigs reduce the transmission of *S.* Typhimurium on the herd level.

In further studies, it has to be investigated if repeated ("booster") immunizations will further reduce the colonization rate in the inner organs.

Conclusion

The findings underline the potency of the vaccine tested to prevent clinical symptoms of salmonellosis and to significantly reduce the colonization of inner organs as well as the shedding of *Salmonella* Typhimurium, which both contributes to an increased consumer protection.

Acknowledgements

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ASSESSMENT BY SIMULATION OF THE EFFICIENCY OF STRATEGIES TO CONTROL BVDV SPREAD WITHIN A DAIRY HERD

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Introduction

The bovine viral-diarrhoea virus (BVDV) is widespread in many countries and induces production losses in infected herds[4]. Different strategies to control infection by the BVDV within a herd are available to farmers: either protection by vaccination, or strategies combining monitoring, screening and elimination of Persistently Infected (PI) animals with biosecurity actions (prevention of virus introduction into the herd and of transmission between animals in the herd). Strategies without vaccination (zoo-sanitary schemes) are generally preferred in areas where the risk of new introduction of the virus in a herd is lowered by collective programmes. The efficiency of control measures can be assessed ex-ante using epidemiological models. In the criteria of interest to evaluate the efficiency of a strategy, the ability to eliminate the virus in infected herds can be measured by the probability of and the time to clearance, and the extent of infection. Among the previously published models aiming at studying the BVDV spread[2,4,6,7,9], two[2,7] studied BVDV control by elimination of PI animals. Both were deterministic model and could not represent variability of expected results. One estimated that maximum age at which PI new-born calves should be detected and removed to obtain clearance was below 11 days of age[2]. This is not applicable in field conditions with existing tests. The second concluded that elimination of PI animals was economically unattractive[7]. Screening for PI animals was based on individual testing of all animals, and did not consider the availability in dairy herds of bulk-milk testing for antibodies or virus in cows.

The objective of the present study was to investigate, by simulation, the expected effect of applicable zoo-sanitary schemes in a dairy herd on duration and extent of BVDV infection, in a context of low risk of new infection.

Material and Methods

A stochastic simulation model was used[10]. It consisted of two processes: one modelling the herd dynamics (demography, structure and management) assuming the dairy herd as a multigroup population (semi-Markov process) and the other modelling the transitions between BVDV statuses (Markov process). An individual-based approach was used to take into account individual characteristics influencing the occurrence of events (movements between groups, transitions between BVDV statuses, vertical virus transmission). The transiently infective animals were assumed to be able to transmit the virus to susceptible animals only in the same group whereas the PI animals were assumed to transmit the virus to susceptible animals both within their group and in other groups.

In the modelled herd, actions to avoid virus transmission from herd's neighbourhood were assumed to be in place. PI animals were assumed to be detected before any movement between herds and not to be sold. In such a context, the most probable remaining origin of virus introduction is the purchase of an immune dam carrying a PI foetus which cannot be detected by available tests. The virus introduction was simulated as the purchase of an immune heifer carrying a PI foetus, 20 days before calving. No virus reintroduction over time was simulated. Four scenarios representing four strategies were studied: (1) no other action, (2) prevention of contacts between animals of different groups of age, (3) test-and-cull of PI animals, and (4) combination of (2) and (3). The prevention of contacts between animals was modelled by setting transmission rates between different groups to zero. The test-and-cull consisted of monitoring the herd, and, in case of a positive result, screening for detecting and eliminating PI animals. Every 6 months, the antibody level in the bulk-milk was measured by an ELISA test. If the percentage inhibition was higher than 60% (corresponding to a prevalence of immune cows higher than 30%[1]), a virus spread was assumed. Then, screening for PI animals was based on consecutive combined tests for antibody and virus detection, defined per category of animals, in order to mimic existing zoo-sanitary schemes. Specificity of antibody ELISA, antigen ELISA and PCR were set to 0.978, 0.99 and 0.99, and sensibilities to 0.969, 0.97 and 1, respectively[1, 3, 8].

The initial herd consisted of 38 cows, 13 bred heifers, 18 heifers before breeding and 3 calves, all of which were susceptible. The virus spread was simulated over 10 years. For each strategy, 600 replications were run.

Effects of strategies on virus elimination considered three categories of criteria:

- *The interval between virus introduction in the herd and detection of infection from bulk-milk antibodies*
- *The occurrence of and time to virus clearance*

Clearance was defined as absence in the herd of any shedding animal or dam carrying a PI foetus. The probabilities of virus persistence within the herd (as opposed to clearance) were represented by Kaplan-Meier curves. The distributions of time to clearance were compared between scenarios, stratifying by time of bulk-milk antibody detection (or level allowing detection in case of strategies with no monitoring). Herds already cleared at time of bulk-milk antibody detection were excluded from this latter analysis.

- *The extent of the infection in the herd*

The total number of contaminated animals in the herd during 10 years was calculated for each replication.

Results

Monitoring bulk-milk antibodies every 6 months allowed the detection of BVDV infection within one year after virus introduction in most cases when there were contacts between groups of animals of different ages, but could also result in very late detection (Table 1). In the latter case, the herd was often already cleared from the virus when seroconversion was evidenced.

Table 1. Number of replications per interval from virus introduction to detection of bulk-milk antibodies and % of replications with herd not yet cleared

	Virus introduction to detection - in days					
	190	370	550	730	910	1090
Test-and-cull only						
# detected	120	135	25	4	4	4
% not cleared	98.3	96.3	84.0	25.0	0	0
Prevention of contacts and test-and-cull						
# detected	0	0	0	2	178	8
% not cleared	-	-	-	0	24.7	0

Clearance occurred earlier with test-and-cull than with do-nothing, but persistence was further reduced by prevention of contacts in the herd (Fig. 1). Extent of infection was only slightly reduced by test-and-cull, whereas prevention of contacts resulted in a drop in the number of contaminated animals (Fig. 2). Test-and-cull mainly reduced time to clearance (Fig. 3 and 4), but, in case of prevention of contacts, for only 7% replications.

Figure 1. Probability of virus persistence for four strategies (600 replications by strategy)

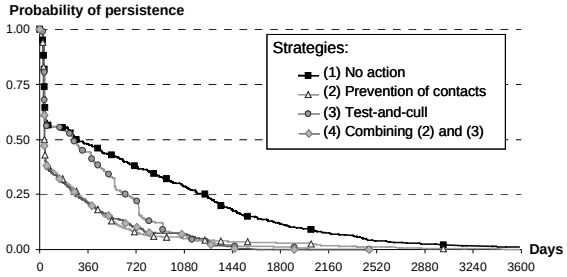
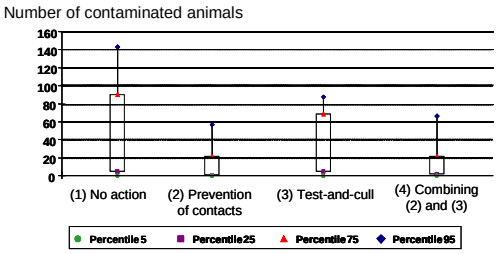


Figure 2. Extent of the infection for four strategies: number of contaminated animals during the simulated 10-year period (600 replications by strategy)



Discussion

After a purchase of a non-PI dam carrying a PI foetus, a zoo-sanitary scheme based on test-and-cull generally reduces persistence of BVDV in a herd, but this effect may be largely delayed due to late detection of infection. If late detected, the herd is likely to be free of PI animals at cows' seroconversion. In Bretagne, PI animals were found in only 28% of seroconverting herds (Joly, unpublished data), suggesting that virus introduction may often have occurred more than one year before. Prevention of contacts between groups appears to be very efficient in limiting both duration and extent of infection, as compared to test-and-cull. Nevertheless, in many commercial herds, total prevention of contacts (assumed here) may not be possible. BVDV infection in herds where virus transmission between groups is only partly prevented could be further investigated.

Figure 3. Time to clearance by time of detection of bulk-milk antibodies for test-and-cull (right) vs. no action (left)

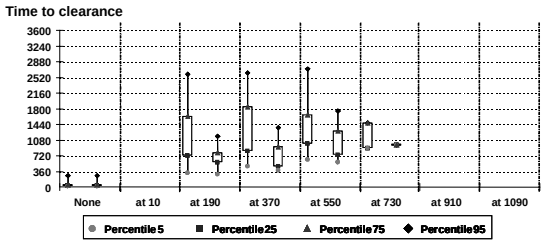
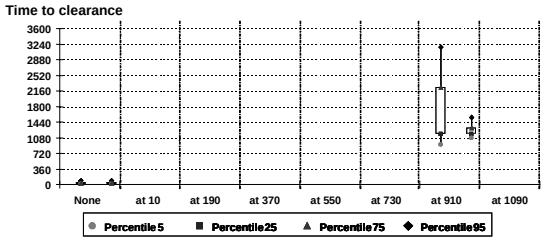


Figure 4. Time to clearance by time of detection of bulk-milk antibodies for prevention of contacts with (right) vs. without (left) test-and-cull



Costs of the test-and-cull strategy can be calculated from the number of laboratory analyses, and losses resulting from BVDV can be estimated from number and category of infected animals depending on the strategy.

Conclusion

Model simulation allows the investigation of how BVDV control strategies interact with herd management and provides relevant data to assess their technical and economic efficiencies in various herd situations. In a context of low risk of virus introduction, zoo-sanitary schemes appear to reduce overall duration of infection, but still have to be evaluated economically.

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RECENT TREND OF BSE IN FRANCE

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Analysing the pattern of the Bovine Spongiform Encephalopathy (BSE) epidemic in France is crucial both for risk analysis and the assessment of control measure efficacy.

Two modelling studies of BSE in France (Donnelly 2002, Supervie & Costagliola 2004) showed, if not the same expected number of total BSE cases, at least the same overall pattern of the annual incidence of infection. Both models showed two distinct peaks of the epidemic. The first one concerned the cohorts of animals born in 89 and the second one concerned the cohorts of animals born in 94 and 95. According to the same models, large numbers of bovines belonging to birth cohorts prior to 1989 could have been infected, whose related BSE cases remained completely unnoticed.

From mid 2000 until July 2001, the surveillance system underwent several changes. In addition to the MRS, a pilot active surveillance program was implemented on cattle at risk (dead on farm, euthanasied or emergency slaughtered) from mid 2000. Systematic screening was extended to all cattle over thirty months of age entering the food chain in January 2001, to all cattle at risk (as defined above) in June 2001, and to all cattle older than 24 months entering the food chain in July 2001. The active surveillance system showed that many BSE cases were not detected or reported by the clinical surveillance system. Before the surveillance of all adult cattle entering the food chain was implemented, the trend of the epidemic was difficult to analyse without the use of sophisticated models because of underreporting

It has been done since then by comparing successive birth cohorts using logistic regression models adjusted for the type of animals (classified in dairy, beef cattle, mixed or unknown) and for the region. Such analyses are made on two separate categories of the cattle population: at risk animals from western France (Bretagne, Basse Normandie and Pays de la Loire regions) tested during the 2nd semester of 2000, 2001 and 2002 (Morignat and others 2004) and cattle in metropolitan France entering the food chain and tested in 2001 and 2002 (La Bonnardière and others 2003). The pattern of the risk of contamination is roughly the same for both populations (Fig 1). It is consistent with the results of the modelling studies: an increasing risk for animals born prior to 1994 and 1995, and a decrease for the following ones. The beginning of this decrease matches with the implementation of the control measures of July 1996, i.e. the ban of specified risk material and cadavers in animal foodstuffs. Since most animals are assumed to be contaminated between the ages of six and eighteen months, this decrease could be imputed, at least partly, to this control measure.

In spite of this dramatic decrease, 76 BSE cases born after this measure have been detected so far whose origin of contamination remains unknown. An epidemiological analysis of these cases is running in order to raise hypotheses of contamination: vertical transmission, consumption of contaminated MBM, due to late or incomplete application of the measures of 1996, consumption of potential other risk materials still allowed as certain animal fats or bicalcic phosphates made from bones and exposure to imported products.

Besides, the mandatory screening of all adult cattle permitted to analyse the geographical risk of BSE (Abrial and others 2004). This was done from the geographical location of the farms in which BSE positive animals detected from July 2001 to July 2003 were raised during their 1st year of life. Born After the Ban (BAB) cases (i.e born between January 1991 and June 1996) and superBAB cases (i.e born between June 1996 and December 2000) were differentiated. Relative risk of BSE in each geographical area was computed in comparison to the national average during the same period of time taking into account the different cattle systems (dairy, beef) and the structural neighbourhood of the geographical areas. France was divided into 1,264 hexagons of 23 square km. The analysis was based on a probabilistic model describing the number of observed cases in each hexagon and the model was fitted thanks to a Monte Carlo simulation. The results (Fig 2) showed a significant spatial heterogeneity for the risk of BSE contamination (at the 1% level), for BAB cases (36 % of the hexagons) and for SuperBAB (19 % of the hexagons). Comparison of the distribution of the risk shows that areas with a higher risk for BAB and superBAB cases were roughly the same, which argues rather for common sources of contamination than for new sources of contamination. However, the origin of SuperBAB cases has to be formally evidenced. Moreover, the future trend of the BSE epidemic in France will have to be monitored precisely in order to evaluate the efficiency of the meat and bone meal ban implemented in November 2000.

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LA BONNARDIÈRE, C., CALAVAS, D., ABRIAL, D., MORIGNAT, E. & DUCROT, C. (2003) Estimating the trend of the French BSE epidemic over six birth cohorts through the analysis of the abattoir screening in 2001 and 2002. *Veterinary Research* **35,** 299-308

ABRIAL, D., CALAVAS, D., JARRIGE, N., DUCROT C. (2004) Spatial analysis of BSE in France. *Preventive Veterinary Medecine*

Figure 1: Summary graph of the risk of BSE in France in the successive birth cohorts

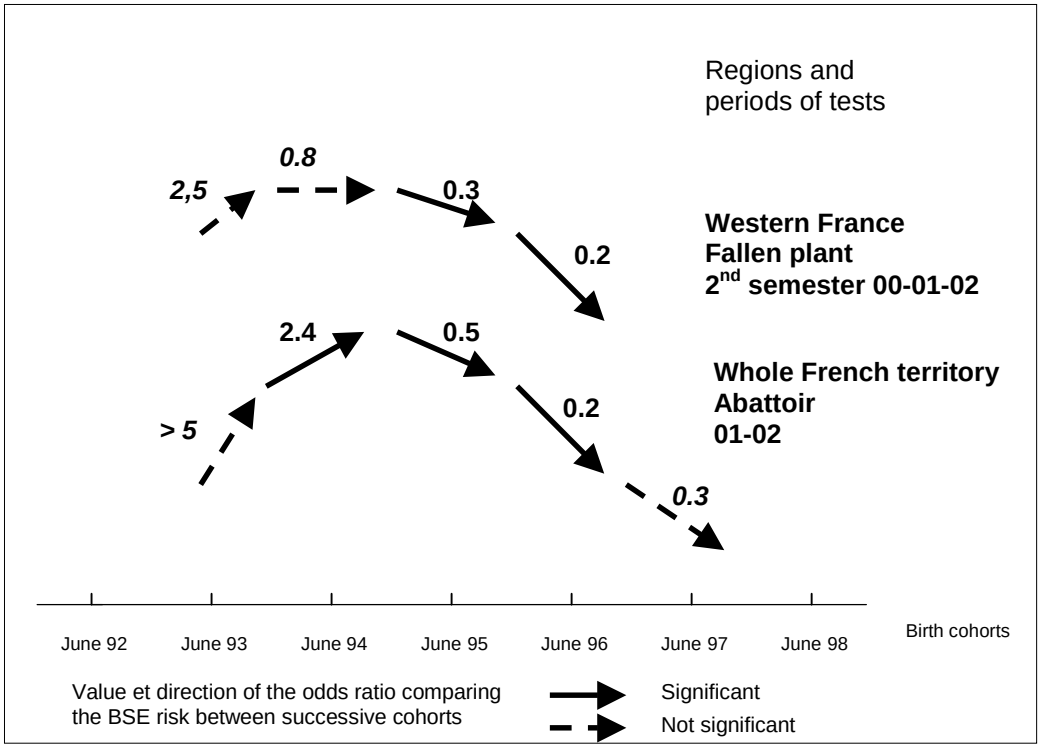
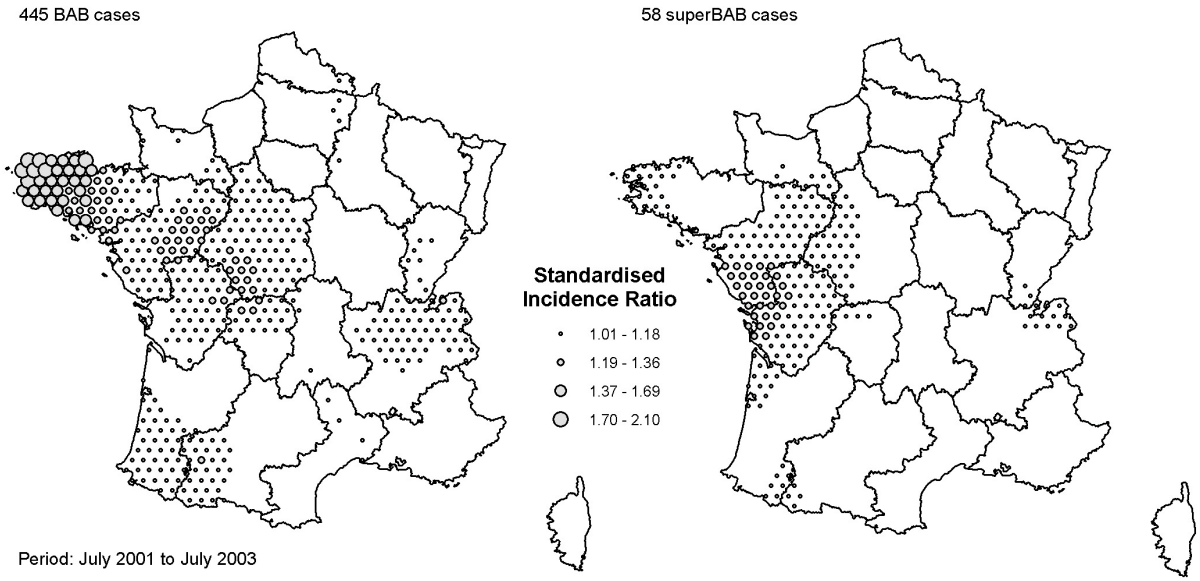


Figure 2: Areas with a relative risk of contamination of BSE (RRC) significantly greater than the national average (p<0.01), for BAB cases (36 % of the hexagons) and superBAB cases(19% of the hexagons



Tools and strategies for fighting diseases

Posters

EFFECTS OF RESPIRATORY DISORDERS ON GROWTH RATE OF NON-WEANED CALVES IN CHAROLAIS COW-CALF FARMS OF PAYS DE LA LOIRE (FRANCE) AND ECONOMIC IMPACT RELATED TO THESE DISORDERS.

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Introduction

In the area of Pays de la Loire, the typical intensive cow-calf farming system relies on the association of a cow-calf rearing unit and a young bull fattening unit. Charolais breed is coming first. Occurrence of respiratory disorders in non-weaned calves in this production system appeared to be quite high [1]. But at the moment, the impact of these disorders is not documented in this production system. Therefore, this study aimed at assessing, the effects of the occurrence of respiratory disorders on growth rates, and the economic results.

Material and Methods

Data originated from a survey carried out in 162 farms of Pays de la Loire. The sample consisted of Charolais cow-calf farms with >30 cows calving per year. The study period was the housing period (from the 15 of September 1999 to the 15 of March 2000). A respiratory disorder was defined as the association of at least one respiratory sign and, in the same calf or another calf of the same pen, at least one general sign the same day or the day before. Farmers were provided with this specific definition. They had to record day to day all treatments for the calves. Data about mortality and growth retardation were also recorded. Finally, after validation, 7496 calves in 156 farms were taken into account for analysis.

Incidence rates of cases (number of cases over the number of calf-days at-risk) were estimated on two different scales: incidence of first cases for the whole study period and herd-level incidence rate. A calf was considered at risk of experiencing a case if (i) alive in the barn, (ii) at least 3 days-old, (iii) non-weaned (or aged <150 days), and (iv) not treated.

Weight-gain data before weaning were available for a sample of 3,516 of these calves located in 111 buildings.

From this sample, a multivariable, GLM, regression model was performed to assess the effects of the occurrence of respiratory disorders on growth rates (proc. GLM, SAS Institute Inc., 1996). Three categories of calves were considered: treated calves, not treated calves in buildings with at least one case and not treated calves in buildings without any cases.

Five groups of farms were identified, based on the Herd-level incidence rate and the incidence of severe repercussions (death and severe growth retardation). For each group, a partial-budgeting simulation was applied to calculate variations in the inputs and outputs of a reference farm without any case. The annual net profit for this farm in the Occurrence zero group was near to 20.000 € in 1999 Within each identified group of farms, average treatment incidence, lethality rate, severe and moderate growth retardation rates were calculated. Numbers of dead animals and of animals with severe or moderate growth retardation were derived considering the 57 viable calves from the 60 calving cows of the reference farm.

Results

Incidence of first cases for respiratory disorder was 1.53 cases for 1,000 calf-days at risk. Compared with non treated calves in buildings without any cases, treated calves had a loss of body weight at 150 day of life of nearly 16, 10 and 8 kg for disorders occurring respectively before 45 days, between 45 and 90 days and between 90 and 150 days of life. Moreover, not treated calves in buildings with cases had a loss of body weight of 5 kg.

Herd-level incidence rate averaged 2.52 treatments for 1000 calf-days at risk. Lethality rate, severe growth retardation rate and moderate growth retardation rate were 6.0%, 7.2 and 2.7 % of the treated calves, respectively. Five groups of farms were identified, based on incidence and severity of repercussions: (1) Zero-Occurrence; (2) Low-Occurrence; (3) Moderate-Occurrence; (4) High-Occurrence; and (5) Severe-Consequences. These groups gathered 21, 22, 17, 28, and 12% of the farms, respectively.

Discussion and conclusion

Our approach allowed to express a global economic impact cumulating extra-costs and estimated losses associated with the occurrence of respiratory disorders in non-weaned calves. Total impact was found quite low, except for 12% of the farms where one fifth of the annual net profit was lost, mainly due to the consequences of mortality. The partial budget approach used only included variation in variable costs from a small number of origins. Not including variation in fixed costs seemed here relevant: *(i)* considering that extra-labour has no opportunity cost in the familial farming system under study; and *(ii)* according to the fact that farm buildings characteristics were not found being frequently risk factors in this survey [2].

Growth retardation on non treated calves in buildings with cases suggest that sub-clinical respiratory disorders may exist to some extend.

Acknowledgements

The authors gratefully acknowledge all the farmers, technicians, students and veterinarians who participated in the data collection and the Région des Pays de la Loire for its financial support to the programme.

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A RAPID SINGLE STEP MULTIPLEX PCR ASSAY FOR THE DETECTION OF *CHLAMYDOPHILA ABORTUS, CHLAMYDOPHILA PECORUM AND COXIELLA BURNETII* FROM RUMINANTS CLINICAL SAMPLES

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Introduction

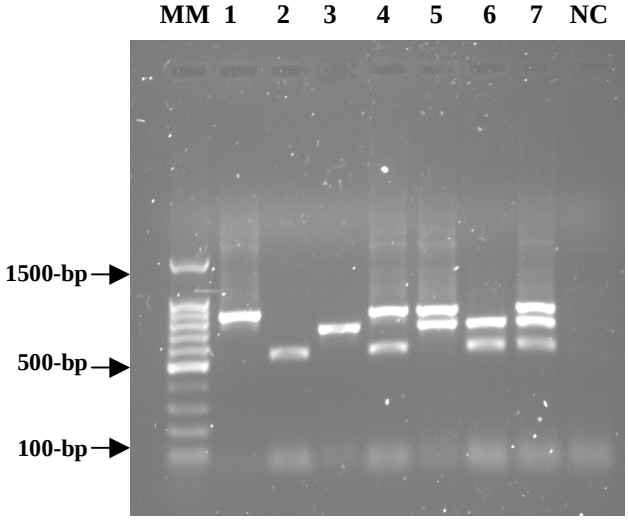
Chlamydiosis and Q fever are important causes of ruminants' abortion around the world (Rodolakis et al. 2004). They are caused respectively by intracellular and gram negative bacterium, *Chlamydophila* and *Coxiella*. Two species of the genus *Chlamydophila* cause diseases in ruminants, *C. abortus* and *C. pecorum* (Rodolakis et al. 1998). The available detection methods such bacteriological examination, culture or serology either lack both sensitivity and specificity or give only retrospective diagnosis. In order to improve *Chlamydophila* and *Coxiella* detection, specific PCR primers were designed and a sensitive multiplex PCR (m-PCR) was developed for rapid simultaneous detection and differentiation of *C. abortus*, *C. pecorum* and *C. burnetii.*

Material and methods

Three primer sets were designed and used to amplify the respective fragment of *C. abortus*, *C. pecorum* and *C. burnetii* reference strains (AB7, iB1 and Nine Mile respectively). As this test will be commercialised by a manufacturer, the sequence of the primers and the experimental protocol could not be given. This m-PCR assay was performed on 257 clinical samples taken from infected ruminant's flocks that have showed problems of abortion diseases.

Results

Fig.1 Multiplex PCR amplification of C. abortus, C. pecorum and C. burnetii reference strains individually and all possible combination. Lane MM: 100-bp ladder, 1: C. abortus AB7, 2: C. pecorum iB1, 3: C. burnetii Nine Mile, 4-6: duplex reactions, 7: triplex reaction, NC: negative control.



PCR reaction performed with the primers, designed in this study, resulted in the amplification of PCR product allowing a specific identification of *C. abortus* (821-bp), *C. pecorum* (600-bp) and *C. burnetii* (687-bp) micro-organisms (Fig.1). Multiplex as well as duplex or single PCR performed on reference strain purified DNA detect as little as 50 bacteria per PCR reaction. Amplification experiments performed with several *C. abortus*, *C. pecorum* and *C. burnetii* strains gave specific PCR product. However, no amplification was noted using DNA from other pathogens suspected to be present into tested clinical samples.

This m-PCR assay was performed on 257 clinical samples and showed that 67 samples were infected by either one of the three pathogens. Two vaginal swabs were m-PCR positive of both *C. abortus* and *C. burnetii* and none of the tested samples was shown to be infected simultaneously with the three pathogens. However *C. pecorum* strain was detected in one vaginal swab taken from aborted ewe and in epididymus of infected ram.

Discussion

Several tests that detect *Chlamydophila* and *Coxiella* antibodies made chlamydiosis and Q fever individual diagnosis tests widely available. However, these tests are not specific and poorly sensitive. Previous works have reported the use of PCR to detect individually *C. abortus* (Laroucau et al. 2001) and *C. burnetii* (Berri et al. 2000) in vaginal swab samples taken after lambing or abortion of infected ruminants. Here, we reported the successful development of multiplex PCR assay for the simultaneous detection and differentiation of *C. abortus*, *C. pecorum* and *C. burnetii*. Amplification experiments performed with both purified genomic DNA of bacteria or with spiked clinical samples showed that this assay was sensitive and specific. The performance of the m-PCR in field study showed that these two infections are widespread within the tested flocks. Two clinical samples were contaminated with both *C. abortus* and *C. burnetii* and the ability of this assay to detect dual infections was therefore known. Furthermore, *C. pecorum* was detected in vaginal swab taken from a female ewe that has aborted showing that this strain could be associated with small ruminant's abortion.

Conclusion

To conclude, we have successfully developed a multiplex PCR that can detect and differentiate three causative agents of ruminant's disease with a good sensitivity and specificity. The diagnosis of chlamydiosis and Q fever may be greatly simplified and performed at low cost. In addition, the result can be obtained rapidly which is helpful clinically if antibiotherapy has to be undertaken.

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THE ERADICATION PLAN OF PRRS IN A FRENCH REGION , "LES PAYS DE LA LOIRE"

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Introduction

One region of France, called "Pays de la Loire", was confronted with PRRS in November 1992.
Having got experience in disease eradication, with the previous plan against Aujeszky's disease, the pig farmers decided together to try to tackle PRRS, and a collective action was right away set up.
This paper describes the methods applied at a regional level, and analyses the results obtained. The pending questions are also mentioned.

Material, history

In the Pays de la Loire region, there are about 2 200 herds and 105 000 sows, on a 20 000 km^2 territory divided into 5 administrative sections(the French departments). The average herd size was about 100 to 150 sows in farrow-to-finish farms.
In November 1992, the first samples that tested positive regarding PRRS were found when controlling imported gilts from Great Britain. The spread of PRRS infection could rapidly be stopped by serological testing in the recipient herds, and removing positive animals.
At that time, National and European Community rules of control measures as well as epidemiological inquiries were lifted.
Not a single PRRS outbreak had been yet reported in Pays de la Loire. A random serological survey was undertaken and carried out, in one third of the total number of herds.
Surprisingly, several appeared to be positive, and the only relationship underlined was the semen from one infected artificial insemination centre. This AI centre was banned.
An exhaustive survey was then settled. New infected herds were found, with clinical signs, and epidemiological links were put in light.
It appeared necessary to define a regional agreement signed by all the members of the pig industry, specifying technical and financial rules. That was done in November 1993, under the aegis of the Sanitary Defence Confederation (GDS). The aim was to control the spread of the infection.
A financial reserve was provided by a bank loan of 430 000 €, in order to give farmers a compensation when destocking their herds.

Methods, the plan

The technical methods used have already been described (1).
The measures were purely sanitary, based on epidemiological considerations, on account of the lack of available vaccine at that time.
The replacement gilts still regularly introduced came from PRRS free herds;
Different tools have been used, among which :
- serological survey in all the farms with breeding sows
- additional tests in herds located 2 Km radius from one infected herd
- The circulation of the virus was assessed in infected herds, with enlarged samples
- A strategy for PRRS virus elimination was adopted according to maintenance or stop of virus circulation (2)
- Infected herds were strictly managed separately from the free ones. The groups of farmers did perfectly comply to the rules .

In addition to these technical aspects, the way the plan was run clearly defined the role of each partner. We decided to work in transparency when analysing technical results. Needless to say that we had constantly to convince some rather reluctant farmers of the validity of the measures.
The plan was run on voluntary basis.

The GDS looked after the general project management.

The veterinary research institute of Ploufragan was the scientific expert. National and regional funding could be found, but the farmer's contribution represented 80% of the global budget.

Results

At the moment (mid 2004) 14 herds still remain infected, among 2200 herds.
The infection rate has never been higher than 2.2 per cent.
From the beginning of the action, 196 herds were detected infected, and 182 cleaned up.
The number of samples per year was about 18 000.
Subsidies for depopulating the infected herds or animals reached 1.3 million €.
The cost of the whole plan, per year , per sow, is about 5 €.

Discussion

On January 1992 the first, French sanitary authorities handed over" the baton" to pig producers about any action regarding PRRS. The producers decided to move forward by themselves.
The Pays de la Loire region includes few independent pig farmers but more than 95 % of the pig farmers are members of groups of producers (co-operatives), for genetic schemes, feed supply and/or animal trade, and these organizations greatly contributed to the plan on the day-to-day basis.
One originality in France is the existence of those farmer organizations specifically dedicated to animal health . They are called "GDS" (for Sanitary Defence Groups). They are gathering by themselves voluntary farmers , and acting collectively against several diseases. For pig producers, the first aim was the eradication of Aujeszky's disease in 1985. This was achieved a few years later. It was a first result at the French scale, and this success, shared by all the regional participants, gave hope and confidence. So, when PRRS stroke, because of this previous experience, a collective plan was rapidly set up.
One difficulty for such a regional and voluntary plan, is to definitely convince the farmers to accept and adopt on their own the required collective measures, instead of having compulsory rules. Eradication decisions and related procedures might have been hard to apply.

Until now, no American PRRS virus strain have been suspected.
So the situation is easier to manage. There is no need for a broad vaccination. Vaccination is mainly used in infected herds as a help to stop the virus circulation.

Conclusion

In" Pays de la Loire", which is not really a densely populated pig area , PRRS eradication plan was successful.
The regional plan started just at the moment when PRRS arrived in the country. We believe that appropriate measures and strict biosecurity precautions since the beginning did avoid a wide spreading,
However it is currently still necessary to keep a strong motivation; we know that nothing is definitely granted in the field of infectious diseases. PRRS virus remains a permanent threat.
A national agreement to officially define and recognize the "PRRS free status" for the herds would be helpful.

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EPIDEMIOLOGY OF SCRAPIE I IN ITALY: AN UPDATE

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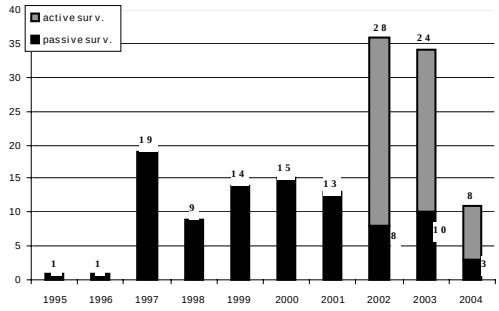
Introduction. Scrapie in sheep and goats is the longest known Transmissible Spongiform Encephalopathy (TSE) and to date it is considered non pathogenic for humans at least under natural conditions, but, under experimental conditions, sheep are easily orally infected by the agent of Bovine Spongiform Encephalopathy (BSE). Therefore the EU priorities small ruminants TSE research, surveillance and control because of theoretical possibility that BSE has entered in the European sheep and goats population, and may pose a threat to public health. The need of effective surveillance of TSE in small ruminants led the EU Commission to launch an intensive EU-wide programme of monitoring; from the 1° January 2002 besides the passive surveillance (PS) has been implemented a scheme of active surveillance (AS) which targets a large sample of healthy slaughtered animals and fallen stock, over 18 months of age.

In Italy, where the small ruminants population is over 9 millions, the disease was reported firstly in 1976. Up to 1990, 25 outbreaks were identified in sheep. After the mandatory reporting system was enforced in 1991 scrapie apparently disappeared till 1995. Aim of this study is to provide data on the descriptive epidemiology of the disease in Italy since 1995.

Material and Methods. The National Reference Laboratory (CEA) is in charge for collection, review, clean-up, analysis and interpretation of national data on scrapie. Crude rates of prevalence and incidence and their rate ratios were obtained from collected data; herd incidence rates (outbreaks per 10,000 herds) by region were computed to show the geographical distribution of the disease.The current study covers the years since 1995-till May 2004; the genotype of the prionic protein was obtained from affected sheep.

Results. 153 outbreaks have been identified (Fig 1). During 2003, in Italy 45.133 animals have been tested in the frame of AS, with 29 positives (16 healthy slaughtered and 13 fallen stock).

Fig 1. The epidemic curve by year of diagnosis



The crude prevalence rate obtained from AS during 2003 is 6.3 cases per 10,000 tests.

Fig 2: Active surveillance in Italy 2003 vs 2002

TSE testing activity:

Fallen stock	6.204 / 6.000* (103,4%)
Healthy animals	38.495/ 60.000* (64,2%)

prevalence (+ives/10,000 test & 95%CI)

	Italy 2003		vs.	Italy 2002	
crude	6.3	(4.2 – 9.2)	vs.	14,1	(10.0 –19.4)
H.anim.	4.2	(2.5 – 6.9)	vs.	9.7	(6.2 – 14,6)
F.stock	19,3	(10.5 –34.8)	Vs.	46.2	(25,9 – 76.3)

*sample size for Italy required by EU

With respect of risk categories, the probability of detecting a case is much higher among fallen stock than among healthy animals. In the first five months of 2004, 11 new outbreaks were identified (8 in the frame of AS and 3 of PS), in the previous years AS detected the most of outbreaks.The number of Italian Regions involved in the epidemic has increased year by year; animals affected are mostly sheep. More than 64,000 animals were subjected to culling or alternatives safeguards procedures on the basis of the Italian legislation in force. The PrPgene genotypes of 194 affected sheep belonging to different breeds, from Italian outbreaks, was distributed as below: 166 ARQ/ARQ, 26 ARQ/AHQ and 2 ARQ/VRQ.

Conclusion

Despite the low number of outbreaks identified, the available data suggest that scrapie is widely spread in Italy; the introduction of active surveillance led to a significant improvement of the scrapie surveillance system based previously just on mandatory reporting. Worthy of note is the low frequency of the allele VRQ in Italian scrapie affected sheep and high frequency of allele ARQ.

Acknowledgements

We thank for their contribution the colleagues of the network of Istituti Zooprofilattici Sperimentali involved in TSE surveillance.

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PRODUCTION OF LONG TERM, LOW-COST SPECIFIC PATHOGEN FREE PIGS

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Introduction

In pig production, most of the people are familiar with the techniques in use when eradicating pathogens. The first technique described was based on surgical methods (Young et *al* 1955). In our facilities at Ploufragan, Specific Pathogen Free piglets have been obtained for several years by hysterectomy. The newborn piglets are raised in sterile isolators. This technique aiming at the production of CD/CD piglets is indeed very efficient but a rather expensive mean to obtain clean pigs (Cariolet et Tillon 1978). In addition, raising piglets in sterile isolators is delicate because of the permanent risk of bacterial contamination (Cariolet et *al* 1987).

In 1979, we decided to build up a specific facility able to maintain a high health status for an entire pig herd. The objective was to obtain SPF piglets through the natural ways in a protected, totally confined piggery. Such SPF piglets could be obtained on a regular base, at a much lower cost. This paper gives some details of the conditions which permitted the maintenance of the high health status in the piggery.

The building design

The piggery is a single piece building with concrete walls. Air inlet is equipped with a filtration system. The air level filtration is referenced as EU 13. The floor is partially (sows) or totally slatted (other pigs). The slurry is removed daily. There is no use of straw.

Biosecurity measures

Only a very limited number of technicians are allowed to enter the building. Normally, these technicians do not visit conventional piggeries. Strict sanitary measures are taken before people may enter this building (shower and total changing of clothes). A specific entrance is devoted to the feed, the latter being prepared in our own feed mill and delivered in bags (Pelleted feed). The entry of pig feed is highly controlled. The feed is conditioned in pellets after being heated to 72°C during 3 minutes. The surface of each bag is sterilized by paraformaldehyde sublimation in a dedicated coffer. All diets are free from antibiotics.

There is no introduction of semen from outside the unit. Replacement boars are periodically introduced. They are all hysterectomy-derived and have been raised in total confinement in our experimental rooms. These rooms are also of level 3 biosecurity with air filtration. The suckling period of 28 days is considered as the quarantine phase. The newborns due to enter the piggery can suck there SPF sows whose own piglets have been removed. After having passed through the required health checks, the piglets are transferred into the post-weaning room of the piggery.

Results concerning infections

Serology regularly demonstrates the absence of the following viral contaminations : Classical and African swine fever, Aujeszky's disease, Porcine Reproductive and Respiratory Syndrome (PRRS), Parvovirus, Porcine Respiratory Coronavirus, Influenza, Porcine Circovirus type 2. Our herd is free from pathogenic bacteria responsible for respiratory diseases, *Mycoplasma hyopneumoniae Pasteurella multocida, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae* and other bacteria like *Haemophilus parasuis* and *Streptococcus suis type 2*. The pigs are also free from digestive pathogens : *Lawsonia intracellularis, brachyspira hyodysenteriae, Salmonella enterica, Listeria monocytogenes and campylobacter*. All these bacteria are searched for on selective media.

Results concerning clinical disease and performance

Health evaluation is first based on daily clinical observation and recordings. In addition, serological controls are carried out twice a year on all the pigs. For some bacteria like *Salmonella* and *Campylobacter*, faeces are sampled once per 7 weeks. The small replacement boars produced by hysterectomy and raised by SPF sows in a quarantine room have undergone the totality of these health checks before their introduction into the piggery.

There is no vaccine used in this unit. The only health problem occasionally encountered is high temperature in sows after farrowing. When this happens, the concerned sows receive antibiotics for 3 consecutive days. Another point to be mentioned relates to some leg problems in the sows. We have to carefully sort the gilts in this respect.

Reproduction and growth performances are rather good. Twenty-six piglets are weaned per sow / year and they reach 100 kg live-weight at 135 days of age on average. Mortality rate from birth (born alive) to weaning is 12,8 % mainly due to euthanasia of light newborns (below 0,8 kg). Mortality from weaning to slaughter is below 1%.

Discussion

Although the general health status of the pigs issued from our piggery was the same as that of piglets produced by hysterectomy and raised first in isolators, the flora borne by the two groups of pigs was not exactly the same. When representatives of both groups were mixed at 6 weeks of age, we could observe some disorders. In particular an outbreak of exudative epidermitis occurred in the hysterectomy-derived. In this case *Staphylococcus hyïcus* was identified.

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A CASE STUDY OF NEONATAL DIARRHOEA IN A FARROW-TO-FINISH PIG FARM

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Introduction

Enteric diseases are the most common infectious cause of mortality and economically significant disease in suckling pigs worldwide (1). Neonatal diarrhoea which occurs within the first few days of birth is primarily caused by bacterial (*E. coli* and *Clostridium*) or viral agents. Since piglets are affected through contaminated environment and faeces of the sow, environmental and management factors are important regarding disease control (1, 2). The purpose of this paper is to present a case history of neonatal diarrhoea which occurred in a 600 sow French farrow-to-finish pig farm.

Material and methods

Case history

The herd had a known history of neonatal diarrhoea which was satisfactorily kept under control with the use of medication. In 2001, pre-weaning mortality was around 6 %. The unit experienced a recrudescence of diarrhoea resulting in pre-weaning mortality of more than 11 % in 2002-2003. The disease affected piglets from 2-4 days of life with mild to profuse diarrhoea. The piglets appeared hairy and were really "poor doing" animals. Diarrhoea occurred in 50 to 100 % of the litters and more often affected gilt's litters than sow's litters.

The farm's health team attempted multiple control strategies involving the use of commercial vaccines (*E. coli* and *Clostridium perfringens*) to the sow at the end of pregnancy and piglet medication without significant positive results.

Laboratory investigations

5 piglets of 2-3 days of age and considered to properly express the problem were submitted to necropsy and laboratory investigations. In all of them, small intestines with liquid yellow contents and oedema of the mesocolon was reported as well as yellowish large intestinal content. Bacteriological examination revealed the presence of *E. coli* and *Clostridium perfringens* (1.10^8/g of digestive content). The search of rotavirus and toxin of *Clostridium difficile* type A was negative.

Clinical investigations relative to farrowing management were carried out. A focus was particularly made on the environment the pigs were offered during the farrowing phase.

- *Farrowing room* : The rapid turn around resulted in only 16 hours rest time. Water supply, cleaning and disinfection procedure, feeding routines and ventilation system were subjected to detailed examination. Only part of them was within normal limits.
- *Management and farrowing practices* : Detailed and repeated examinations of 3 batches of sows and piglets revealed that cross-fostering concerned about 70 % of litters (23 % of piglets). This practice was applied without regard to parity.

Sow's body condition was evaluated with a method regularly used in our unit. It was scored on a scale from 1 to 5 according to the sow stoutness and the skin look (1: very thin, rough hair, abscesses, to 5 : good condition). 25 % of the sows were below the target score before farrowing.

Advices given and improvements : On the basis of these investigations several changes were implemented.

- *Farrowing room* : The pits of each farrowing room were emptied and washed between batches. The disinfectant was changed for another that included a sporicidal target in its spectrum.
- *Management practices* : Dry sow feeding strategy was re-evaluated to improve sow feed intake. 4 and 3 weeks before parturition, an attempt to immunise the piglets consisted in feeding back the pregnant sows with the small intestine and its contents removed from a piglet showing typical diarrhoea. Around farrowing (1 day before until 4 days after farrowing) medication (spiramycine and oxytetracycline) was added to the diet of the sows and gilts according to the manufacturer's recommendations. It was advised to reduce cross-fostering and to take into account sow's parity.

Once the changes were implemented 3 batches of sows and their piglets were followed from farrowing to weaning. Diarrhoea and fostering were daily recorded the first two weeks after farrowing.

Results and discussion

In the 3 considered batches, diarrhoea occurred in 23 (6/30), 31 (6/32) and 20 % (5/30) of litters and mainly affected piglets from gilts and second parity. In the same time, cross-fostering was reduced to 40.28 and 26 % of the litters. Pre-weaning mortality reached 11.4%, 7.6 % and 9.6 % for the 3 batches respectively and was kept under 10 % 2 months thereafter. This mortality rate was not attributable to diarrhoea but mainly to non infectious causes (overlaying and splayleg). Sow's body condition was improved during gestating period. The results suggest that management and husbandry changes coupled, when appropriate to sow's medication around farrowing, positively contributed to the control of the neonatal diarrhoea. Sow's medication may have reduced sow's excretion of faecal pathogens including *E. coli* and *Clostridium* and subsequently might have limited the contamination of the environment. Since diarrhoea mainly occurred in the litters of gilts, we can suppose that the reduction of fostering may have limited the spread of the disease among sow's litters. The feedback of diarrhoeic piglets intestine to gilts and sows pre-farrowing is known to be an effective technique to confer a passive immunity to piglets towards digestive disorders (2). Mild diarrhoea still affected first parity litters but the piglets at weaning showed a much better aspect than before.

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DIAGNOSTIC SURVEY ON CONTAGIOUS EPIDIDYMITIS OF RAMS IN PIEDMONT (ITALY)

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Introduction

Brucella ovis is the causative agent of contagious epididymitis of rams. It produces a clinical or subclinical disease in sheep that is characterised by genital lesions in rams and the main consequence of the disease is reduced fertility.

The disease is world-wide distributed and in Europe has been reported in France, Germany, Hungary, Romania, Russia, the Slovak Republic, Spain, but probably occurs in most sheep-raising countries (1) and in Northern Italy.

The existence of clinical lesions (unilateral or, occasionally, bilateral epididymitis) in rams may be indicative of the existence of infection, but laboratory examinations are necessary to confirm the disease. Laboratory confirmation may be based on direct (bacteriological isolation of *B. ovis* from infected tissues) or indirect methods (Complement Fixation Test, CFT). Molecular biological methods, such as polymerase chain reaction is being developed.

In 1998, after having done researches in order to establish the prevalence of epididymitis in rams on national territory, we pointed out in 2003, many positive serologic reactions to *B. ovis*.

Flocks on which we have diagnosed the infection, were officially brucellosis free since 1996 and usually go to mountain pasture during summer in regional territories.

Material and Methods

We have tested 634 animals by serological test (CFT).

Two positive rams were slaughtered and their samples were further processed with other laboratories researches: anatomo-pathological testing to elicit the presence, on these two animals, of the purulent orchids-epididymitis. Further more we used bacteriology and molecular biology techniques on tissue samples (spleen, testicles, epididymus) considering the difficulties in isolating *Brucella ovis*.

Serology - We used technique according to bibliography (5) and optimised by National Health Superior Institute.

Bacteriology – Tissue were macerated in sterile saline in a Stomacher. The whole material was inoculated onto selective medium developed by Farrell (7), with the addition of 10% horse serum and incubated at 37°C in an atmosphere containing 5% CO_2 for 15 days. All colonies resembling Brucella were sub-cultured onto blood agar medium with 5% sterile ovine blood and incubated for a further 2 days before re-examination. If Brucella was suspected using Stamp's staining (3), then the colonies were identified to species by classical techniques (2)

Molecular Biology (PCR) – We used primers according to bibliography (4,6), while the amplification protocol was optimised by our Laboratories.

Results

28 animals among 634 were resulted seropositive, both males and females. Bilateral orchid-epididymitis (ascessual form) was detected at necropsy.

Bacteriological testing has elicited the presence of *Corynebacterium* spp in both rams and the presence of *Brucella* spp in one of two rams. Species identification by PCR done in Brucellosis Reference Centre Laboratories, confirmed our diagnosis and the presence of *B. ovis*.

Molecular biology has detected *Brucella* spp on two examined rams.

Biochemical tests	*B.* spp (other than *B. ovis*)	*B. ovis*
Catalase	+	+
Oxidase	variable	-
Urease	variable	-
Mobility to 37°C	-	-
Mobility to 20°C	-	-
Lactose fermentation	-	-
Haemolysis	-	-
Nitrate reduction	+	-
Indole production	-	-
H_2S production	variable	-
CO_2 requirement	variable	+

Table n. 1: Biochemical differences between B. spp and B. ovis

Discussion

However, indirect diagnosis based on serological tests is preferred for routine diagnosis.

The demonstration of the existence of genital lesions (bilateral orchid-epididymitis) by palpating the testicles of rams was indicative of the presence of *B. ovis* infection in this flock. However, this clinical diagnosis is not sensitive enough because only about 50% of rams infected with *B. ovis* present epididymitis (5).

Moreover, the clinical diagnosis is extremely unspecific due to the existence of many other bacteria causing clinical epididymitis, e.g. *Corynebacterium* spp.

Conclusion

Bacteriological and molecular biology methods let us to confirm the presence of B. *ovis* on two seropositive rams. This case-report is very important for evaluation of *B. ovis* presence in Italy.

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PREVALENCE OF ENTERITIS IN GREEK RABBITRIES

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Introduction

The importance of rabbit meat as source of food for human consumption is well established long time ago. However, over the last years its nutritious value is well understood by consumers, especially after the last food "scandals" that affected other sectors of animal origin food production. Rabbit meat belongs to white ones, thus it is a particularly healthy animal origin protein source that makes it the consumers' first option. In Greece, the development of rabbit production began during the last decades and just recently started to be intensive.

The incidence of digestive system's problems is high in rabbits. Among them, enteritis comes first, as they constitute almost the 80% of the digestive system diseases. This is a very important inhibiting factor to the growth of enterprises that deal with the intensive production of rabbit meat (3).

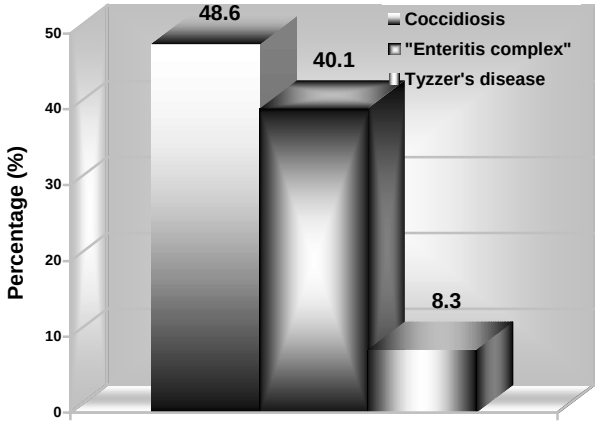
Over the last years there has been noticeable progress in the clarification of the aetiology of pathological findings of the digestive system of rabbits, which were characterized as "non-specific" enteritis. Generally, enteritis could be categorized depending on the causative agent in dietetic, bacterial, viral, parasitic, enteritis of unknown cause and in enteritis from antibiotics due to the injudicious use of certain antibiotics. Apart from the various pathogenic microorganisms, the role of several not contagious factors in the appearance of enteritis in rabbits is also important. Among these factors housing conditions and management practices applied in rabbitries are significant. Moreover, the irrational and non-hygienic nutrition causes changes in the composition of the normal microbial flora of the intestine, as well as an increase of number and spread of several pathogens (1, 2).

Prevalence of enteritis in Greek rabbitries

The incidence of rabbit enteritis constitutes the most important problem in industrial greek rabbitries. This was concluded from the cases of rabbits that came from greek rabbitries and were treated in the Clinic of Productive Animal Medicine of the School of Veterinary Medicine of the Aristotle University of Thessaloniki over the last 6 years. The diseases that more often appear are: coccidiosis, «enteritis complex» and particularly the mucoid enteropathy, as well as in a smaller percentage the Tyzzer's disease.

The incidence of coccidiocis is noticeable in a significant number of rabbitries. Factors, as the non appropriate microclimate of the stable and the housing conditions of rabbits (e.g. low temperatures, particularly when it is combined with increased levels of humidity, non-hygienic nutrition, sudden dietary changes, stress) increased the incidence of coccidiocis. Furthermore, the diagnosis was complicated when the feed used contained coccidiostats, because several coccidian strains can become resistant to these drugs.

Figure 1: Most common enteritis of rabbits in Greek rabbitries for the period 1998 - June 2004



The mucoid enteropathy as part of the «enteritis complex» was observed in older rabbits and associated with mucus production in the intestine. In those cases, the mortality was lower than the classic "mucoid enteritis" which is common in young rabbits, just after weaning age which is the age when the caecal microflora is becoming established and the animals are most vulnerable. The mucoid enteropathy was noticed in rabbitries that did not feed a high fibre diet or used excessive quantities of grains, proteins and fats in the diet, as well as when sudden dietary changes, stress or injudicious application of certain antibiotics occurred.

The Tyzzer's disease was observed in rabbitries with low health status animals, in which various stress factors occurred and an improper disinfection programme was applied. Clinical signs were acute in the young rabbits and high mortality rates were observed at this stage, while the older ones developed chronic weight loss.

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RABBIT PRODUCTION IN GREECE: THE MOST COMMON DISEASES

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Introduction

The continuous consumers' demand for rabbit meat made the need for further investigation of this sector of greek livestock production crucial regarding its size, and mainly concerning the pathological situations that it deals with. Thus, it becomes possible to achieve the first steps for the assurance of the production of enhanced hygienic quality rabbit meat which will fulfill the consumers' demands, while it will also contribute to the improvement of the greek rabbit production (3).

During the last 6 years, there has been an attempt from the Clinic of Productive Animal Medicine to specify the major diseases, which occur in the intensive greek rabbit production.

The most common diseases in Greek rabbitries

From this study it was found that most common are the diseases of the digestive system, especially enteritis and coccidiosis, followed by respiratory system diseases such as pasterellosis. The occurrence of parasitic dermatoses, in most of the cases due to mites, is also high, while neurological and neuromuscular disorders are quite common. Other diseases, due to various causative agents were observed, but in a very low incidence (Figure 1).

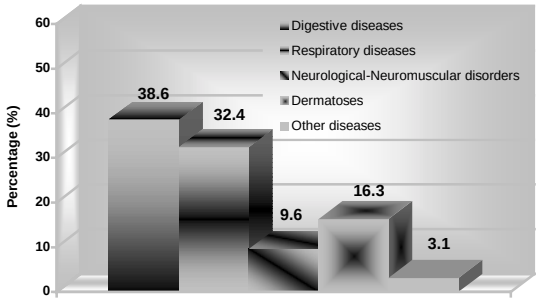
The majority of the greek rabbitries suffer from the chronic form of pasterellosis which results in large economic losses. Various clinical signs observed such as anorexia and therefore in weight loss, cases of pneumonia and pleuritis, as well as reproductive disorders leaded to negative effect in the productive parameters. The lack of application of on farm biosecurity measures such as improper quarantine procedures for the incoming new animals in the farm leads to the introduction of pasterellosis in naïve farms or to the entry of new strains of *Pasteurella multocida* in already infected farms.

The different types of parasitic dermatoses caused by various parasites consist a widespread pathological finding in almost all greek rabbitries. Especially ear mites (*Psoroptes cuniculi*) seem to have the largest occurrence, followed by sarcoptic mites (*Sarcoptes scabiei var. cuniculi*) infection. Nevertheless low incidence of Demodex mites (*Demodex cuniculi*) and fur mites (*Cheyletiella parasitovorax*) can be observed. The above-mentioned dermatoses were usually observed in overpopulated rabbitries with lack of appropriate disinfection and parasite control programmes.

The most common digestive disorders referred to coccidiosis, «enteritis complex» and particularly the mucoid enteropathy, as well as in a lower incidence the Tyzzer's disease. The digestive diseases had a negative impact on farm productivity, especially when inappropriate housing conditions and dietary programme coexisted.

The main incidence of neurological and neuromuscular disorders referred to torticollis and paresis/paralysis. The most frequent cause of torticollis was otitis media/interna caused by Pasteurella multocida and Staphylococcus spp. Mainly, the cases of paresis/paralysis occurred due to spinal trauma and "splay leg" (especially in young rabbits up to few months of age).

Figure 1: Most common diseases of rabbits in Greek rabbitries for the period 1998 - June 2004



Furthermore, since there is no real implementation of a proper on farm veterinary management programme in greek rabbitries (under full supervision and responsibility of the farm veterinarian), this resulted in the appearance of several health disorders due to unsuitable housing conditions (e.g. temperature, humidity, inappropriate ventilation systems, pen density), nutritional deficiencies and injudicious use of veterinary pharmaceutical preparations (1, 2). Moreover, the improper attempts in the reproduction and genetics section leaded to the decline of the productivity rates and the health status of the animals.

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FACTORS AFFECTING POST-WEANING MORTALITY ON FARROW-TO-FINISH INDUSTRIAL PIG FARMS IN GREECE: II. INFLUENCE OF HUMAN FACTOR

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Introduction

Highest degree of intensification in pig production requires highly controlled animal microenvironment, and thus increased dependence on man. Human factor may influence pig production either directly (source of zoonotic pathogens) or indirectly as microbial carrier, stress-inducer, manager and care taker. Therefore, critical participation of specialized personnel to each stage of production will expected to have serious impacts on welfare, productivity and health status of pigs (1). The present study investigates the role of stockperson, veterinarian and farmer, the three most important categories in primary pig production, on post-weaning mortality of farrow-to-finish industrial farms in Greece.

Materials and Methods

The study was carried out on 27 farrow to finish industrial farm over 150 sows (with a total of 17,740 sows under production which represents 23.29% of the population in industrial farms over 150 sows or 14.41% of the overall sow population in Greece). The selection of farms was based on criteria such as full or part-time veterinary consultation, existence of production records and history of collaboration with our institutions involved in the study. Data concerning the biosecurity measures in each farm were collected by questionnaires addressed to farm veterinarians. The influence of human factor-related risk factors on post-weaning mortality in over 349,785 weaned piglets (actual capacity of sampled farms) had been investigated. These factors include: a) veterinarian type of employment (part-time or full-time), b) hours of farmer's activity on farm (more or less than 4 hours per day), c) ratio of sows:stockpersons (more or less than 70 sows per stockperson) and d) stockpersons' education level (with or without high school education). The chi-square analysis was performed in order to determine the associations between mortality rate and risk factors.

Results and Discussion

The results of the study showed, that there were significantly ($P \leq 0.05$) higher death rates in weaning pigs when: a) veterinarian occupied on part-time basis, b) farmer's activity on farm was less than 4 hours per day, c) the ratio of sows:stockperson was lower that 70 and d) stockpersons were of low educational level (Figures 1, 2, 3, 4).

Although the above results do not necessarily imply that these risk factors were the direct causes of the increased mortality, however they do indicate areas where further attention should be warranted by researchers and farmers.

Figure 1: Post-weaning mortality rates (%) under different types of veterinarian's employment

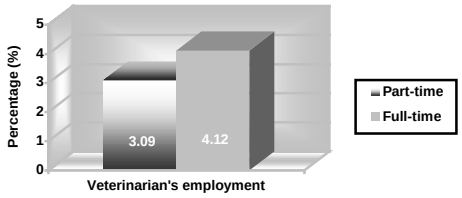


Figure 2: Post-weaning mortality rates (%) under different hours of farmer's activity on farm

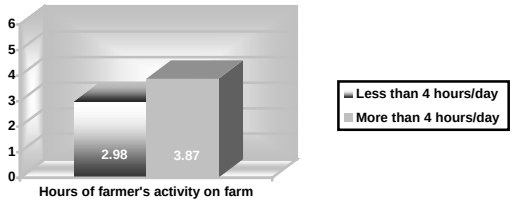


Figure 3: Post-weaning mortality rates (%) under different ratio of sows:stockpersons

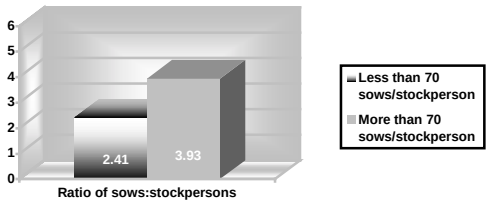
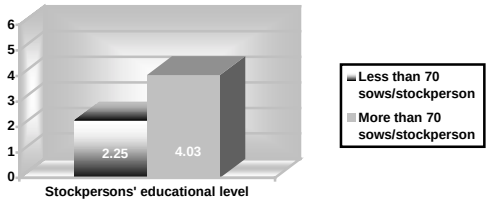


Figure 4: Post-weaning mortality rates (%) under different stockpersons' education level



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PERFORMANCE CHARACTERISTICS OF A NEW PSEUDORABIES VIRUS PRVgB ANTIBODY ELISA TEST

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Introduction

Currently, IDEXX PRV antibody screening tools consist of the HerdChek* PRV Antibody Test Kit for Screening and PRV Antibody Test Kit for Verification. In order to simplify the task of screen-testing herds, a single PRVgB antibody test has been developed. The PRV highly conserved genes gB or gD have been recommended to be used for PRV testing in swine[1]. This new PRVgB antibody test gives an option of either short (60 minutes) or long (overnight, ON) protocols for laboratory flexibility. The performance of the PRVgB antibody test compared to the current HerdChek PRV antibody test kits is reported in this study.

Materials and methods

The following PRV antibody ELISA test kits were used in this study: IDEXX HerdChek PRV Antibody (Screening) and IDEXX HerdChek PRV Antibody (Verification). Occasionally, the IDEXX HerdChek PRV gI (=g1) Antibody Test Kit was also used. Characterized swine sera were obtained from a variety of sources in the United States and in the European Union.

The PRVgB assay is a blocking format ELISA test. The biological reagents for the antibody test described in this manuscript consist of: PRV antigen immobilized on polystyrene microtiter plates, a PRVgB-specific antibody enzyme conjugate, and two control sera (positive and negative) for antibodies against the PRVgB antigen. The cutoff S/N values for the new PRVgB assay using the short protocol are: negative as greater than 0.70, positive as less than or equal to 0.60, and suspect as greater than 0.60 and less than or equal to 0.70. The cutoff S/N values for the new PRVgB assay using the overnight protocol are: negative as greater than 0.60, positive as less than or equal to 0.50, and suspect as greater than 0.50 and less than or equal to 0.60.

Sensitivity was assessed by testing temporal bleeds from nine pigs inoculated with PRV Shope strain and two pigs inoculated with a PRV modified live vaccine. Sensitivity was also tested using a PRV reference serum from the OIE ADV Reference Laboratory in Maisons-Alfort, France. Finally, the apparent sensitivity of the PRVgB test was measured using a set of 189 positive field sera.

Specificity was determined by testing several hyperimmune sera acquired from the National Veterinary Services Laboratory (NVSL), Ames, Iowa, which were produced monospecifically against other swine viruses. Negative population samples from the United States, France and Germany were also evaluated. And, during field trials held in three different laboratories in Germany, a total of 999 field negative sera were tested.

Results and discussion

Sensitivity

Both the PRVgB and the current HerdChek PRV Antibody Test Kits correlated very well in their detection of seroconversion time. Most of the temporal pigs were positive in both assays by Day 7. This demonstrates that the PRVgB test has a comparable sensitivity for early detection of seroconversion following exposure to PRV.

The European Union requirement for PRV screening assays states that the OIE SEAgB reference serum must be detected as positive at a dilution of greater than or equal to $1:2^2$. Both the PRVgB (short protocol) and the current HerdChek PRV Antibody Test Kits detected the OIE SEAgB reference serum as positive at 1:16; and in the overnight protocol, the PRVgB detected 1:32 as positive (Table 1). This demonstrates that the PRVgB test has adequate sensitivity to be used as a screening tool for herd surveillance.

Table 1: PRVgB Sensitivity Study on OIE SEAgB reference sera

	Diluted in PBS	PRV Screen S/P	Result	PRV Verification S/P	S/NHC	Result	PRVgB (Short Protocol) S/N	Result	PRVgB (Overnight Protocol) S/N	Result
OIE SEAgB reference	1:2	3.057	+	2.944	14.71	+	0.111	+	0.051	+
	1:4	2.123	+	2.024	13.51	+	0.147	+	0.073	+
	1:8	0.877	+	1.046	9.12	+	0.243	+	0.090	+
	1:16	0.574	+	0.450	5.13	+	0.470	+	0.132	+
	1:32	0.238	-			-	0.702	-	0.198	+
	1:64	0.073	-			-	0.988	-	0.514	S
	1:128	0.010	-			-	1.070	-	0.719	-
Cutoff:										
Positive:		S/P >=0.40		S/P >=0.40; S/NHC >=1.80			S/N <=0.60		S/N <=0.50	
Suspect:							S/N >0.60–0.70		S/N >0.50–0.60	

For the 189 field positive samples, the current HerdChek PRV antibody and PRVgB tests showed 98.94% agreement (with both the PRVgB short and overnight protocols). Three different samples were observed to be discrepant between the two tests. The first discrepant sample was PRV screen- and verification-positive, suspect in the PRVgB short protocol (0.613 S/N), and positive in the PRVgB overnight protocol (S/N 0.171). The second discrepant sample was positive on both PRV screening and verification, negative on PRVgB short protocol (S/N 0.754), and suspect in the PRVgB overnight protocol (S/N 0.579). The third sample was positive on both PRV screen and verification, positive on PRVgB short protocol (S/N 0.586) and suspect on PRVgB overnight protocol (S/N 0.505).

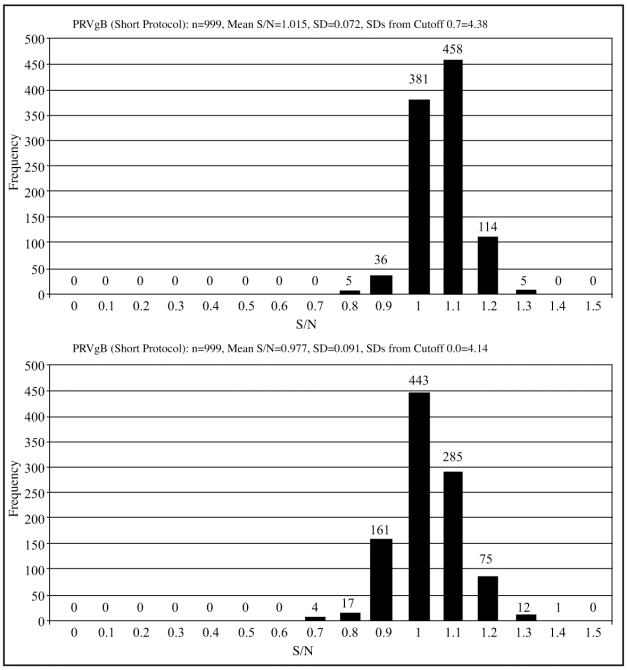
Specificity

Nine of the hyperimmune sera produced by NVSL against swine influenza virus (H1N1) and (H3N2), porcine adenovirus, porcine parvovirus, porcine reovirus, porcine rotavirus, encephalomyocarditis, hemagglutinating encephalomyelitis, and Transmissible Gastroenteritis virus did not cross-react on the PRVgB assay.

The PRVgB (short protocol) showed 99.38% agreement and 99.48% specificity compared to the current HerdChek PRV Antibody Test Kits performed on a total of 960 sera from negative field populations. Seven animals were observed to be discrepant among this set. The two discrepant animals from the U.S. sample set included: one sample that was positive on PRV screen (S/P 0.552) and negative on PRV verification (S/P 0.385 and S/NHC 1.868) was positive on PRVgB (S/N 0.398), and a second discrepant was negative on PRV screen (0.091) and suspect on PRVgB (S/N 0.684). From the European sample set, one discrepant animal tested as positive on PRV screen and verification, and as negative on PRVgB and PRVgI (S/N 0.817 and 1.044, respectively). The second EU discrepant sample was

positive on PRV screen and verification, positive on PRVgB (S/N 0.524) and negative on PRVgI (S/N 1.078), and is considered to be a potential PRVgI vaccinate. The fifth through seventh discrepants were all negative on PRV screen (S/P values of 0.336, 0.314 and 0.141), suspect on PRVgB (S/N values of 0.612, 0.660 and 0.690) and negative on PRVgI (S/N values of 1.083, 1.105 and 1.096). During field trials in eastern regions of Germany (considered to be free of PRV), the specificity of PRVgB test was determined to be 100% (short protocol) and 99.90% (overnight protocol). The single discrepant animal had an S/N of 0.569 (overnight protocol). The frequency distributions of all German PRV-negative populations tested during the field trials are presented in Figure 1. Therefore, in these studies, the PRVgB specificity ranged from 99.48 to 100%. The field testing data from Germany demonstrate that in PRV-free status herds, the specificity is expected to be very good, with our specificity results being between 99.90 and 100.0%.

Figure 1: PRVgB Kit Lot 2755-34B on All German PRV-Negative Population



Conclusions

The IDEXX PRVgB test described above demonstrated a high level of sensitivity and specificity. Further, it showed a good correlation to the current HerdChek PRV Antibody Test Kits (for screening and for verification). Therefore, this new PRV assay is validated as an effective tool for the testing of PRV antibodies in swine serum.

Acknowledgments

We appreciate the help we received in the form of swine serum samples from: the National Veterinary Services Laboratory, Ames, Iowa; and Dr. Howard Hill, Dr. Joseph Connor, Dr. Fernando Osorio and staff at the University of Nebraska at Lincoln, Nebraska.

Sera from the EU were provided by: Professor Bernard Toma at the OIE ADV Reference Laboratory at Maisons-Alfort, France; and the Wusterhausen ADV Reference Laboratory, Germany.

During our German field testing, we are indebted to the help of: Dr. Siegfrid Lange and staff at Bad Langensalza Diagnostic Laboratory; Drs. Korber, Mewes, Gehrmann and staff at Stendal Diagnostic Laboratory; and Drs. Volker Pohle, Hermann Nieper and staff at Leipzig Diagnostic Laboratory. These three facilities were gracious in providing laboratory space and serum samples.

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COLONIZATION OF NEONATAL PUPPIES BY *STAPHYLOCOCCUS INTERMEDIUS*

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Introduction

In dogs, *Staphylococcus (S.) intermedius* has been considered as one of the major coagulase-positive species of Staphylococcus. Besides its role as commensals on mucosal surfaces and the skin, *S. intermedius* is also involved in several diseases of dogs like pyoderma and septicaemia in new borne puppies (e.g. Münnich et al., 2002). For effective disease control information about the source and the route of infection are important.

In the present study we investigated the colonization of neonatal puppies by *S. intermedius*. Further potential sources of *S. intermedius* and the survival of this germ in the environment were assessed.

Material and Methods

Fourteen bitches and their litters, 55 puppies in all, were included in the study. The animals were sampled at a number of sites (buccal mucosa, anal mucosa, vulva) using swabs, moistened in sterile PBS. The bitches were sampled 1 week prepartum and then, together with their puppies within 24 h postpartum and also 2, 4, 6 and 8 weeks after the whelping day. Furthermore milk samples of the bitches were investigated.

Swab samples were also taken from the flour and the walls in the kennels. The survival of *S. intermedius* on surfaces common in kennels (wood, steel, tiles) was studied.

From one bitch and their puppies *S. intermedius* strains isolated at two weeks after whelping were differentiated by RAPD technique. Furthermore S. intermedius strains isolated from a 2nd litter of the same bitch were included in this investigation. The DNA for these investigations were isolated by using the "GenomicPrep Cells and Tissue DNA Isolation Kit" (Amersham Pharmacia Biotech). RAPD analysis was done by using the "Ready To Go RAPD Analysis Kit" (Amersham Pharmacia Biotech) as recommended by the manufacture.

Results

- Bitches

Seven days before whelping *S. intermedius* was isolated from 5 of the 14 bitches. One day postpartum 12, two weeks postpartum 13, four weeks postpartum 12, six weeks postpartum 10 and eight weeks post partum 5 of the 14 bitches were positive for *S. intermedius* (Figure 1).

- Puppies

Within 24 h after birth *S. intermedius* was isolated from 42 of the 55 puppies. *S. intermedius* was isolated from 54 of the 55 puppies two weeks after whelping. Four weeks postpartum 33, six weeks postpartum 33 and eight weeks postpartum 32 puppies were tested positive for *S. intermedius* (Figure 1).

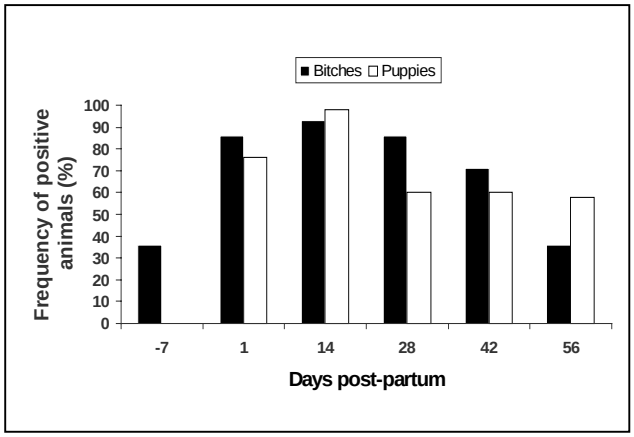
- RAPD-Analysis

Three isolates from the bitch and two isolates from the puppies expressed identical fingerprinting profiles. Further a second litter of the same bitch was investigated 8 month later. Here, we found also strains with identical fingerprinting profile between both litters.

- Survival in the environment

For all tested materials half-life periods up to several weeks were found.

Figure 1: Frequency of S. intermedius positive animals



Discussion

This study indicated that the process of birth is related to a higher frequency of colonisation of bitches with *S. intermedius*. Furthermore our results demonstrated that colonization of puppies by *S. intermedius* is a gradual process that starts almost immediately after birth and is increasing until the second week after whelping. Similar findings were reported by Matsumoto et al. (1976) and Allaker et al. (1992).

Main source for the colonisation of the puppies is the bitch. Furthermore surfaces in kennels contaminated by *S. intermedius* can serve as source, due to the considerable survival of this germ in the environment.

Acknowledgements

The authors would like to thank Mrs. Gnädig and Fiedler for technical help in the laboratory.

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Genetic resistance to diseases

Oral Communications

MOLECULAR APPROACHES TO DISEASE RESISTANCE

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Introduction

Genetic resistance to infectious diseases has been a subject of many controversies. The finding in the last 30 years of a number of polymorphisms in genes strongly influencing the outcome of the infectious process in various species has given a definitive response to the question of the existence of genetic resistance to infection. Looking for such genes has now been largely recognized has a powerful tool for the analysis of host/pathogens interaction mechanisms. In domestic species, direct selection of resistant populations is considered as a promising strategy against a number of infectious diseases. In these species, the use of genetic resistance should have advantages in a number of cases. The first idea coming to mind concerns its use against diseases for which we do not have any other possibility, neither vaccine nor therapeutics having been found. Transmissible Spongiform Encephalopathies (TSE) may represent the archetype of such diseases. The second area of major interest for the use of genetic resistance in farm animals are diseases due to a variety of pathogens using similar mechanisms to invade the host and/or to determine lesions as in the case of gastrointestinal nematodes. A third major goal of this approach is the possibility to decrease the carrier state in contaminated animals. Carriage of infectious pathogens by animals without clinical sign is largely responsible for the transmission to human of a number of infectious diseases. In the present communication devoted to the interest of using molecular tools for the discovery and the use of resistance genes in domestic animals, we will take a number of examples from studies on these pathogens of concern both for farm animals and human.

Observation of the variability in mouse lines and natural populations

Among the first reports establishing the existence of a genetic component in resistance to infectious diseases are the pioneer papers of Webster (1933) and Gowen (1948) who compared the relative susceptibility to salmonellosis or viral infections of a number of mouse lines. Although they crossed together some of these lines and suggested through F2 analysis the possibility of discrete genes controlling susceptibility to diseases, they lack markers to perform co-segregation experiments and thus failed to formerly demonstrate their existence. Protein polymorphisms evidenced by electrophoresis provided such markers at the beginning of the sixties. Combined with segregation analysis of well defined phenotypes related to disease susceptibility, they allowed the location of some genes of resistance to disease in inbred lines of mice, e.g. the *Ity*, *Bcg* and *Lsh* genes (which were considered as identical or closely linked on mouse ch 1) respectively controlling mouse susceptibility to *Salmonella typhimurium*, *Mycobacterium bovis* and *Leishmania donovani* (Rosenstreich et al, 1982), the *Lps* gene (mouse ch 4) regulating the host response to the lipopolysacharide component of bacterial membranes (O'Brien, 1981), the Xid gene (mouse ch X) involved in the regulation of antibody synthesis (O'Brien, 1981). These two genes are also involved in susceptibility to Salmonellosis and several other bacterial diseases. The Mx gene (mouse ch 16) that controls mouse susceptibility to several viral infections was also identified at the beginning of the eighties (review by Staeheli, 1990). In the same time, comparison of selected lines or breeds, or analysis of the variability observed in natural populations suggested the existence of discrete genes controlling the susceptibility to some infectious or parasitic diseases of farm animals (reviewed in Lantier and Vu Tien Khang, 1988). However, very few genes could be identified, essentially because of the very low number of genes or markers available on the farm animals genetic maps. One example of such a gene primarily detected in farm animals by the Neuropathogenesis Unit (Edinburgh) is the *SIP* gene controlling the duration of Scrapie incubation period in cheviot sheep (Foster et al, 1988).

Direct cloning from protein data: the example of the PrP gene

Scrapie is a fatal, progressive sheep and goat neuropathology. It belongs to the group of Transmissible Spongiform Encephalopathy (TSE) of animals and human. These diseases are characterized by the accumulation in or close to vacuolar lesions of the PrP, the protease resistant protein (Goldmann et al, 1991) or prion protein (Carlson et al, 1991). By cloning the PrP gene from a cDNA library using hybridisation with oligonucleotides designed from the reverse translation of the PrP protein sequence, Oesh et al (1985) evidenced that the PrP protein corresponds to the pathological isoform of a post-translationaly modified host encoded protein. The genes controlling the duration of the scrapie incubation period in mice (sinc) and sheep (sip) are thought to be identical to the PrP gene (Moore et al, 1998). A number of observations has shown the cosegregation of polymorphisms of the PrP gene and of the scrapie incubation period in mice and sheep (Carlson et al, 1991, Goldmann et al, 1991, Clouscard et al, 1995). In human, the polymorphism of the PrP gene also influences individual susceptibility and clinical features of the disease. The PrP encoding gene is highly conserved in mammals and is also found in birds (Lee et al, 1998). The profound effect of mutations of the PrP gene on sheep susceptibility to scrapie (Elsen et al, 1999, Andréoletti, 2000) and the importance of BSE for human health has lead several groups in GB, Netherland and France to propose the selection of resistant sheep as a

new mean to control sheep TSE, i.e. both scrapie and an eventual BSE strain that could have accidentally been transmitted to a sheep flock. Such a prophylactic action is presently under application in these three countries.
However, the PrP gene polymorphism does not explain the total variability observed in natural or experimental TSE. Several groups are consequently now looking for other genes influencing the TSE pathogenesis in the vicinity of the PrP locus (Moore et al, 1999) or on the whole genome (see the QTL approach bellow).

The reverse genetic approach

The search for Genetic markers of resistance to disease and comparative mapping. The tools that now permit the reverse genetic approach (or positional cloning approach) to clone and sequence the located and phenotypically identified genes became available during the eighties. Polymorphism of nucleotide sequences such as RFLP (restriction fragment length polymorphisms) could be detected using specific digestion of the DNA through restriction enzymes and then hybridisation techniques (Southern blot) using molecular probes. Such polymorphisms are much more frequent than protein polymorphisms, allowing a considerable enhancement of the number of available genetic markers in chromosome region of interest. This approach was applied to the precise location of the *Ity/Lsh/Bcg* genes on mouse chromosome 1 (Schurr E, 1990). The enrichment in genetic markers of region of interest also led to the concept of the conservation through the evolution of a number of chromosomal segments. This concept of comparative mapping has become of major importance for the study of genetic resistance to infectious disease allowing the transposition to large animals and human of information from location on the mouse genome of susceptibility genes. For example the mouse chromosome 1 fragment carrying the *Ity/Lsh/Bcg* genes (cloned as *Nramp1*, see bellow) has been shown to be partially conserved in human (Schurr et al, 1990) and domestic animals including birds (Pitel et al 1994, Cellier et al, 1996, Hu et al, 1995, Girard-Santosuosso et al, 1997). Because the genetic maps of human and model genomes are the object of constant developments, this concept of comparative mapping has now become a powerful mean to get genomic information and speed up gene identification in less studied species.

Positional cloning and conservation of resistance genes. The discovery of the PCR technology gave to the molecular biologist a number of new genetic markers such as the microsatellites and SNPs (single nucleotide polymorphisms). These new tools facilitate the definition of small chromosomal regions surrounding genes of interest. The larger the analysed segregating population is, the smaller is the interesting chromosomal fragment, thus facilitating the application of molecular strategies of physical mapping. A positional cloning approach using a large mouse population segregating for genetic markers of the *Ity/Bcg/Lsh* genes allowed the group of Ph Gros and E Skamene to define a small region surrounding this locus. The use as one of the parental line of a feral mouse line increased the probability to observe polymorphisms at each of the available genetic marker. Cloning of large genomic fragment combine with an exon trapping strategy allowed this group to identify the mouse *Nramp1* gene (Vidal et al., 1993) as a candidate gene. In inbred mouse lines, a polymorphism of this gene was found to be responsible for the resistance (*Nramp* $^{Gly\ 169}$) and the susceptibility (*Nramp* $^{Asp\ 169}$) to intracellular pathogens (Vidal et al., 1993, 1995; Malo et al.,1994). However the definitive demonstration of the *Nramp1* gene being responsible for the resistance/susceptibility to *Salmonella*, *Mycobacteria* and *Leishmania* only came from gene inactivation and reconstitution in knock out mice (Vidal et al, 1995). Because of their potential economic and health interest, the identification in laboratory rodents or human of genes influencing the outcome of infectious diseases prompted research groups working on farm animals to look for the existence of such genes in these species (Staeheli, 1990, Lantier et al, 1990, Malo et al, 1995, Feng et al, 1996, Qureshi et al, 1996, Barthel et al, 2001). The availability of genomic sequences greatly facilitated this work. Hypothesising sequence conservation between species, a number of research groups have for example cloned the NRAMP1 gene in bovine (Feng et al, 1996), sheep (Bussmann et al 1998), and chicken (Hu et al, 1995). This fruitful approach has been extended to a variety of disease susceptibility genes and species. Thus the TolR4 (formerly the mouse Lps gene) has been shown to influence the outcome of salmonella infection in mice and chicken (Hu et al, 1997, Qureshi et al, 1999), probably through the activation of an adaptative immune response by recognizing a conserved microbial structure, and to participate to the mouse pulmonary resistance to *Pasteurella pneumotropica* in conjunction with *Nramp1* and the MHC class II genes (Chapes et al, 2001).

The Quantitative Trait LOCI (QTL) approach

The development of genetic map in domestic animals (Barendse et al 1997) was a prerequisite for the considerable development in such species of the QTL approach, or "Genome scan". First applied to mouse studies (Lander and Schork, 1994), this approach corresponds to generalization of the molecular tools and statistical methodologies used in studies looking for the cosegregation of genetic marker and a candidate gene. Based on the analysis of a progeny from a parent heterozygous both for the marker and the candidate gene, it evaluates for each locus the statistical difference at the phenotypic level between individuals receiving one or the other of the two alleles of the genetic marker. In mammals, the simplest system may result from the progeny analysis of a back cross between a F1 from two inbred line with different susceptibility to a given disease and the disease susceptible parent line. In the case of a single gene with a dominant resistance allele there will be a statistically significant relationship of the resistance phenotype with the linked genetic marker allele from the resistant parental line. Similar analysis can be extended to the analysis of F2 population or of progenies from crosses between outbred populations (Haley et al, 1993, LeRoy and Elsen, 1993). In the case of resistance to disease in domestic animals a QTL approach has been able to define a new salmonella resistance gene in chicken (SAL1, Mariani et al, 2001). In the case of larger

animals with long generation intervals such as ruminants, the cost and the duration of such experiments that requires large sized populations has led to protocols devoted to the identification of QTL for a variety of unrelated characteristics: wool traits, carcass quality and salmonella resistance in sheep (Moreno et al, 2001; Ponz et al, 2001) or milk quality, fertility and parameters of mastitis susceptibility in bovines (Zhang et al, 1998). Another possibility might be to first define chromosomal region of interest in mice in order to limit the expensive work of genotyping. As an example of such strategy, QTL analysis have been performed in mice with the aim to identify regions of the genome outside the PRNP gene that support the variability observed in natural populations infected by the agents of the bovine or ovine TSE. Four complementary studies have been published (Manolakou et al, 1998, Stephenson et al, 2000, Llyod et al, 2001, Moreno et al, 2003); they were based on complementary experimental protocols (Scrapie or BSE agent, laboratory or feral mouse lines). A dozen of new QTL were detected, some of them by several studies. Although this result confirms that other genes than the PRNP one are influencing the duration of the incubation period, their identification through a positional cloning approach remains a long process. The relative importance of each of these QTL in ruminant populations cannot be inferred from mouse studies. One can imagine that two possibilities should be explored. The first one is to confirm the existence of QTL in ruminant chromosomal regions homologous to the one defined in mice through familial analysis. Such a protocol is presently in progress in a sheep flock naturally infected by the scrapie agent (Elsen et al, 1999). The second complementary strategy consists in researching candidate genes in these chromosome regions.

The candidate gene approach and the analysis of mechanisms of resistance to infection

The major difficulty encountered by such approach is linked to the size of the putative QTL region. As for positional cloning, the first step consist in accumulating new markers in the target portion of the genome in order to better define the QTL location. Simultaneously, one can have a look on genes potentially concerned with the disease resistance/susceptibility phenotype. Of special interest in this case are the genes involved in immune mechanisms related to the infectious process. Beside the genes already identified as “disease susceptibility” genes such as the already mentioned NRAMP1 or PrP genes, polymorphisms of effector or regulatory molecules may affect the efficiency of the host immune response against viral, bacterial or parasitic diseases. Pathogenesis studies with knock out mice in which one or several of the interferon (IFN) genes themselves or of the genes coding for the receptors to this cytokines family have been eliminated illustrate the central role of interferon in host mechanisms of resistance to a number of parasitic, bacterial or viral diseases (Samuel, 2001; Dessein et al, 2001). The role of Interferon gamma in human susceptibility to Mycobacterial diseases is the object of a continuous interest, genetic deficiency in this cytokine or in its receptor inducing mortality in children infected or even vaccinated with *Mycobacteria* (Abel et Casanova, 2002, Dupuis et al, 2000). However, the elucidation of the mode of action of this cytokine family is difficult to determine because of the number of regulatory and effector molecules activated by interferons. The Mx proteins are among the few effector of the interferon with known antiviral activities. The Mx gene has been described a long time ago as controlling mouse susceptibility to a number of viral infection in mice. It corresponds to a highly conserved family of interferon (IFN) responsive genes that code for structurally related nuclear and cytoplasmic proteins collectively referred to as Mx proteins. The Mx1 and Mx2 murine genes show a high degree of sequence similarity and are both located on chromosome 16 (Staeli, 1990). Ortholog genes have been cloned in fish, birds, mammals (including farm animals, Ellinwood et al, 1998) and human (Hefti et al, 1999) and a number of polymorphisms have been identified. Recent results using transgenic mice suggest that Mx proteins have antiviral properties on their own and should represent an interesting molecule in term of improvement of the resistance of farm animals to a number of viral diseases.

The expression pattern approach

The emergence of new tools and approaches for study of the host pathogens interactions, including functional genomic, should lead to further insights into the structure-function relationship of a number of genes of susceptibility to diseases and to the identification of key components of the disease resistance mechanisms, which could represent target for an effective selection for multi-resistance to pathogens of importance in animal populations. The major advantage of these new molecular biology techniques is their possibility of simultaneous application to large numbers of samples. According to each technology, one can compare large numbers of individuals, laboratory rodents strains or cell lines submitted to various stress conditions and test for the expression of thousands of target molecules or genes. Approaches such as the hybridisation of differential mRNA on high-density oligonucleotides arrays have been applied to the “profiling” of the host response to variations of physiological and pathologic conditions. However one of the difficulties of these approaches resides in the definition of criterions used to classify genes as “regulated”, *i.e.* to distinguish real activation from back-ground and minimize the number of false positive. This is a complex problem when comparing outbred animals submitted to a variety of natural uncontrolled stimuli. In order to simplify experimental model, one may use animal lines in controlled environment or *in vitro* culture systems. This approach has been developed by the group of C. Nathan to investigate the macrophage response to Interferon gamma (IFNg) and/or *Mycobacterium tuberculosis* (Mtb) infection (Ehrt et al, 2001). Macrophages are both a target cell for intracellular pathogens and the “chef d’orchestre” of the induction of the immune response in the early phase of the infectious process. Their activation increases phagocytosis, synthesis of inflammatory and regulatory mediators, and production of the bactericidal derivatives of Nitric Oxyde (NO) and Reactive Oxygen Intermediate (ROI). These bactericidal compounds,

respectively encoded by genes NO-synthase (iNOS) and phagocyte oxydase (Phox), play an essential role in host defense against a variety of pathogens. Microarray experiments reported in the paper from Ehrt et al (2001) showed that macrophage activation induced the suppression or the induction of a total of about 2000 genes. Mtb mimicked or synergized with IFNg rather than antagonized its action, confirming the central role of this cytokine in the resistance to intracellular pathogens. However the same strategy applied to macrophages deficient in iNOs and phox reveales that these two enzymes or more probably the cascade of their products help orchestrate the profound remodelling of the transcriptome that underline macrophage activation, suggesting a modified view of signal transduction by protein-protein interaction relays.

Conclusion

Understanding of infectious disease pathogenesis requires identification and characterization of host/pathogen interactions. Through evolution, a series of innate immune defense mechanisms have evolved to protect the host against the constant threat of microbial injury and direct the development of specific adaptive immune responses (Qreshi et al, 1999). Genetic analyses of host resistance in animal models or natural populations submitted to high infection pressure have provided new insights in the mechanisms of host immune response and demonstrated the feasability of selection for disease resistance in domestic animals. Rapid advances are now being made in the integration of dense genetic maps and complete sequence of model and human genomes. Comparative genomics and sequence analysis will play an increasingly important role in facilitating the transfer of new knowledge from the best known models to farm species of economic importance. However, farm animals may have also a pivotal role to play in this knowledge acquisition through their particular capacity to be both a target species of veterinary importance and animal models for other organisms including human. As such, they should beneficiate of the application of the new technologies of functional genomic.

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SPREAD OF INFECTIOUS DISEASES AMONG DIFFERENT DEGREES OF RELATIVES

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Introduction

The distribution of an infectious disease over an animal population and its evolution through time are the results of the dynamic interactions of the host and pathogen systems. These interactions may be represented in the form of mathematical functions specified by parameters that quantify the rates at which processes evolve. Various types of epidemic models have been formulated depending upon the characteristic of the infection (3). Among others, the SIS model is appropriate for infectious disease for which no permanent immunity occurs after recovery. The initials SIS refer to the movement of a typical animal through the two states of the disease: Susceptible – Infectious. An animal in the state *S* is healthy but susceptible to become infected with the disease upon exposure to the contagious agent. Upon infection, it enters the state *I* and remains in it until recovery to the *S* state.

Usually, SIS models treat populations as homogeneous in the sense that an *I* animal is equally likely to infect any *S* animal and all *S* animals are equally susceptible to infection by any *I* animal. However, it must be recognized that animals are more or less resistant to a same infective dose because of genetic and non-genetic differences. It seems likely that the genetic factor behind resistance to infectious disease is a combination of a number of genes, each having a small contribution to the disease relative risk (8).

Both, deterministic and stochastic modeling approaches exist. Deterministic models are based on ordinary differential equations and capture the essential relationships among the different components. However, an infection may be initiated in a small population, and under such conditions, a stochastic model that allows for inherent fluctuations may yield qualitatively different behavior.

In this paper, we extend homogeneous deterministic and stochastic SIS models to investigate the impact of genetic heterogeneity in the spread of a bacterial infectious disease.

Material and Methods

Let a population of density N constituted of g groups of cows sharing the same kinship degree i such that $N = \Sigma_i N_i$ (i = 1, 2, … g) and $p_i = N_i / N$ is the proportion of pairs of relatives of the i^{th} kinship degree ($\Sigma_i p_i = 1$). Each i^{th} group is constituted of S_i susceptible cows and I_i infected ones, with $S_i + I_i = N_i$. The initial conditions (t = 0) are specified by $S_i(0) = s_0$ and $I_i(0) = i_0$.

The deterministic form of the SIS model is for the i^{th} group of relatives:

$$dS_i/dt = \Delta - \mu S_i + \gamma I_i - \lambda_i k [SI_i]$$
$$dI_i/dt = \lambda_i k [SI_i] - (\gamma + \mu + \varepsilon) I_i$$
$$dN_i/dt = \Delta - \mu N_i - \varepsilon I_i$$

where Δ is the constant replacement rate, μ is the natural culling rate, γ is the recovery rate, ε is the culling rate due to the infection, k is the contact rate between cows, λ_i is the probability that any one contact will transmit infection and $[SI_i]$ is number of encounters between an infected cow and a susceptible one. As a measure of the susceptibility of a cow to infection, λ_i is a function of the degree of relatedness between cows in contact within the i^{th} group (a_i), the heritability of the resistance to infection (h^2), and the average population transmission probability (λ_0): $\lambda_i = h^2 (1 - \lambda_0) a_i + \lambda_0$.

In the stochastic framework, the spread of a SIS infectious disease is modelled as a Markovian continuous-time model (1). The infinitesimal transition probabilities in the interval (t, t + dt) are defined by:

$Pr[(S_i, I_i)_{t+dt} = (s + 1, i) \mid (S_i, I_i)_t = (s, i)] \sim \Delta\, dt$
$Pr[(S_i, I_i)_{t+dt} = (s - 1, i) \mid (S_i, I_i)_t = (s, i)] \sim \mu\, s\, dt$
$Pr[(S_i, I_i)_{t+dt} = (s, i - 1) \mid (S_i, I_i)_t = (s, i)] \sim (\mu + \varepsilon)\, i\, dt$
$Pr[(S_i, I_i)_{t+dt} = (s + 1, i - 1) \mid (S_i, I_i)_t = (s, i)] \sim \gamma\, i\, dt$
$Pr[(S_i, I_i)_{t+dt} = (s - 1, i + 1) \mid (S_i, I_i)_t = (s, i)] \sim \lambda_i\, k\, [si]_i\, dt$

where Δ, μ, ε, γ, λ and k have the same meanings as in the deterministic model. The Gillespie algorithm was selected for the stochastic simulation. This discrete-event simulation technique makes time steps of variable length, based on the transition probabilities and numbers s and i . In each iteration, random numbers are generated to determine the time and the type of the next transition. Upon the execution of the selected transition, the populations are altered accordingly and the process is repeated (6).

Deterministic and stochastic models were illustrated by modelling bovine mastitis spread on a dairy farm with 5 different groups, each composed of 20 relatives of the i^{th} degree with a_i = 0, ½, ½², ½³ and ½⁴. Models were implemented by introducing a single infected cow in each group and typical proportion of infected quarters was computed. Default values for the parameters were derived from the literature on *S. aureus* quarter infection and on culling strategies in dairy cattle (4, 7, 9,10): $\lambda_0 = 2 * 10^{-2}$, $\gamma = 4 * 10^{-3}$, $\varepsilon = 0.005$, $\mu = 7 * 10^{-4}$, $h^2 = 0.05$. The replacement rate was chosen to insure the initial disease-free equilibrium: $\Delta = \mu S_{i(t=0)}$. As no information was available on the average number of contacts per unit of time made by a quarter, it was assumed constant and directly proportional to the number of quarters initially present in each group of relatives.

Results

For each group of relatives, the deterministic model has two equilibrium points: the disease-free equilibrium with $I_i = 0$ and $S_i = \Delta/\mu$ and the endemic-disease equilibrium with $S_i = (\gamma + \mu + \varepsilon)/(\lambda_i k)$ and $I_i = [(\Delta \lambda_i k) - \mu (\gamma + \mu + \varepsilon)]/(\mu + \varepsilon) \lambda_i k$. The Jacobian matrix evaluated at both equilibria showed the endemic-disease equilibrium is always stable but the disease-free equilibrium is stable if $R_{0i} < 1$ with $R_{0i} = [\lambda_i \Delta k]/[\mu (\gamma + \mu + \varepsilon)] < 1$. As the same

set of coupled equation was applied to all groups of relatives, the R_0 for the whole population is $R_0 = \Sigma_i\ p_i\ R_{0i}$ for i = 1, 2, … g. This global R_0 gives the total average number of new infective cows in the population produced by one infective during the mean (death-adjusted) infective period (5).

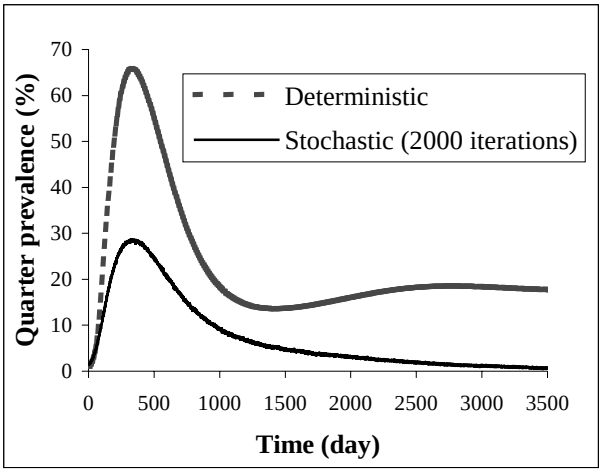
The relationship between R_0 and the proportion of relatives is given by

$$R_0 = [\Delta/\mu]\ [k/(\gamma + \mu + \varepsilon)]$$
$$[\lambda_0\ (1 - h^2\ \Sigma_{i=1}\ a_i\ p_i) + h^2\ \Sigma_{i=1}\ a_i\ p_i].$$

Then, in a population composed of unrelated and relatives of one type (a_i>0), the maximum proportion of relatives tolerable to have no or minor epidemics (R_0<1) is:

$$p_{Max} = \{1 - \lambda_0\ [\Delta/\mu]\ [k/(\gamma + \mu + \varepsilon)]\}/$$
$$\{(1 - \lambda_0)\ a_i\ h^2\ [\Delta/\mu]\ [k/(\gamma + \mu + \varepsilon)]\}$$

The results of the simulation with the deterministic and stochastic models for the *S. aureus* infection are illustrated in the following figure:



Discussion

The central question is whether or not, and under which conditions, an infectious disease will spread in a population when the degree of susceptibility is related to the degree of relationship between susceptible and infected animals. Given the assumption of fitness declining with increased inbreeding, the probability of an epidemic will be minimized if the population is composed only of unrelated animals but this is not an absolute constraint (2). Indeed, the global R_0 can be made less than 1 for different population structures. For example, in a population composed of related and unrelated of the i^{th} type, the maximum proportion of related cows (p_{Max}) admissible to keep R_0<1 can be computed and will increase if h^2 decreases. This is particularly interesting for the control of infectious disease for which h^2 is usually low.

Other assumptions underline the model such as equal contact amongst animals of different genotypes and constant average infectiousness per infective animal. But these are an obvious starting point for developing any general theory and more realistic models may be developed.

Conclusion

Methodologies exist to help breeders to make appropriate breeding choices to limit the transmission of an infectious disease based on the knowledge of parameters characterizing the infection (γ, ε), the population demography (Δ, μ) and the genetic composition (λ_0, a_i, h^2) of the population.

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BREEDING PIGS RESISTANT TO *ESCHERICHIA COLI* F18 IN THE FIELD – A PROGRESS REPORT FROM SWITZERLAND

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Introduction

Postweaning *Escherichia (E.) coli* diarrhoea and enterotoxaemia (oedema disease) cause significant loss mainly in newly weaned pigs. Out of 125 pigs in this age group examined post mortem in 1999, 39% died from *E. coli* diarrhoea and 27% from enterotoxaemia (4). In both diseases, the pathogenesis is characterized by at least two essential steps, I. colonisation of the small intestine and II. production of one ore more toxin(s). Adhesion of the bacteria to the intestinal wall is an indispensable prerequisite of colonisation. Adhesion depends on the higly specific interaction between bacterial fimbriae and host receptors. The two types of fimbriae F4 and F18 dominate in postweaning disorders. The type F4 comprises three antigenic variants and the type F18 the two variants F18ab and F18ac (7). In a Swiss survey of post mortem diagnoses with 125 pigs over two weeks of age, F4 was detected in 49%, F18ab in 41% and F18ac in 10% of the cases (8).

Receptor activity can be demonstrated either *in vivo* after oral challenge based on clinical outcome and faecal shedding of the organism, or *in vitro* by microscopic observation of intestinal villi, enterocytes or enterocyte brush borders after incubation with the fimbriated bacteria. The *in vitro* technique requires the pig to be killed. Both variants of fimbriae F18, i.e. F18ab and F18ac, bind to the same receptor (3,7).

Receptors for each of the two fimbrial types are not present in every individual pig. Adhesion is inherited in one locus with adhesion dominating over non-adhesion (1). The gene specifying for the F18 receptor forms part of the malignant hyperthermia syndrome (MHS) linkage group located on chromosome 6 (9) and is closely linked to or even identical with the fucosyl transferase 1 (*FUT1*) gene (6). In this gene, a guanine (*G*) / adenine (*A*) polymorphism at position 309 is highly correlated with the receptor genotype. This polymorphism allowed to develop a diagnostic DNA test based on PCR-RFLP for use in live pigs. *FUT1* genotypes *G/G* and *G/A* are highly correlated with adhesion and genotype *A/A* with non-adhesion of *E. coli* with fimbriae F18 (6).

The excellent concordance between *FUT1* genotypes and results of microscopic adhesion tests has been confirmed in several laboratories and populations of pigs. Strikingly, the *A* allele coding for resistance has not been detected in 20 out of 21 Chinese native breeds (10). In a recent Danish study (5) the microscopic adhesion test showed complete concordance with *FUT1* genotypes, but some pigs with the *A/A* genotype were not perfectly protected against a heavy challenge with a diarrhoeagenic F18 positive *E. coli.*

In Switzerland, the diagnostic DNA test has been available to pig breeders in the field for about 8 years. We therefore decided to collect and present data on the changing frequencies of the *A* and *G* alleles as well as on the strategies applied by the breeders and the disease status in pure *A/A* herds.

Material and methods

Data on *FUT1* genotypes of all pigs examined were obtained from the blood typing laboratory of the Swiss Federal Institute of Technology. Genotypes of AI boars were provided by the Swiss AI stations, a branch of SUISAG at Sempach. Nucleus breeders and two breeding organisations provided the names of breeders who had bought breeding stock of *A/A* genotype. These breeders were sent a questionnaire asking about the breeding strategy, the present status of the herd regarding F18 resistance, occurrence of oedema disease and post-weaning *E. coli* diarrhoea, and eventual positive or negative side-effects of breeding for resistance.

Results

In the blood testing laboratory between 400 and 800 pigs are examined per year. In samples from Swiss Large White pigs the frequency of the $FUT1^A$ allele increased from 0.31 in 1996 to 0.52 in 2004, whereas in Swiss Landrace samples it started from 0.07 and reached 0.24.

The number of AI boars with the *A/A* genotype increased from 8 in 1999 to 25 in 2004; only one out of the latter belonging to Swiss Landrace. However, more Landrace boars will soon be at disposition, since the frequency of the *A/A* allele in Landrace boars has been raised from 0 in 1996 to 0.21 in 2004.

The questionnaire was answered by 58 breeders who had bought *A/A* breeding stock. In only 12 herds all boars and sows were of the *A/A* genotype. Three out of the 12 pure *A/A* herds were nucleus herds. Most other nucleus herds know the genetic status of their pigs, but do not see a need to replace genetically valuable stock precipitately. Out of the 12 pure *A/A* herds, eight had suffered before from either oedema disease or post-weaning diarrhoea; all of them reported to be free from clinical disease now. Three herds had never suffered from problems, and one owner did not respond to this question.

Several owners of herds of mixed resistance genotype reported severe post-weaning diarrhoea proven or suspected to be caused by *E. coli* with fimbriae of the type F4. There were no consistent answers to the question addressing side effects (e.g. health or reproduction) of breeding *A/A* pigs.

Discussion

The data presented show a steady increase of the $FUT1^A$ allele in the Swiss pig population. Most of this increase is due to the decision of the geneticists at the central genetics management and the AI adminstration to

systematically favour selection of carriers of the *FUT1*^{A} allele. In constrast, only a minority of the breeders are concerned about the frequency of the resistance allele in their herds.

The evaluation of the effectivity of breeding for resistance in the field is hampered by the fact, that the true incidence of post-weaning *E. coli* F18 diarrhoea cannot be determinded without laboratory investigations. More than half of this complex disease is caused by enterotoxigenic *E. coli* with fimbriae F4. A similar *in vivo* diagnostic test for genetic resistance against *E. coli* diarrhoea caused by F4 strains appears highly desirable. Anyway, none of the pure *A/A* herds previously affected by oedema disease had problems after replacement by *A/A* breeding stock. There was no indication that break-throughs of resistance occurred in the field. Hypothetically, toxigenic *E. coli* might be able to produce a mutant adhesin overcoming the lack of receptor in pure *A/A* herds.

Care must be taken that other important genetic traits are not negatively influenced in the process of breeding for disease resistance. Candidate litters in nucleus herds must be identified early to allow blood typing before castration of the male piglets is done. With this proceeding breeding for resistance does not slow down genetic progress for other traits.

The data available were not suitable to detect side-effects on production traits. An early analysis in the Swiss pig population had not revealed any impact of the *FUT1* genotypes on meat production traits (C. Stricker, personal communication). In Germany, Binder et al. (2) found no sigificant effect of the *FUT1* genotype on meat production and meat quality traits. Their investigation was based on 813 German Landrace, 576 Piétrain and 68 German Landrace pigs raised in a testing station. Care was taken by these authors to separately look at effects of the *FUT1* and the *MHS* loci. In a program for resistance breeding, the linkage of the *FUT1* gene with the *MHS* gene may lead to a concomitant change in the incidence of the MHS syndrome depending on the genetic situation in the pig population concerned.

Genetic disease resistance has the great advantage over other preventive measures that investment is mainly limited to nucleus herds, and that return in terms of reduced pig loss and lower cost for medical treatments will potentially flow for decades.

Conclusion

The still limited field experience indicates that breeding for resistance against *E. coli* with fimbriae F18 is feasible and has no unwanted side-effects, if it is practiced with due vigilance.

Acknowledgements

The cooperation of breeding organisations and herd owners is gratefully acknowledged.

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F2-FAMILIES FROM EUROPEAN AND CHINESE BREEDS AS SUITABLE MODEL FOR THE MAPPING OF DISEASE RESISTANCE IN SWINE.

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Introduction

Detecting and improving disease resistance by traditional phenotype selection can hardly separate environmental and genetic effects. Thus the aims of modern genomic approaches are to shift the selection criteria from phenotypically expressed disease status to allele status at the DNA level and to get insight into the molecular mechanisms of resistance/susceptibility against certain infectious dieseases. Mapping of disease resistance loci by linkage analysis needs to adress three major requirements: a) an informative disease model and a suitable inbred line, i.e. founder populations differing as much as possible in their inherited degree of resistance against the disease of interest and in their marker phenotypes, b) informative disease records, gained under a highly standardized environment, c) linkage maps, moderately populated with informative markers.

Our current disease models are dealing with the *Pseudorabies Virus (PrV)*, *Sarcocystis miescheriana* and *Salmonella typhimurium*. European breeds like Pietrain or Large White and the chinese Meishan breed as founders, and their F2-crosses have proved a suitable and informative animal model. The present work describes significant differences in resistance/susceptibility between founder breeds in the three disease models, the status of phenotypic and genetic evaluation of the F2-crossbreds, and the way to the mapping of disease resistance loci as QTLs.

Material and Methods

Animals and pretesting: Purebred chinese Meishan pigs were available from a herd of the University of Stuttgart-Hohenheim. They were compared with commercial Large White or Pietrain pigs to check for suitability regarding potential disease models in clinical studies (PrV: Federal Research Center for Virus Diseases of Animals [BFAV] in Tuebingen; S. miescheriana: experimental station "Unterer Lindenhof" of the University of Stuttgart-Hohenheim; S. typhimurium: Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig).

F2-families: Significant differences have been found between european and chinese breeds for all three models; so, F2-families were set up to detect responsible disease resistance loci as QTLs by linkage analysis, starting with PrV and S. miescheriana.

F2-crossbreds were produced at the experimental station "Unterer Lindenhof". Litters were housed and fed under standardized conditions.

Challenge, sampling and investigation: At an age of around 12 weeks, piglets were challenged at the BFAV (*PrV*) or the Dep. of Swine Diseases in Giessen (*Sarcocystis*), rsp.

All F2-pigs were clinical examined and sampled to describe onset, course, degree and outcome of disease as accurately as possible.

Statistical analysis: Variance of clinical, haematological and clinical-chemical traits have been analysed with the Statistical package for Social Sciences (SPSS/Pc).

In the case of *Sarcocystis*, heritabilities for these traits have been evaluated with the CVE-version 4.2.5 (Groeneveld).

Genotyping: 110 microsatellite markers were selected from the public maps, based on their position, ease of scoring, and informativity. Markers were evenly spaced on the 18 porcine autosomes and the pseudoautosomal region of the X-chromosome to give maximal marker-intervals of less than 40 cM. 85 of them were established for genome-wide linkage mapping in PrV. In case of Sarcocystis, establishing of amerkers and genotyping has just started.

Linkage and QTL-analysis (PrV): Linkage was analysed with the software package CRIMAP. Clinical and genotyping data were combined, and QTL-analysis was done according to an interval mapping strategy with a monolocus regression analysis (Haley et al., 1994).

Results

Significant differences in resistance/ susceptibility between founder breeds were obvious in all three disease models.

PrV: The PrV model showed significant differences in resistance/susceptibility to PrV between european Large White and chinese Meishan pigs. After intranasal challenge with a highly virulent PrV-strain, all pigs developed clinical signs, e.g. fever from days 3 to 7 p.i. All purebred Large White pigs, all F1 and 75% of the F2 generation developed neurological symptoms at days 5 to 7 p.i. and died or had to be euthanized. The purebred Meishan pigs and 25% of the F2-pigs did not develop any signs of neurological disorder and reconvalescended until days 8 or 9 p.i..

Rise in temperature in F_2-animals started two days p.i.. At day three, two groups of different temperature response could be distinguished: one showing a quick rise, reaching temperatures of about 41°C, and a second group rising slowly and staying beyond 40.5°C. Temperature profiles and the appearence/non-appearance of neurological symptoms were not correlated.

QTLs (fig. 1) for appearence/non-appearence of neurological symptoms were found on chromosomes SSC9, SSC5 and SSC6. Together they explained 84% of the F2-response. Further significant QTLs, associated with immune-dependent rise in body-temperature have been found on chromosomes SSC2, 4, 8, 10 and 11 (Reiner et al., 2002b).

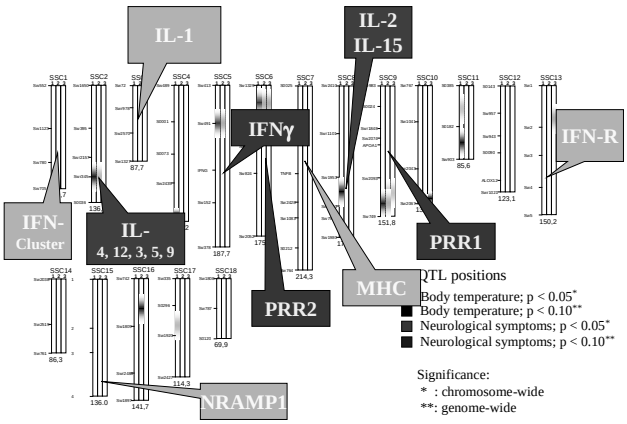

Fig. 1: Genome-wide mapping results for QTLs on body temperature and neurological symptoms in F_2-pigs after intranasally challenge with 10^5 pfu of the PrV-strain NIA3 at the age of 12 weeks. QTLs are indicated as marked areas on the chromosomes.

S. miescheriana: Significant differences appeared in clinical, serological, haematological and parasitological findings of Pietrain (PI) and Meishan (ME) founder piglets. after challenge with S. miescheriana. The major discriminating period post infection (p.i.) was between days 42 and 45 (Reiner et al., 2002a). Severity of signs was negatively correlated with specific immunoglobulin titres during the first 3 weeks p.i. and positively with the load of merozoites in muscle tissues, the latter being 20 times higher in PI than in ME. Sarcocystis-specific variances in F2-pigs showed significant shares of additiv-genetic variance, with moderately up to high heritabilities, supposing the existence of significant disease resistance loci in this model. Genotyping experiments for QTL-analysis have just been started.

S. typhimurium: The status of elucidation of the Salmonella-model still is at its beginning. After an oral challenge of purebred weaners, clinical diarrhoe scores decreased faster in Meishan piglets than in commercial hybrid piglets.

Discussion

PrV: QTLs found in this study point to gene effects on a) the appearance/non-appearance of neurological symptoms and b) QTLs for temperature course and thus immunological response after challenge with PrV. Major QTLs on SSC9 and SSC6 are linked with the loci PRR1 and PRR2. Both receptor proteins are involved in adsorption and penetration of the PrV to the cell in rodent models. Initiation of infection by alphaherpesviruses requires a cascade of interactions between different viral and cellular membrane components. Linked QTLs presented in our study stimulate to investigate these genes more specifically. Specific immunology against herpesviridae seems to be sustained by the Interleukin 12 (IL12) – INFg-pathway. The IL12-gene is located within an interleukin cluster on SSC2, close to a region associated with a QTL on temperature course p.i.. Further QTLs are linked with the INFγ-locus (SSC5).

Since our study elucidates genetic differences in resistance/susceptibility against PrV between Meishan and Large White pigs, these genetic diverse breeds are informative to elucidate the role of host defense against PrV in swine.

S. miescheriana: The present work describes clinical, clinical-chemical and haematological data, with definite clues for genetically determined differences in resistance/susceptibility against this protozoan parasite. The data highlight the suitability of this model to further analyse chromosomal regions, candidate genes and thus the molecular basis of host-parasite interaction.

S. typhimurium: More detailed studies are needed to define more precisely differences in resistance/ susceptibility against this very important pathogene and to examine if a specific F2-approach would have enough power to find associated disease resistance loci.

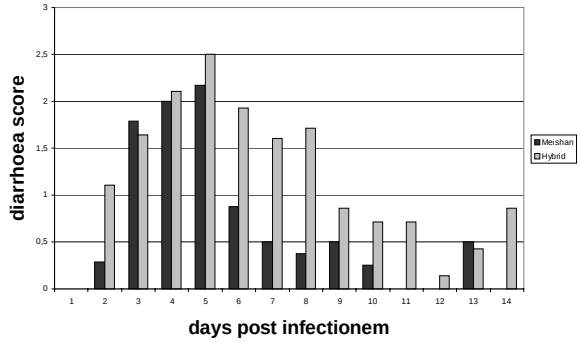

Fig. 2: Diarrhoea score of Meishan and Hybrid pigs after oral challenge with S. typhimurium.

Conclusion

Our current disease models are dealing with the *Pseudorabies Virus*, *Sarcocystis miescheriana* and *Salmonella typhimurium*. Elucidation status of the models is quite different, but all three models show that european breeds like Pietrain or Large White and the chinese Meishan breed make a suitable and informative animal model for disease resistance in differing kinds of host-parasite interactions. Exemplary, the PrV-model shows the way from clinical differences in resistance/ susceptibility against a specific disease towards disease resistance loci. Further research, including fine mapping of candidate genes and the evaluation of responsible gene variants may lead to a better understanding of pathogenesis and to a better prophylaxis based on less suceptible pigs.

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IDENTIFICATION OF CHROMOSOMAL REGIONS ASSOCIATED WITH RESISTANCE/SUSCEPTIBILITY TO VHSV IN DOUBLED HAPLOID RAINBOW TROUT (*O. mykiss*): FIRST RESULTS

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Introduction

Viral haemorrhagic septicaemia (VHS), caused by Egtved rhabdovirus, is one of the diseases that constrain rainbow trout production in Europe. Different studies support the existence of genetic variation within trout populations for the susceptibility to the disease. The present study aimed at detecting quantitative trait loci (QTLs) affecting resistance in the species, as a first step for future prospects in selective breeding and increased knowledge of genetic architecture of disease resistance in fish.

Material and Methods

Selection of homozygous grand-parents for alternative resistance

Experimental fish originated from the experimental INRA SY strain. Mitotic homozygous gynogenetic rainbow trout were screened using an *in vitro* test for resistance, the VREFT value (viral replication in excised fin tissue), which we previously showed to be correlated with resistance to waterborne challenge against the virus (Quillet *et al.*, 2001). 'Resistant' (R) and susceptible (S) individuals were selected on the VREFT values, and their expected resistant/susceptible status was further checked by performing progeny testing.

Production of doubled haploid experimental progeny

Clonal F1 hybrids were produced by intercrossing the selected R and S parents. F1 females were then reproduced by mitotic gynogenesis, according to Ditere*at al*. (1993), in order to produce the experimental progeny. Two non related haplo-diploid families (F98 and F00) were produced in two different years and used in the present study. Experimental fish were reared under controlled conditions (recirculated tap water unit).

Phenotypic testing of resistance to VHSV

Waterborne challenge. Resistance tests to VSHV were conducted with waterborn challenges, according to Dorson *et al*. (1991) on 4 to 5-months old juveniles (mean weight: 1.2 to 1.6g). Infected fish were kept for 2h in a 5 x 10^4 pfu ml^{-1} virus suspension with vigorous aeration after stopping the water supply. For each family, 1100 to 1200 progeny, distributed into 9 or 10 replicated aquaria were challenged, and a control group was mock-infected. Mortality was monitored twice a day until a plateau was reached (34 days in F98 and 58 days in F00). Total mortality was 92.6% in F98 and 79% in F00. Dead fish were immersed in absolute ethanol for further DNA extraction. At the end of the period of survey, surviving fish were sacrificed (lethal dose of anaesthetics), and immersed in ethanol.
In vitro test for resistance. A sample of 300 F00 progeny not challenged against the virus was kept for further growth. When they were 16-months old, fish were anaesthetized, and individually PIT-tagged. Anal and/or pelvic fin were clipped and processed as described in Dorson and Torhy (1993) to perform a measure of replication in excised fin tissue (measure VREFT-1). Seven months later, a random sample of 100 fish were scored again (measure VREFT-2).

Genetic markers and source of DNA

Genetic markers. Microsatellites previously developed for salmonids were utilized. They were amplified with PCR conditions optimised for each microsatellite, and were run on polyacrylamide gels, using fluorescent labelled primers. Alleles were detected using a FM-BIO II, Hitachi fluorescence scanner. Microsatellites used to test association with resistance were chosen from linkage information in the families, in order to allow a even genome scan whenever possible.
Source of DNA. Selective genotyping was used to test association with challenge issue. In a first step, the earliest dead fish (10% of challenged progeny) and the survivors (all survivors in family F98, and 10% of the challenged fish in family in F00) were sampled from each aquarium (sample 1). Fish were examined for every chosen locus, and a test for association between genotype at the marker and challenge issue was performed. When the test was suggestive ($P<20\%$), a second sample was examined (sample 2, including the next 10% to die, and 10% 'resistant' taken at the end of the mortality curve in F98, or among survivors in F00) in order to confirm the result.
Association with VREFT values was also tested in F00 family, for the most significant loci detected in challenge analysis ($P<0.01$). The test was performed for the two sets of measurements (VREFT-1 and VREFT-2).

Test for linkage and association studies
Simple association between allele at any given locus and challenge issue was assessed within each family using the χ^2-test (1dl). The null hypothesis is that there is no association between the genotype and the phenotype, and that the 2 parental alleles are equally represented in R and S progeny. A P value less than 0.05 was taken as significant at a point-wise level. A Bonferroni correction for the number of tested loci was used to calculate a genome-wise significant level ($P<0.05$ was taken as significant). A joined association study was then performed combining data from the 2 families. In that case, it was assumed that R grand-parents both transmitted the same allele coded 1, and that susceptible grand-parents both transmitted the same allele coded 0.
Association between genotype and VREFT was tested using one-way Anova performed on log-transformed VREFT values (comparison of mean values of progeny having inherited alternative allele).

Results

Genomic regions associated with response to challenge

Twenty-nine out the 31 linkage groups of the species could be scanned. The mean number of markers examined per linkage group was 2.8 in F98 and 3 in F00.

Table 1. Significant microsatellites for association with challenge issue in the two experimental families

Marker	LG[a]	Point-wise P value (P<0.05)	Experiment -wise level significance
Family F98			
OmyFGT18/1 TUF	II	0.025	
Ocl1UW	XI	0.012	
Omy7DIAS		0.006	
Omy14INRA	XV	0.015	
OMM1131	XXVII	0.028	
Ots108SS1	XXXIX	0.033	
Ssa124NVH	XXXI[b]	<0.0001	YES
Family F00			
OmyFGT18/1 TUF	II	0.009	
Omy18 INRA	VIII	0.002	
Ssal14DU	XIV	0.027	
Omy77DU	XVI	0.037	
OMM1013		0.016	
OMM3000	XXVI	0.024	
OMM1053	XXXI[c]	<0.0001	YES
OMM1080		<0.0001	YES
Joined analysis			
OmyFGT18/1 TUF	II	0.0004	YES
Ocl1	XI	0.023	
Omy7DIAS		0.002	
OMM1030	XII	0.034	
OMM1034	XIX	0.049	
OMM1023	XXII	0.039	

[a]: the linkage group (LG) are numbered according Nichols et al. (2003). Synteny was inferred from common microsatellites with published maps (Sakamoto *et al*., 2000; Nichols *et al*., 2003). In a number of linkage groups, linkage in females was inferred from published information on male linkage maps.

[b]: locus not yet mapped in the family at time of impression. Position inferred from unpublished data (Danzmann, R. Guyomard, pers. comm.)

[c] : putative location (lod =1.4, loci informative in one family only for the construction of the map). OMM1080 and OMM1053 totally linked.

Family F98. Analysis of sample 1 revealed 31 suggestive loci out of 92 analysed. After analysis of sample 2, 7 loci located on 6 different linkage groups remained significant, of which one was significant at the experiment-wise level (Ssa124NVH). The allele of the R grand-parent at that locus was inherited by 71% of the resistant fish.

Family F00. Twenty-four loci out of 86 examined were suggestive in sample 1. After analysis of sample 2, 8 loci located on 6 different linkage groups remained significant at a point-wise level, of which two were significant (OMM1080, OMM1053, totally linked). For these loci, 87% of survivors inherited the allele of the R grand-parent.

Joined analysis. Three new putative linkage groups were detected. Association of LG II loci with challenge issue was confirmed (Table 1).

Genomic regions associated with VREFT values in family F00

The effect of the allelic origin on the values of VREFT was tested for 3 markers (Table 2). The two clustered markers (OMM1053 and OMM1080) displayed high association with VREFT-1. Fish that carried the allele from the R grand-parent exhibited lower VREFT values, an evidence for a higher level of resistance. Yet, the effect of the couple of loci was found to be not significant on VREFT-2 values.

Table 2. Test for association with VREFT values in family F00 at the 2 dates of measurement (Values are the probabilities of allelic effect in the Anova model)

Marker	LG[a]	VREFT-1	VREFT-2
OmyFGT18/1 TUF	II	ns	ns
Omy18INRA	VIII	ns	ns
OMM1053	XXXI[b]	<0.0001	ns
OMM1080		<0.0001	ns

[a]: labelled as in Table 1.

[b] : putative location (lod =1.4, loci informative in one family only for the construction of the map). OMM1080 and OMM1053 totally linked.

Discussion - Conclusion

The study supports the existence of several chromosomal regions of trout genome associated with resistance to VHSV (maximum of 13 putative regions). Two linkage groups (LGII and LGXXXI) were highly significant, and common to both families. Associations with markers expected to belong to LG XXXI were found to be particularly tight, although VREFT values gave some inconsistent results, which question the biological and immunological significance of the trait. The finding of DNA markers associated with resistance can help in marker assisted selection, and is a step towards a better knowledge of disease resistance mechanisms in fish, and future development of efficient vaccines or therapeutics.

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SELECTING FOR INCREASED OR DECREASED RESISTANCE TO *SALMONELLA* CARRIER STATE IN FOWLS.

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Introduction

In France as in other countries, *Salmonella* remains a major cause of human disease related to food consumption. In one third of cases, the serotype responsible for human food poisoning is *Salmonella enteritidis* (4). Poultry products are the main source of human *Salmonella* infections, mostly because chickens may be asymptomatic carriers (*i.e.* remain contaminated by *Salmonella* for several weeks without showing any symptom that could help to their detection). Both caecal and ovarian *Salmonella* carrier-states may be involved in human contamination. While the latter may result in vertical transmission of *Salmonella* and in yolk contamination, the former is responsible for a horizontal transmission of the bacteria and for human disease through contamination of the egg shell at the oviposition and of the carcass during evisceration. In both cases, the existence of asymptomatic carriers dramatically complicates the prophylaxis of this disease.

Food safety could potentially benefit from an increase in the genetic resistance of fowls to the *Salmonella* carrier-state (i.e. a better ability of animals to clear *Salmonella*), which can be measured by the persistency of the bacterial infection after inoculation. To address this question, experimental models of infection were defined in chicks (5) and adult hens (11). Using these models, the heritability of resistance was estimated at 0.20 in young birds (3) and more than 0.35 in laying hens (1). These results show that the *Salmonella* carrier-state is partly genetically controlled and strongly suggested that selection for increased resistance to *Salmonella* colonisation and excretion could be efficient and reduce the risk of foodborne *Salmonella* infection. A selection experiment has therefore been realized for four generations to test the feasibility of such a genetic improvement and obtain genetic models that should be very helpful in understanding the mechanisms of resistance. The goal of this work is to present the first results of this experiment, which are both responses to selection and estimated genetic parameters.

Material and methods

The base population consisted of 79 animals sampled from a layer-type line. A divergent selection on resistance was carried out. Moreover, from the second generation on, breeders from adult and young lines were distinguished *i.e.* two resistant and two susceptible lines have been selected. Chicks were inoculated at one week of age and resistance assessed five weeks later by caecal contamination as in (5). According to the rate of clearance, *Salmonella* was either present in almost all animals or in a small proportion of them. In the former case, resistance was assessed by the level of contamination and coded in a quantitative trait by the logarithm of the number of c.f.u. per caeca and, in the latter, as an all-or-none trait (i.e. presence or absence of *Salmonella*). In adult laying hens, contamination of caeca, spleen, liver and ovary was assessed, as described in (11), and a total of five traits were thus considered, i.e. presence/absence of *Salmonella* in each of these four organs as well as presence/ absence of the bacteria in either spleen, liver or caeca. The latter was coded "1" if at least one organ was found positive and "0" in the other cases. Selection for or against chicken resistance was performed on the mean genetic value (EBV) for both traits.

As inoculated animals could no more be kept for reproduction, sib-selection has been performed. A total of 3817 animals were measured, among which 1408 at the adult age and 2409 at the younger age. In the latter, resistance could be measured as an all-or-non trait in 690 animals and in a quantitative trait in 1719 chicks. Genetic parameters were estimated using REML and VCE software (10) in one hand, Gibbs Sampling in the other one.

Results

For the lines selected on chicken resistance, no significant difference in level of contamination could be observed. At the opposite, a significant difference (of about 10% in incidence) was observed when presence or absence of *Salmonella* was considered. Clear and significant differences were observed between the lines selected in a divergent way on resistance to the adult carrier-state: percentages of contaminations differed by about 20% for liver, spleen and caeca.

For all traits, significant heritabilities were observed. All of them were higher than 0.20. The ability of chicks to clear *Salmonella* (*i.e.* presence/absence of *Salmonella*) appeared to be genetically independent of the level of contamination. A major and unexpected result was the estimated negative genetic relationships between adult and chick resistance.

Discussion

Indeed, the large and unpredictable variations in *Salmonella* clearance do not facilitate selection at a younger age, especially as level of contamination and presence/absence of *Salmonella* are uncorrelated. One solution could be to slaughter a representative sample of animals at regular intervals in order to find out the relevant *post inoculation* interval at which animals should

be slaughtered and resistance be assessed as an all-or-none trait with an optimal contamination rate (of about 50%), as suggested by Duchet-Suchaux (personal communication). But such a strategy would complicate the organization very much. It was therefore decided to slaughter animals at a given interval and use all available information. In addition to sib-selection and a technical problem at the third generation, this feature probably explains why no difference in level of contamination could be observed yet in spite of favourable heritability coefficients.

Adult selection seems to be, at least until now, more efficient. Further generations should allow us to confirm this result. More importantly, estimated genetic correlations between presence/absence of *Salmonella* in different organs appeared to be positive and of average value, which is favourable for selection : for example increasing resistance to spleen contamination should also result in a decreased ovary contamination. This result is also in favour of considering the global contamination of animal, since it is more precisely assessed and combines several traits, all of which are positively correlated.

All these results confirm that selection for reduced carrier state is possible. It stresses the importance of a precise definition of the trait, *i.e.* of the *Salmonella* strain, the dose to inoculate, the organ to be tested, the interval *post inoculation* and so on, as the genetic control may be strongly dependant of those conditions. In a longer term, it will be very interesting to compare these lines in different conditions or after contamination with different bacterial strains.
Though promising these results may seem, selection for increased resistance would be very difficult to implement since experimental infections, which are both very expensive and time consuming, are required. Identifying the underlying genes could make it possible to alleviate the need of such measures. Indeed, the effects on resistance to *Salmonella* carrier-state of several genes were already demonstrated or suggested (2, 6, 7, 8, 9). Such an experiment can also be used to test their interest in commercial stocks.

Conclusion

This study confirmed that resistance at both ages and in all organs exhibited a genetic background which may profitably be used as an additional mean of prevention of human food poisoning. It also emphasized the importance of the choice of the selection criteria.

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Genetic resistance to diseases

Posters

SARCOCYSTIS MIESCHERIANA: PHENOTYPIC AND GENETIC CHARACTERIZATION OF A DISEASE RESISTANCE MODEL IN SWINE.

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Introduction

We investigated the usability of *S. miescheriana*, as a model for disease resistance in porcine protozoan infection. With carnivores as definite hosts, *S. miescheriana* causes acute and chronical phases in susceptible pigs, leading to the formation of sarcocysts in skeletal and heart muscle tissues. In naturally infected swine, mild infections do not usually cause clinical signs, but weight gain and meat quality may be reduced over the whole fattening period. When we tested Pietrain and chinese Meishan pigs for differences in susceptibility/resistance against sarcocystosis, clinical signs, loads of merozoites in muscle tissues and specific immune response after oral challenge discriminated well between both breeds (Reiner et al., 2002). Aiming towards the molecular basis of sarcocystis-susceptibility as a model for host-parasite interaction, we have set up a F2-crossbred model from both founder breeds. The present work describes clinical, clinical-chemical and haematological data, with definite clues for genetically determined differences in resistance/susceptibility against this parasitosis.

Material and Methods

A number 139 F2-crossbreds set up from chinese Meishan and european Pietrain breeds as founders, were challenged orally with a dose of 50,000 sporocysts per animal at an age of 12 weeks. The crossbreds were clinical examined on days 7 to 1 ante infectionem (a.i.), and on days 0, 7, 12 to 14, 21, 28, 35, 42, 45, 49, 56, 63 and 70 post infectionem (p.i.). Blood samples were collected on days 0, 14, 28 and 42 p.i., to be screened for a broad range of relevant haematological and clinical-chemical parameters. Each animal served as its own control and was further compared to a set of F2-control animals and to purebred Meishan and Pietrain pigs, each challenged and controlled.

Variance of traits was analysed with the Statistical package for Social Sciences (SPSS). Heritabilities for clinical, haematological and clinical-chemical values were evaluated with the CVE-version 4.2.5 (Groeneveld).

Results

Clinical effects of *S. miescheriana* infection can be best clarified by the deviations of body temperatures after challenge from baseline a.i.. During second schizogony, a significant fever peak formed, with no differences between founder breeds and a smaller deviation in F2 pigs. A second fever peak became visible during 6th week p.i. in Pietrain and a part of the F2, but not in Meishan pigs. Numbers of merozoites, which had developed during the chronical state of infection until day 70, varied significantly between founder breeds and crossbreds, with Pietrain pigs showing 20 fold higher loads per g of muscle tissue than Meishan pigs (fig. 1). Merozoite loads of the F2 crossbreds were found to be at the average level between their founder breeds. Variance within F2 pigs was mainly explainable by additive genetic effects, leading to heritabilities in the range of 0.75.

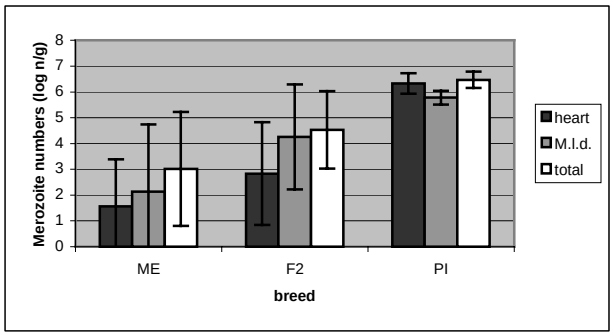
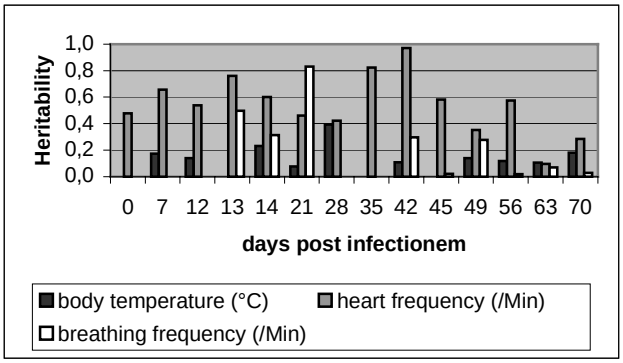


Figure 1: Numbers of merozoites per g of heart muscle or musculus longissimus dorsi (M.l.d.) and total numbers of both of Meishan (ME), F2-crossbreds (F2) and Pietrain (PI) pigs (x±s).

Some clinical and clinical-chemical parameters showed moderate to high heritabilities too, mainly during acute (days 12-14) and chronical sarcocystosis (days 42-45).

Figure 2: Heritabilities for clinical traits, depending on days post infectionem, as estimated from F2-crossbreds.



Discussion

Clinical, clinical-chemical and haemato-logical data produced a complex picture of sarcocystosis in swine, with definite clues for genetically determined differences in resistance/susceptibility against this parasite.

Conclusion

The data highlight the suitability of this model to further analyse chromosomal regions, candidate genes and thus the molecular basis of host-parasite interaction in sarcocystosis.

Acknowledgements

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Air quality in animal houses

Oral Communications

INTRODUCTION - EFFECTS OF AIRBORNE POLLUTANTS AND FACTORS AFFECTING CONCENTRATIONS IN LIVESTOCK BUILDINGS

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Introduction

The main airborne pollutants found in piggery buildings are ammonia, carbon dioxide, dust particles, viable and non-viable microorganisms, and their components. Airborne particles in piggery buildings consist of animal skin, hair, dried urine, faeces, bedding material, microorganisms, grain and other particles. Airborne particles usually act as a vector for pathogenic bacteria, viruses, endotoxins, odorous material, gases (including ammonia) and liquid substances. Viable bacteria and viruses carried by dust particles may have a greater ability to survive and cause infection in animals and transfer infection into neighbouring livestock buildings. Airborne microorganisms attached to airborne particles are often described as 'viable' airborne particles (Hartung and Seedorf 1999). The finer fraction of the biologically active airborne material referred to as 'bioaerosol' and this fraction could remain in suspension in the air for long periods (Hartung and Seedorf 1999). Endotoxins are the cell-wall components of gram-negative bacteria and these compounds are released after the death of the bacteria.

One of the important gaseous pollutants present in livestock buildings is ammonia (Hobbs *et al.* 1999). Most ammonia originates from urine and faecal material produced by the animals and as a result of chemical/biological breakdown of the waste material (Groot Koerkamp *et al.* 1998). Pigs consume a substantial amount of protein and other nitrogen containing material as part of their diet. However, a large percentage of the nitrogen consumed is excreted and ammonia is produced as a result of bacterial breakdown of the dietary nitrogen (Groot Koerkamp *et al.* 1998). Carbon dioxide (CO_2) in piggery buildings is mainly produced by the animals as a result of normal respiration and a smaller amount is produced as a by-product of bacterial breakdown of waste material (Ni *et al.* 1999b).

Previous publications revealed that significant amounts of airborne pollutants can be found in the airspace of piggery buildings (Seedorf *et al.* 1998; Takai *et al.* 1998; Chang *et al.* 2001) (Table 1). Piggery managers, scientists and building engineers are concerned with sub-optimal air quality, as high airborne pollutant concentrations could potentially affect the environment through emission and human and/or animal health via biological effects.

Table 1. Concentrations of different pollutants measured in previous studies.

Pollutant	Range of concentration	Reference
Ammonia	5-18 ppm	(Groot Koerkamp et al. 1998)
Inhalable particles	0.63-5.05 mg/m^3	(Takai et al. 1998)
Respirable particles	0.09-0.46 mg/m^3	(Takai et al. 1998)
Respirable endotoxins	74-189 EU/m^3	(Seedorf et al. 1998)
Total airborne bacteria	Approx. 10^5 cfu/m^3	(Seedorf et al. 1998)

Emission issues

Generally, it is accepted that airborne pollutant emissions have to be reduced from livestock buildings in order to minimise potential damage to the environment (Seedorf *et al.* 1998; Takai *et al.* 1998) and the transmission of pathogenic microorganism between buildings and farms (Hartung *et al.* 1997). It is also well documented that particles, endotoxins and airborne microorganisms emitted from livestock buildings could get into waterways via run-offs and leakage after rain (Barber 2002). In many developed countries, nutrient enrichment and bacterial contamination of waterways is a major concern for the livestock industries, eg. UK (Hooda *et al.* 2000). Particles in livestock ventilation air have been implicated in transporting odour (Hartung *et al.* 1986; Hoff *et al.* 1997; Bottcher 2001). Different odourants are absorbed on particle surface and then desorbed in large local concentrations, potentially creating secondary emission sources around livestock buildings (Takai *et al.* 2002). Additional odorous components might also be produced by bacterial activity within the particles as a result of the different microorganisms breaking down the organic content of the dust particles (Martin *et al.* 1996).

In general, livestock production is heavily implicated in significantly contributing to atmospheric ammonia emissions (Arogo *et al.* 2003). In pig production the major ammonia emission sources are the buildings, effluent lagoons and manure application areas (Aneja *et al.* 2000; Nicholson *et al.* 2002). Some relationship between ammonia and odour emission is also assumed but not proven consistently (Ognink and Groot Koerkamp 2001). Further research on the relationship between ammonia and odour emission is necessary in order to understand the importance of emitted ammonia in odour formation and also to potentially use ammonia measurements as an indication of odour levels. Ammonia emitted from livestock buildings is also implicated in creating opportunities for the formation of very small (PM 2.5) particles, which in turn will have public health consequences (Arogo *et al.* 2003). Excess ammonia deposited in sensitive ecosystems and leached into waterways can cause harmful algal blooms, decrease

water quality, change soil pH and directly affect the constitution of native flora (Arogo *et al.* 2003).

Carbon dioxide (together with methane and nitrous oxide) emitted from livestock buildings is considered to be part of 'Greenhouse gas' emission load of the piggery operation (Sommer and Moller 2000). Greenhouse gas emissions from agriculture operations are increasingly scrutinised by environmental authorities.

Human and animal health effects

The quality of working environment within piggery buildings is heavily influenced by the concentration of different pollutants in the air. Epidemiological studies related to the health of farm employees have revealed that workers in piggery buildings might be exposed to pollutant concentrations which could contribute to the development of occupational respiratory diseases (Crook *et al.* 1991; Dutkiewicz 1997). Pig farmers have a high prevalence of wheezing, symptoms of chronic bronchitis and decline in lung function which could be related to exposure to airborne pollutants in livestock buildings (Schwartz *et al.* 1995; Mackiewicz 1998; Laitinen *et al.* 2001).

Studies have demonstrated the effects of sub-optimal air quality on production efficiency (Donham and Leininger 1984; Donham 1991; Urbain *et al.* 1994; Donham 2000). There is evidence that airborne microorganisms, their products and/or components are capable of triggering immune responses and physiological changes in livestock, such as the activation of the immune system (Cargill *et al.* 2002). In turn, this could result in a reduction in feed intake, as well as a diversion of protein and energy away from the development of muscle tissues (Kelley *et al.* 1987; Klasing *et al.* 1987; Klasing and Barnes 1988). Other studies demonstrated the synergic effect of ammonia and other airborne pollutants (such as endotoxins and dust) on animal health and production efficiency (Gustin *et al.* 1994; Urbain *et al.* 1996; Wathes *et al.* 2002a; Demmers *et al.* 2003). Researchers in the UK also demonstrated the behaviour tendency of pigs preferring to avoid high ammonia concentrations, if given a choice (Smith *et al.* 1996; Jones *et al.* 1998; Jones *et al.* 1999; Wathes *et al.* 2002b). Studies conducted in Australia demonstrated that pigs reared in clean environment with better air quality grew faster than pigs living under "normal" commercial conditions (Banhazi and Cargill 1998; Cargill *et al.* 1998; Black *et al.* 2001).

Carbon dioxide levels regularly encountered in piggery buildings do not pose any treat to human or animal health (Banhazi and Cargill 2000). However, CO_2 is of great interest to livestock managers as CO_2 is widely used to estimate ventilation rates of livestock buildings. High CO_2 levels are an indication of reduced ventilation rates in buildings (van't Klooster and Heitleger 1994).

Factors affecting airborne pollutant concentrations

Understanding the factors affecting airborne pollutant concentrations is important, as this knowledge is the first important step in reducing and controlling the internal concentrations of these pollutants. In turn, controlling internal concentrations will enable livestock managers to control emissions. Understanding the factors influencing the internal concentrations of carbon dioxide is also important as such knowledge will enable livestock managers to control ventilation rates of piggery buildings.

Many studies, examining the influence of environmental factors on particle concentration in piggery buildings have demonstrated a circadian dependency. Airborne particle (including viable particle) concentrations are typically higher during the day than night (Pedersen 1993; Seedorf *et al.* 1998; Pedersen *et al.* 2001). The difference in particle concentrations with time of day is related to the higher level of animal activity during daytime (Cargill et al. 1997; Pedersen 1993). Viable particle concentrations within piggery buildings are reportedly influenced by the stocking density and stocking rate of pigs (Cargill and Banhazi 1996). Air humidity strongly influences the condition of the air and therefore the density and the size of the suspended particle concentrations inside piggery buildings. Several studies in piggery buildings have demonstrated a relationship between respirable particle concentration and relative humidity (Butera *et al.* 1991; Ellen *et al.* 2000). Ventilation also has a complex effect on the concentration of air pollutants. The ventilation system is designed to facilitate the elimination and transportation of particles outside the building via exhausted air (Duchaine *et al.* 2000; Wang *et al.* 2000). However, the turbulence associated with increased ventilation favours the re-suspension of settled particles. The ability for ventilation systems to resuspend particles is influenced greatly by the hygienic conditions encountered within livestock units (Gustafsson 1999). The bedding is a major source of particles in livestock buildings and its characteristics would affect particle concentrations (Ellen *et al.* 2000; Barnett *et al.* 2001).

A number of studies concerning the influence of environmental and management factors on ammonia concentrations in piggery buildings have demonstrated an effect of pen hygiene. Ammonia concentrations are typically increasing with increased level of pen floor contamination (Aarnink *et al.* 1996; Aarnink *et al.* 1997; Ni *et al.* 1999a). Ammonia concentrations within piggery buildings are reportedly influenced by the characteristics and management of the effluent system as well as the pH of the slurry (Hörnig *et al.* 1999; Jensen 2002). Turbulence associated with increased ventilation favours the volatilisation of ammonia from exposed sources, such as manure pits and contaminated pen floors (Ni *et al.* 1999a). However, improved pen cleanliness will limit the opportunities for volatilisation and will therefore greatly improve the effectiveness of the ventilation system. It was reported that it is more likely to find high gas concentrations (both CO_2 and ammonia) in buildings housing younger animals as those buildings usually have reduced ventilation levels to save heating cost (Donham and Popendorf 1985). Investigations undertaken in Sweden revealed that ammonia concentrations in pig and poultry houses are influenced by location of air inlets and outlets, stocking rate of the animals, air flow rate and time intervals between manure removals (Gustafsson

1997). While the effects of air in/outlet locations will have little implications for typically naturally ventilated Australian piggery buildings, the other factors identified will likely to have consequences for ammonia levels even under the hot/dry Southern Australian climatic conditions.

Discussion

Based on the results of previous studies, it is evident that improving air quality in livestock buildings could produce significant benefits, including reduced environmental damage, improved production efficiency and better working environment for farm employees. A number of management, environmental and housing factors have been demonstrated in separate studies to interact and influence the concentrations of airborne pollutants within and emission from piggery buildings. However, these factors have not been evaluated considering all factors simultaneously. In addition, most of the studies conducted in relation to airborne pollutants in livestock buildings were mainly concerned with the concentrations and/or emissions measured (Wathes *et al.* 1998). Very few studies have attempted to model and therefore explain the variation observed in concentrations. Therefore, a comprehensive study was needed to investigate the interaction between different air quality parameters and housing/management features in order to determine the key factors affecting the internal concentrations of airborne pollutants in piggery buildings, to predict and ultimately reduce the concentrations and emissions of these airborne pollutants. A number of articles published as part of this series report on the outcomes of such an investigation (Banhazi *et al.* 2004c; Banhazi *et al.* 2004a; Banhazi *et al.* 2004b).

Conclusion

1. Improving air quality could reduce environmental damage, improve production efficiency and workers health.
2. A number of factors have been identified in previous studies to affect the concentration of airborne pollutants.
3. Few studies have attempted to model the variation observed in pollutant concentration.

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FACTORS AFFECTING THE CONCENTRATIONS OF AIRBORNE PARTICLES IN AUSTRALIAN PIGGERY BUILDINGS

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Introduction

Airborne particles in piggery buildings consist of animal skin, hair, dried urine, faeces, bedding material, microorganisms, grain and other particles (Wathes 1994). High airborne particle concentrations could potentially effect production efficiency, human and/or animal health and the environment (Takai *et al.* 1998). A number of previous studies have demonstrated that different management, environmental and housing factors influence the concentrations of airborne particles within piggery buildings (Gustafsson 1999). However, these factors have not been evaluated simultaneously using a statistical modelling approach. Therefore, a comprehensive study of air quality in piggery buildings was designed and used to determine the key piggery design and management factors that affect the internal concentrations of airborne particles in piggery buildings.

Material and Methods

The detailed methodology of the study was described by another paper in this series, so only a brief outline is given here (Banhazi *et al*. 2004). The concentration of respirable and inhalable particles was determined gravimetrically using standard cyclone dust sampler and "seven-hole" sampler (SKC Inc., Pennsylvania), respectively. The dependent variables of interest were inhalable and respirable particle concentrations and the log-transformed data was analysed using a general linear model procedure (SAS 1989). The results from this analysis presented are based on Least Squares Means of fixed effects and best-fit slopes of covariates, where relevant.

Results

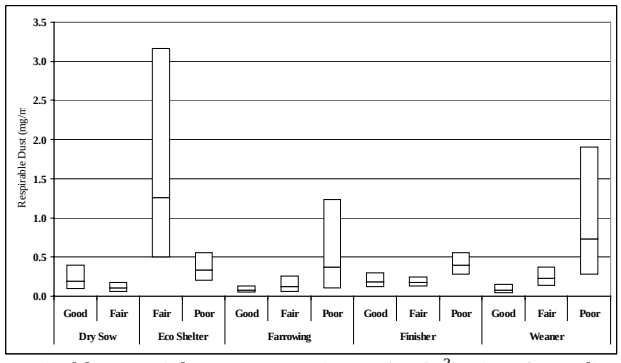
The main factors identified to affect the concentrations of inhalable and respirable particles are summarised in Table 1. Selected results from the GLM analysis are shown in Figure 1-2. Generally temperature and sow numbers (indication of farm size) displayed a positive, while increased airflow a negative relationship with airborne particles (Figure 2.). The effect of humidity varied in different buildings and the effects of temperature and airflow interacted with management (Figure 1 and 2.).

Table 1. Significant effects associated with inhalable and respirable particles (P<0.01).*

Inhalable particles (R^2 = 0.726)	Respirable particles (R^2 = 0.689)
Seasons	Building type x hygiene
Number of sows	Number of sows x seasons
Building type x temperature	Humidity x building type
Airflow^2 x management	Air flow x management
Building size x management	Temperature x management
	Humidity x management

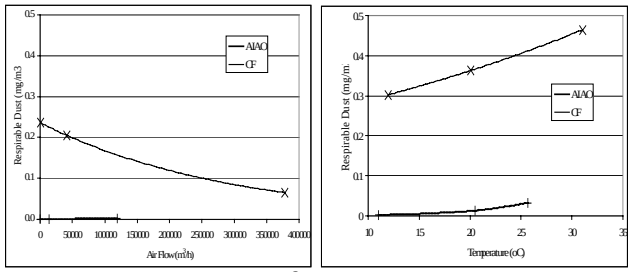
*(*Effects involved in interactions were retained as main effects.)*

Figure 1: Effects of floor hygiene/building type interactions on



respirable particle concentrations (mg/m^3) in Australian piggery buildings (LS means with 95% confidence intervals).

Figure 2: Effects of temperature and airflow on respirable



particle concentrations (mg/m^3) in Australian piggery buildings (estimated slopes).

Higher inhalable particle concentrations were measured in winter (2.95 mg/m^3) in piggery buildings compared to summer (1.68 mg/m^3) and the effect of sub-optimal floor hygiene was significant and varied in different buildings (Figure 1-2.). The models developed explained approximately 70% of variation in both traits.

Discussion

A number of important factors affecting both the respirable and inhalable particle concentrations inside pig buildings were identified in this study (Table 1.). The increased humidity in the air had a reduction effect on respirable particle concentrations in deep-bedded shelters (DBS). Increased humidity would increase coagulation of particles generated from the bedding and their weight would also increase as they absorb water resulting in increased settling rate (Ellen 1999). Building type and cleanliness interacted (Figure 1) and interestingly in DBS the effect of pen soiling appeared to be beneficial. It was hypothesized, that soiling would increase the "adhesiveness" of bedding material, creating a reduction effect by trapping smaller particles and might also increase humidity inside the buildings. In farrowing, weaner and grower/finisher buildings pen soiling had a negative affect on respirable particle concentrations. It is also interesting to note that pen hygiene was not identified as a significant effect for inhalable particles. It is widely accepted that larger (inhalable) particles are mainly generated from the feed, therefore their

concentrations would not be affected greatly by the hygienic condition of pens (Cargill *et al.* 2002).

In general, winter ventilation rates of piggery buildings are lower than summer rates to maintain shed temperature. Therefore, for inhalable particles, an increase has been demonstrated in winter compared to summer (Table 1). The effect of season on respirable particles was more complex as it interacted with sow numbers (farm size) (Table 1).

The size of farm (as described by the number of all sows on the farms) had a significant effect on both inhalable and respirable particle concentrations (Table 1). Inhalable particle concentrations were strongly and positively associated with sow numbers. However, the effect of sow number on respirable dust was more complex. It has been hypothesised that on larger farms, due to work pressures, less time is available for cleaning and general maintenance of the environment of the pigs. The reduced hygiene and/or increased intervals between cleaning episodes creates an ideal environment for higher dust concentrations in buildings on larger farms (Cargill and Banhazi 1998).

Generally, temperature had a positive correlation with both inhalable and respirable particles (Figure 2). As temperature increases, piggery buildings tend to become a drier environment, creating greater opportunities for particle generation (Takai *et al.* 1998). Because of increased temperature, respirable particle concentrations increased dramatically in CF buildings, but also slightly in AIAO buildings. Inhalable particle concentrations were also significantly effected by temperatures, but the relationship was more complex due to interaction with buildings type (Table 1).

Based on the results of the study, improving pen hygiene, reducing excess temperature and improving ventilation should be considered as the main recommendations for Australian piggery buildings. Treatment of bedding materials in deep-bedded shelters is also advisable to reduce the opportunities for particle generation (Banhazi *et al.* 2002). Larger farms might also need to pay extra attention to air quality issues.

Conclusion

1. Deep-bedded shelters showed high inhalable and respirable particle concentrations.
2. Respirable particle concentrations were higher in pig buildings with poor pen hygiene.
3. Inhalable and respirable particle concentrations increased with increasing temperatures.
4. Particle concentrations decreased with increasing ventilation rates and in summer, increased as the size of the farms increased.

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FACTORS AFFECTING THE CONCENTRATIONS OF GASES IN AUSTRALIAN PIGGERY BUILDINGS

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Introduction

One of the important airborne pollutants present in livestock buildings is ammonia, and livestock production has been implicated in significantly contributing to atmospheric ammonia emissions (Arogo *et al.* 2003). High ammonia concentrations and emissions could potentially affect production efficiency, the environment and human and/or animal health. Carbon dioxide (CO_2) in piggery buildings is mainly produced by the animals and CO_2 is of great interest to livestock managers, as it is widely used to estimate ventilation rates of livestock buildings. A comprehensive study of air quality in piggery buildings was used to determine the key piggery design and management factors that affect the internal concentrations of these gases in piggery buildings.

Material and Methods

Numerous piggery buildings (160) were studied and information relating to the engineering and management factors influencing pollutant concentrations in the study buildings was collected. The concentration of ammonia and CO_2 was measured using the Multi-Gas Monitoring (MGM) machine. The collected data was log-transformed and analysed using a general linear model procedure (SAS 1989). The detailed methodology of the study was described by another paper in this series (Banhazi *et al.* 2004).

Results

The significant factors identified from the analyses are summarised in Table 1 and selected results from the GLM analysis are shown in Figures 1-2.

Table 1. Significant effects associated with ammonia and carbon dioxide concentrations (P<0.01).*

Ammonia (R^2 = 0.214)	**Carbon dioxide (R^2 = 0.507)**
Hygiene	Building type
Management x season	Season
Building size X season	Height of ventilation opening
	Ventilation control of ridge opening
	Ceiling height of shed x Ventilation control of wall openings
	Ridge vent height x Ventilation control of wall openings

*(*Effects involved in interactions were retained as main effects.)*

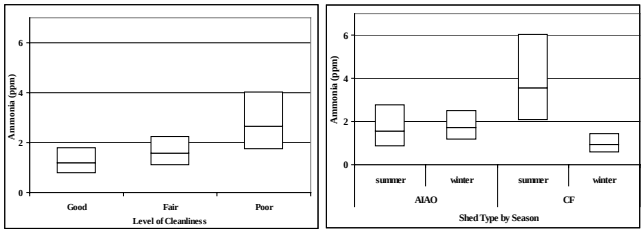

Figure 1: Effects of floor hygiene and pig flow management/seasons interaction on ammonia concentrations (ppm) in Australian piggery buildings (LS means with 95% confidence intervals).

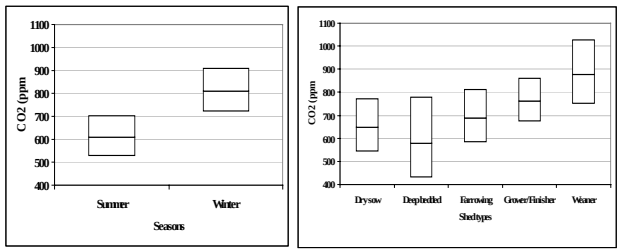

Figure 2: Effects of seasons and shed types on carbon dioxide concentrations (ppm) in Australian piggery buildings (LS means with 95% confidence interval).

The model developed for ammonia concentrations accounted for 21% of variation in the data. Ammonia concentrations were shown to increase as hygiene level decreased. Ammonia concentrations were higher in summer in continuous flow buildings (Figure 1). In terms of factors affecting carbon dioxide concentrations, seven main factors and two interactions were identified as being highly significant (Table 1).

Discussion

The important factors affecting both ammonia and carbon dioxide concentrations inside pig buildings were identified during the analysis (Table 1.). The effect of pig management by season interaction on ammonia (Figure 1) confirms the results of previous studies demonstrating the positive effects of all-in/all-out (AIAO) management on surface hygiene (Cargill *et al.* 1998). In AIAO buildings the effect of season is minimal, as the level of hygiene is probably also unchanged throughout the seasons and in winter no significant elevation in ammonia concentrations was observed in continuous flow (CF) buildings either. However, in summer time an increased ammonia concentration was detected in CF buildings. It is assumed that pen surface hygiene might be poorer in CF buildings compared to AIAO buildings and during summer more ammonia evaporates from the contaminated pen floors (Figure 1). Pigs also tend to soil pens more readily at higher temperatures (Aarnink *et al.* 2001). Therefore, summer appears to be a high-risk period for elevated ammonia concentrations in CF buildings. The significant effect of pen floor (surface) hygiene on ammonia concentrations (Figure 1) was an important finding of the study and confirms the results of previous studies (Aarnink *et al.* 1997; Ni *et al.* 1999).

The type of building had an effect on measured carbon dioxide concentrations (Figure 2). Deep-bedded shelters (DBS) recorded the lowest and weaner buildings the highest CO_2 concentrations. These results agree with expectation and relate to the general ventilation levels maintained in these types of buildings. Under Australian climatic conditions, weaner facilities are the most environmentally controlled buildings, while DBS are

essentially open structures with close to maximum ventilation all year around. Dry sow facilities are loosely regulated environments in terms of thermal control, as it is generally believed that these large animals are able to deal with extreme temperatures (Lorschy *et al.* 1993). Therefore, ventilation levels in dry sow buildings tend to be generous and these buildings are ventilated as much as possible. Surprisingly, farrowing buildings had lower CO_2 levels measured than grower/finisher buildings, indicating that these buildings are less rigorously controlled than grower/finisher facilities. However, in Australia, the majority of pig producers tend to rely on localised heating and microclimate provision, rather than whole building thermal control to provide ideal environment for the newly born piglets (Houszka 2002).

As expected, CO_2 concentrations were higher in winter compared to summer (Figure 2). The increased ventilation rates used in summer in piggery buildings would result in reduced CO_2 concentrations in all buildings (Banhazi *et al.* 2001).

A negative relationship between the size of ventilation inlet (width or the distance between the lower and the higher end of air inlets) and carbon dioxide concentrations has been demonstrated. It is easy to see that as the air inlet size is increased the ventilation rate is also increasing and therefore the carbon dioxide concentration would decrease. Naturally ventilated buildings with larger air inlet are more intensely ventilated resulting in low carbon dioxide concentration rates. However, the overall quality of ventilation is influenced by other factors, such as air flow patterns, airflow control as well as by the quantity of air moved through the buildings.

Based on the results of the study, improving pen hygiene could be considered as the most important recommendation for ammonia reduction. The current practice of managing buildings using all-in/all-out strategy with thorough cleaning of the facilities between batches of pigs is advisable (Cargill *et al.* 1997). In terms of ventilation, Australian buildings are generally very well ventilated, as high ventilation rates are needed to control the thermal environment. However, the air quality improvement capacity of increased ventilation might be limited.

Conclusion

1. The ammonia concentrations were higher in pig buildings with poor surface hygiene.
2. Ammonia concentrations were the highest in summer in continuous flow piggery building.
3. Weaner sheds had the highest while deep-bedded buildings had the lowest carbon dioxide concentrations, indicating the level of ventilation.
4. Winter carbon dioxide concentrations were higher in all sheds compared to summer.
5. The size of air inlets negatively correlated with carbon dioxide concentrations.

Acknowledgements

This study funded by the Australian Pork Limited was part of a large collaborative project between the South Australian Research and Development Institute, Agriculture Western Australia, Agriculture Victoria and the Queensland based Swine Management Services. We wish to particularly acknowledge the contribution of pig producers involved in the study and Mr M. Militch of Cameron Instrumentation who assisted with the project instrumentation. We also would like to sincerely thank Dr C. Cargill, Dr B. W. Hall, Dr J. Black, Dr P. Glatz, Prof. C. Wathes and Prof. J. Hartung for their professional advice, and Dr S. Dreisen, Dr G. Marr and Mr H. Payne for their efforts of coordinating the data collection in different states. The important contributions of all technical officers (Mr R. Nichol, Ms S. Koch, Mr P. Daniels, Mr J. Weigel, Mr S. Szarvas and Ms A. Kefford) involved in the study are also gratefully acknowledged.

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FACTORS AFFECTING THE CONCENTRATIONS OF AIRBORNE BACTERIA AND ENDOTOXINS IN AUSTRALIAN PIGGERY BUILDINGS

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Introduction

The airspace of intensive piggery buildings is filled with a mixture of airborne bacteria, bacterial products and other pollutants such as particles and gases (Wathes *et al.* 1983). Different microorganisms are readily generated within animal buildings and after the death of gram-negative bacteria endotoxins are released. Previous publications revealed that significant amounts of airborne bacteria and endotoxin can be found in the airspace of piggery buildings (Seedorf *et al.* 1998). Piggery managers, scientists and building engineers are concerned with sub-optimal air quality, as high airborne bacteria and endotoxin concentrations could potentially affect the external environment, production efficiency, human and animal health/welfare. Different management, environmental and housing factors have been demonstrated in separate studies to influence the concentrations of different pollutants within piggery buildings (Attwood *et al.* 1987). However, these factors have not been evaluated simultaneously and very few studies have attempted to explain the variation observed in concentrations. Therefore, a comprehensive study of air quality in piggery buildings was designed to determine the key piggery design and management factors that affect the internal concentrations of respirable endotoxin and airborne bacteria in piggery buildings.

Material and Methods

The detailed methodology of the study was described by other papers in this series (Banhazi *et al.* 2004). The filters used to measure the concentration of respirable particles were analysed to establish the endotoxin concentrations in the dust samples using a commercially available Limulus Amoebocyte Lysate (LAL) test. Sampling of airborne bacteria was carried out using a standard Anderson sampler. The dependent variables (airborne bacteria and endotoxin concentrations) were log-transformed and analysed using a general linear model procedure (PROC GLM) (SAS 1989). The results from this analysis presented are based on Least Squares Means of fixed effects and best-fit slopes of covariates, where relevant.

Results

The results are shown in Figure 1 and 2.

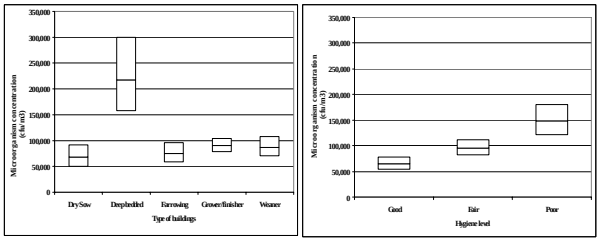

Figure 1: Effects of building type and floor hygiene on airborne bacteria concentrations (cfu/m^3) in Australian piggery buildings (LS means with 95% confidence intervals).

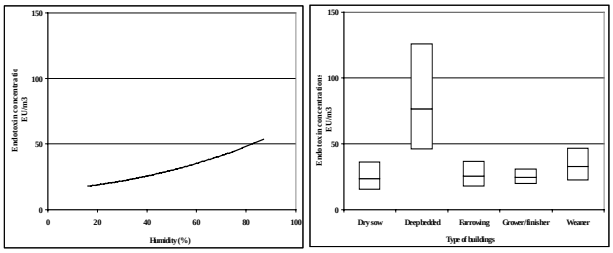

Figure 2: Effects of humidity and building type on endotoxin concentrations (EU/m3) in Australian piggery buildings (LS means with 95% confidence interval and estimated slope).

For airborne bacteria concentrations, the two main effects identified were building type and the level of hygiene (Figure 1.). For endotoxin concentrations, humidity levels and the type of buildings were shown to be significant (Figure 2.). The highest total airborne bacteria (2.17 x 10^5 cfu/m^3) and respirable endotoxin (76.26 EU/m^3) concentrations were detected in deep-bedded shelters (DBS).

Discussion

Several key factors affecting the both viable airborne bacteria and respirable endotoxin concentrations inside pig buildings were identified (Figure 1-2.). The type of building had a highly significant effect on both total bacteria and respirable endotoxin concentrations. DBS recorded the highest airborne bacteria and endotoxin concentrations (Figure 1-2.). The presence of bedding materials were clearly the main reasons for the high airborne bacteria and endotoxin concentrations measured in these buildings, as the effects of different bedding materials have been demonstrated in other species too (Banhazi *et al.* 2002c). Particles, containing endotoxins and airborne bacteria are readily generated in DBS under the dry Australian climate. Further studies on the relationship between the quality and management of bedding and endotoxin/airborne bacteria concentrations would be useful. Examining potential treatment and improved management of bedding materials could help to develop methods to reduce viable particle generation and endotoxin production in DBS. Improvement in air quality in DBS could potentially lead to additional production efficiency improvements in pigs housed in shelters, as well as reduced environmental impact of the operation and reduced health risks for both humans and animals.

The effect of pen floor hygiene (essentially pen cleanliness) on bacteria concentrations was a significant finding of the study. In previous studies (Aarnink *et al.* 1996), the concentrations of ammonia in piggery buildings were strongly associated with the extent of soiling of solid surfaces in pigpens. The current study however, demonstrated a direct link between hygiene level of pen floors and bacteria concentrations of air in livestock buildings. This association between pen hygiene and air quality arises as viable airborne particles and endotoxins are

readily generated from dried faeces on pen floors. Faeces dried on the skins of the animals can also become a major dust/bacteria source. It is evident from the results that sub-optimal hygiene in traditional buildings is one of the main causes of high bacteria concentrations and therefore the improvement of pen hygiene can significantly contribute to healthier environmental conditions externally and in piggery buildings.

This study found that humidity affected endotoxin concentrations (Figure 2). Increased humidity in the air prolongs the survival time of different bacteria (Zucker *et al.* 2000). Generally, the natural half-life of airborne gram-negative bacteria ranges between a few minutes and perhaps an hour. After this time airborne bacteria die naturally and release increased amounts of endotoxins into the air. This finding has implications for dust reduction methods, such as spraying of oil/water mixtures. Spraying the floors of pig pens with a mixture of oil and water has been demonstrated by a number of authors to be an effective way of reducing dust (Takai *et al.* 1995; Banhazi *et al.* 2002b). However, this technique might generate higher endotoxin concentrations, due to the effects of humidity on endotoxins (Figure 2). This result might also explain the lack of positive effects of particle reduction on production efficiency from experiments using oil-spraying (Banhazi *et al.* 2002b). Additional experiments under controlled conditions would be required to understand these effects and possible interactions.

Based on the results of the study, improving pen hygiene is considered to be the most practical recommendation. This study demonstrated that dried faecal material deposited on pen floors is an important source of particles and airborne bacteria. Therefore, this source of airborne pollution should be reduced as much as possible by controlling dunging patterns and improving the hygienic conditions of pens (Banhazi *et al.* 2002a).

Treatment of bedding materials in DBS is also advisable to reduce the opportunities for particle generation (Banhazi *et al.* 2002c). However, care must be taken to ensure that during bedding material treatment the humidity levels are not increased as it might have an adverse affect on endotoxin concentrations. Therefore, further experiments are required to study humidity levels associated with treatments such as oil-spraying and determine the net benefit of such techniques.

The general control of humidity levels in piggery buildings would also be desirable. However, apart from reducing evaporative sources, such as leaking water lines and controlling dunging behaviour of pigs, there is little opportunity to practically control humidity levels in piggery buildings.

Conclusion

1. DBS showed the highest airborne bacteria and respirable endotoxin concentrations amongst all buildings types, 2.17 x 10^5 cfu/m3 and 76.26 EU/m3, respectively.
2. Bacteria concentrations were higher in pig buildings with poor pen hygiene.
3. Endotoxin concentrations increased with humidity levels.

Acknowledgements

This study funded by the Australian Pork Limited was part of a large collaborative project between the South Australian Research and Development Institute, Agriculture Western Australia, Agriculture Victoria and the Queensland based Swine Management Services. We wish to particularly acknowledge the contribution of pig producers involved in the study and Mr M. Militch of Cameron Instrumentation who assisted with the project instrumentation. We also would like to sincerely thank Dr C. Cargill, Dr B. W. Hall, Dr J. Black, Dr P. Glatz, Prof. C. Wathes and Prof. J. Hartung for their professional advice, and Dr S. Dreisen, Dr G. Marr and Mr H. Payne for their efforts of coordinating the data collection in different states. The important contributions of all technical officers (Mr R. Nichol, Ms S. Koch, Mr P. Daniels, Mr J. Weigel, Mr S. Szarvas and Ms A. Kefford) involved in the study are also gratefully acknowledged.

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COMPARING NATURAL TRACER GAS METHODS WITH A SIMPLIFIED CONSTANT RELEASE TRACER GAS METHOD INSIDE A NATURALLY VENTILATED BROILER LIVESTOCK HOUSE

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Introduction

The need to accurately quantify livestock house emissions is increasingly becoming important as the effects of bioaerosols on local environments are recognised and pressure on industry and governments to reduce greenhouse gas emissions increases. The most important aerial pollutants originating from livestock houses are odours, gases, dust, micro-organisms and endotoxins, collectively known as bioaerosols, which are emitted via building exhaust systems into the environment. To quantify bioaerosol emissions, it is necessary to calculate the ventilation rate. The complexity of this task depending on the type of ventilation system installed.

Livestock housing ventilation systems are generally of 2 types, the more common natural ventilation (Louisiana) and the internally regulated mechanical ventilation systems. Continuously quantifying ventilation rates from mechanically ventilated livestock houses with fixed inlets and outlets, is relatively simple, however, to achieve the same level of accuracy in Louisiana type livestock houses with continuously interchanging air exchange surfaces (inlets and outlets) totally dependant on the weather and thermal conditions requires state of the art technology and is much more complex. This paper compares the application of the less complicated and cheaper CO_2, heat mass balance methods (natural tracer gas methods) with a simplified and sophisticated tracer gas constant injection method (artificial tracer gas) in a Louisiana type broiler house.

Because of the high costs of tracer gas, limitations of technical equipment and difficulties in fulfilling the requirements of the tracer gas method in large Louisiana type livestock houses, being (1) constant inter-exchange surfaces i.e dosing at inlets and sampling at outlets and (2) good mixing of air in selected envelope, a simplified method was devised. This involved the construction of a section within the livestock stall and measuring exchange rates under constant transverse wind conditions, hence reasonably constant inter-exchange surfaces can be achieved and with sufficient wind speeds good mixing of the section air with the tracer gas. The aim is that the exchange rate conditions of the section are representative of the whole livestock stall, during the measurement period, hence the ventilation rate calculated for the section is representative for the whole house.

Material and Methods

The Louisiana livestock house is situated in northern Germany. The livestock house runs from north to south and is relatively large 120m length by 16m width and 6.2m height. The livestock house produces around 40 000 broilers per grow out cycle and its internal climate is regulated by side wall curtains and chimney baffles automated by an internal computer based on temperature and humidity parameters. Ventilation rate measurements with tracer gas were conducted over a period of 2 weeks in the winter.

Three methods were used for calculating the ventilation rates. The carbon dioxide mass balance and temperature/humidity mass balance models (CIGR. 1984), with ventilation rates calculated according to 24hr averages. These natural tracer gas methods were compared with the constant injection tracer gas method (VDI. 2001). A section was created within the livestock house (Fig.1) and was partially separated from the livestock house with curtains (internal volume ≈ $1/10^{th}$ total livestock house volume), here the temperature and humidity sensors, gas sampling and dosing equipment were installed. The curtains only very basic covered ≈ 47% of the livestock house width profile, were quickly installed and did not hamper farm management operations.

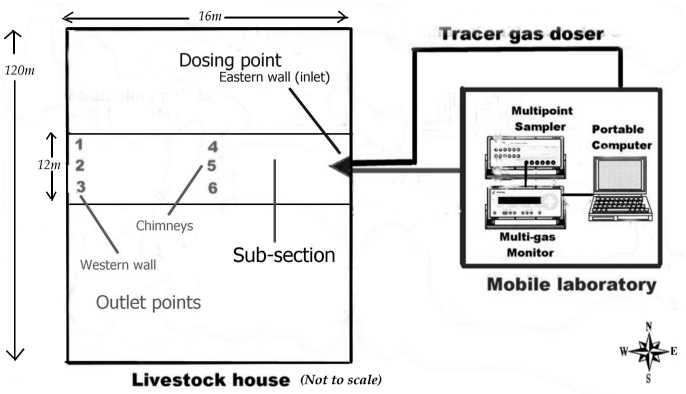

Figure 1: Livestock house experimental section set up schematic

The tracer gas was injected from the prevailing wind side at a height of ≈1,7m (dosing rate ≈ 3-15 ml/sec) and air samples were obtained from the chimney openings (5.7m high) and lee side windows (1.7m), all contained within the sub-section (Fig.1). The majority of measurements were conducted when transverse easterly winds were blowing, therefore, inter-exchange surfaces generally remained fixed.

The Innova 1309 sampling and dosing unit was used in conjunction with the Innova 1312 multi-gas monitor and 7620 Tracer gas monitoring system program. The tracer gas Sulfur Hexafluoride (SF_6) was used and all measurements were stored and calculated in Excel. The multi-gas monitor was set up to measure CO_2 and SF_6 from each of the 6 sample locations, with a whole sampling cycle including sample analysis and cell flushing lasting ≈10 minutes.

The external weather parameters were measured from a UK TOSS weather station, where temperature, humidity, wind speed and wind direction values were recorded hourly. Rotronics sensors measuring temperature and

humidity were also installed at some locations within the experimental sub-section.

Results and Discussion

The basic principle behind the mass balance models used in these calculations are based on the following relationship.

Ventilation rate = Production or volume release of tracer / Tracer concentration inside envelope – Tracer concentration outside envelope

The hourly air exchange rate results with the three methods were found to be very inconsistent (Tab.1). The temperature/humidity method was shown to perform better than the CO_2 method. The very high exchange rates calculated with the CO_2 method (in excess of 50/hour) are unrealistic under such winter conditions. The average hourly CO_2 values (concentration difference between inside stall and ambient levels) failed to exceed the recommended difference of 200ppm for accurate exchange rate calculations (Pedersen *et al*., 1998), causing these high air exchange rate results. The instruments were checked before the experiment and were performing correctly. These low concentrations could be due to the well ventilated atmosphere and/or perhaps the animal density was not large enough.

On the other hand, inside and outside stall temperature and humidity values were above the recommended inside and outside stall level differences (> 2°C for temperature and > 0.5kg $x10^{-3}$ for humidity) for accurate exchange rate calculations (Pedersen *et al*., 1998). Although, the exchange rate values were lower than the tracer gas method they do seem reasonable and were similar to the recommended exchange rate (Büscher. 1998), which is based on winter install conditions and bird weight.

Table 1: Calculated exchange rates

24 hour Air Exchange rate calculations for CO_2, Temperature/Humidity and Tracer gas methods

Date	Air exchanges/hr CO_2 method	Air exchanges/hr Heat method	Air exchanges/hr Tracer gas method	Recommended Air exchange rate/hr (Büscher. 1998)
07-08.11.03 (17-17hr)	63,5	3,2	14,7	2,4
08.11.2003 (00-00hr)	66,8	3,3	17,7	2,4
08-09.11.03 (12-12hr)	66,1	3,1	15,1	2,4
08-09.11.03 (17-17hr)	61,3	3	13,1	2,4
11-12.11.03 (15-15hr)	67,5	3	11,3	2,4
12.11.2003 (00-00hr)	70,2	2,9	10,4	2,4
13-14.11.03 (12-10hr)	51,8	2,3	1	2,4

The tracer gas method recorded higher exchange rates than the heat balance method and much lower than the CO_2 method. It can be assumed that the actual exchange rate would have been lower than the calculated tracer gas values because of tracer gas escape from section. Also, instrumental drift and gas sampling/analysis time (≈ 10 mins for complete cycle) would be additional sources of error. However, the higher ventilation rates seem realistic because of the wind direction and velocity. An overall average hourly wind speed of $3.2ms^{-1}$ was recorded with a median wind direction of 89.9° (where, 90° is a direct easterly transverse wind) and an average wind direction standard deviation of only 5.6°. It is recognised that wind speeds above $2ms^{-1}$ are required for sufficient transverse wind ventilation, but not too high resulting in short circuiting air flow and insufficient mixing of livestock house air with the tracer gas (Demmers *et al.*, 2001). Therefore, under these conditions the inter-exchange surfaces were reasonably fixed, clearly tracer gas escape from the section would have occurred, however, under such conditions the magnitude would have been reduced. The tracer gas exchange rates from the 7^{th}-12^{th} were correlated with the wind speed, with a $r^2 = 0.67$ $p<0.000$, suggesting a direct relationship between exchange rate and wind speed, as expected. This confirms the functioning of the method. The average hourly wind speed recorded in the last measurement period 13^{th}-14^{th} was only 0.3 ms^{-1}, thus well below $2ms^{-1}$, and the wind direction over the period was irregular recording a standard deviation of 116.5 degrees, therefore the air inter-exchange surfaces were variable, thus the effectiveness of the method under these conditions was reduced, however an air exchange rate of 1 per hour although low, is not entirely out of range.

Because of the nature of ventilation in Louisiana stalls, validating the calculated exchange rates for all methods is not possible. All techniques are subjected to errors depending on season, building design, materials etc. However, the correlation between air exchange rates and wind conditions with the tracer gas method is an important tool for confirming air exchange rate values with wind conditions yielding additional valuable information the other methods can not provide. On the otherhand, the mass balance methods are very cheap and easy and in this case the heat balance method provided relatively good results, the problem is there is even less certainty with mass balance results than well performed tracer gas techniques.

Conclusion

The carbon dioxide method performed poorly, however the heat balance recorded results within range of the recommended values and the tracer gas method. It could be assumed that the real exchange rate would be closer to tracer gas method results in comparison with the heat balance technique.

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SAMPLING AND DIFFERENTIATION OF AIRBORNE MOLDS IN ANIMAL HOUSES

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Introduction

Airborne molds in animal houses may affect the health of animals and working personal in many ways (Singh, 2004). They can cause a number of different types of illness, through the production of spores, mycotoxins and VOC emissions, for example:

- Allergic reactions (rhinitis and asthma)
- Hypersensitivity pneumonitis
- Infection, for example Aspergillosis
- Inflammation from fungal cell wall components
- Immune disorder and cancer from mycotoxins
- Irritation from fungal VOCs.

In this study the concentration and the genus composition of airborne molds was determined in different animal houses. Further the inflammation inducing potential of molds was characterized.

Material and Methods

In pilot experiments different aerosol samplers [KROTOW slit-sampler, 6-stage ANDERSEN cascade-impactor, REUTER centrifugal air sampler (RCS, firm Biotest GmbH), all-glass-impinger (AGI-30)] and culture media (SABOURAUD-Glucose-nutrient agar, Bierwürze-Pepton-nutrient agar, Bengalrot-Chloramphenicol-nutrient agar, Dichloran-Glycerin-nutrient agar) were tested for their ability to collect airborne molds in animal houses.

Based on the results of the pilot experiments the concentration of airborne molds were studied in cattle, sheep, pig and poultry houses. Grown molds were stained with cotton blue and identified microscopically using morphological characters.

The inflammation inducing potential was characterized from mold species that were frequently isolated from the airborne state of animals houses by using human whole blood cytokine response. The test procedure is described in detail elsewhere (Zucker, 2004). Briefly, samples of interest are incubated with diluted blood from healthy human donors. After contact with relevant structures monocytes release proinflammatory signal molecules such as interleucin-1ß (IL-1ß). IL-1ß release is quantified by ELISA measurement.

Results and Discussion

- Pilot experiment

The highest collection efficiency was achieved with the Andersen sampler and the Dichloran-Glycerin-agar. Therefore, the Andersen sampler and the Dichloran-Glycerin-agar were used for all further investigations.

The sampler was operated at an airflow of 28.3 l/min 1 m above the ground in the center of the animal houses. The concentrations of microorganisms in 1 m³ of air were calculated from the colony count and airflow, and expressed as cfu/m³. Further the Andersen sampler was used to determine the aerodynamic size of the isolated molds.

- Concentration and genus composition

On average the total amount of airborne molds ranged from 1,8 x 10^2 to 7,5 x 10^3 cfu/m³ in cattle barns, from 2,3 x10^2 to 5,6 x 10^3 cfu/m³ in pig houses and from 7,2 x 10^2 cfu/m³ to 1,2 x 10^5 cfu/m³ in poultry houses. That concentration are comparable to those reported from other investigations in animal houses (e.g. Gemeinhardt und Wallenstein, 1985; Cormier et al., 1990).

Aspergillus, *Penicillium*, *Cladosporium* and *Scopulariopsis* were the most frequently identified mold genera followed by the genera *Alternaria* and *Wallemia* (Table 1). Some genera isolated in this study are known to induce allergic reactions in humans and animals. However to access the "allergic potential" of an bioaerosol the concentration of the specific allergens should be determined. Therefore it is of interest how the number of culturable molds correlates with the concentration of specific allergens, e.g. the number of airborne *Aspergillus fumigatus* and the concentration of Asp f 1 or the number of *Alternaria alternata* and the concentration of Alt a 1.

Table 1: Number of investigated air-samples positive for different genera of molds

Stable	n	*Pen.*	*Asp.*	*Sco.*	*Cla.*	*Alt.*	*Wal.*
Cattle	37 (100%)	30 (81%)	35 (95%)	14 (38%)	36 (97%)	20 (54%)	13 (35%)
Pig	22 (100%)	20 (91%)	22 (100%)	20 (91%)	20 (91%)	13 (59%)	8 (36%)
Poul-try	20 (100%)	19 (95%)	19 (95%)	15 (75%)	12 (60%)	10 (50%)	13 (65%)
Sheep	26 (100%)	25 (96%)	25 (96%)	20 (77%)	15 (58%)	6 (23%)	26 (100%)

n = number of investigated samples, *Pen. = Penicillium, Asp. = Aspergillus, Cla. = Cladosporium, Sco. = Scopulariopsis, Alt. = Alternaria, Wal. = Wallemia*

- Aerodynamic sizes

Most of airborne *Aspergillus*, *Penicillium*, *Cladosporium*, *Scopulariopsis* and *Wallemia* are able to penetrate into the lungs, and a considerable part of *Aspergillus*, *Penicillium* and *Wallemia* can even penetrate into the alveoli. In table 2 the percentage of airborne molds that are able to penetrate into the lung and into the alveoli is shown.

Table 2: Percentage of airborne molds found on stage 6 and stage 3-6 of the Andersen sampler

Stable	Stage of the Andersen sampler 3-6	6
Cattle	71,4%	2,9%
Pig	69,3%	2,6%
Poultry	52,0%	5,3%
Sheep	77,7%	5,1%

Stage 3-6 penetration into the lung, Stage 6 penetration into the alveoli

- Inflammation-inducing potential

In table 3 the minimal concentrations of different microorganisms as well as lipopolysaccharides (cell wall component of gram-negative bacteria) and ß-Glucans (cell wall component of gram-positive bacteria and molds) are shown that are necessary to induce IL-1ß in the human whole blood assay. The potency of molds to induce inflammation is similar to that of gram-positive bacteria, but clearly less than that of gram-negative bacteria. However, it should considered that bioaerosols from animal houses almost always contain different microbial components. Since lipopolysaccharides and glucans activate macrophages/monocytes via different pathways (Takeuchi and Akira, 2001) it is possible that both substances exhibit synergistic effects after simultaneously stimulation.

Table 3: Minimal concentration of different microorganisms and cell wall components of microorganisms that are necessary to induce IL-1ß in the human whole blood assay

Microorganism	Concentration
Aspergillus fumigatus	$3{,}3 \times 10^6$ cfu/ml
Penicillium puberulum	$1{,}6 \times 10^6$ cfu/ml
E. coli D12/4/99	$8{,}8 \times 10^2$ cfu/ml
Staphylococcus xylosus	$1{,}1 \times 10^6$ cfu/ml
E. coli-LPS (O111:B4)	10 ng/ml
Zymosan	100 µg/ml
Laminarin	1 mg/ml
Curdlan	1 mg/ml

Conclusions

In the air of animal houses regularly mould genera/species can be found which might lead to allergic diseases. Furthermore cell wall component of fungi are able to induce inflammatory reactions after inhalation. However, their inflammation-inducing potential is low compared to endotoxins.

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AIR QUALITY INSIDE PIG BUILDINGS IN RELATION WITH PHYSIOLOGICAL STAGES AND SEASON

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Introduction

In pig facilities, the quality of the atmosphere is an essential aspect of the management of the production. Because animals are very sensible to temperature and air speed, pig farmers have to control these physical parameters of the ventilation (temperature, ventilation rate…) in order to obtain the best performances. Nevertheless, air quality integrates more parameters than physical ones. Ammonia, dust and odour are added to physical parameters noticed before. Buildings involved in this study were considered representative of French pig breeding conditions. Values of air quality given in the following pages could not be applied for others breeding conditions than these described in this paper.

Material and Methods

Animals and husbandry: In this study, three physiological stages were considered: sows in farrowing rooms (FW), piglets in post-weaning rooms (PW) and pigs in growing-finishing rooms (GF). Farrowing rooms were fully slatted floor with metallic slats at the back of the sow for easier removal of slurry and plastic slats for the rest of the pen. Fresh air was entered room via a perforated ceiling and the air extraction was under-floor extraction. Sows entered rooms one week before piglet's birth and stay a total of 5 weeks. Ventilation rate applied per sow during the cold period was 110±21 m^3per hour and 263±43 m^3 per hour during the hot period. Average ventilation rate was 188 m^3per hour per sow and average ambient temperatures were 26±2°C and 23±1.2°C respectively for the hot and the cold period. Concerning piglets in post-weaning rooms, the average weaning weight was 8.7±1.0 kg. Time of presence can vary between five and seven weeks. Average weight at the end of the post-weaning period was 25.3 ±1.9kg. Rooms were fully concrete slatted floor with fresh air entering via a ceiling or via lateral entries. Extraction was under-floor. Surface per piglet was around 0.35 m^2. Ventilation rate applied in post-weaning rooms were 13.5±3.2 m^3 per hour for the cold period and 22.2±4.6 $m^3$for the hot period. Average ventilation rate was 18.6±6 m^3 per piglet per hour. Average ambient temperature was 26±1°C for the cold period and 27±1°C for the hot period. For growing-finishing rooms, average pig weigh was 27.5±11.4kg at the entry and 103.5±5.9kg at the end of the GF period for an average time of 105 days. Rooms were fully slatted concrete floor with storage of the slurry in deep pit. Fresh air entered via a perforated ceiling and air exhaust was under-floor extraction with or without cheminey. Pigs were fed *ad libitum* with a multiphase diet. Average surface per pig was 0.7 m^2. Ventilation rate was 24±7 m^3per hour per pig for the cold period and 35±11m^3per hour per pig for the hot period. The average ventilation rate was 31±11m^3 per hour per pig.

Dust concentration: dust concentration was measured using gravimetric method and expressed in milligrams per cubic meter. Measurements were achieved 1 meter above the floor at the middle of the corridor. Time sampling was between one and three hours.

Ammonia: Concentrations were measured in the room atmosphere and in the extracted duct. In the ambiance, ammonia concentration was measured in the centre of the corridor 1 meter above the floor with passive diffuse tubes. Ammonia concentration was expressed in ppm. In the extracted duct, concentration was measured by bubbling method.

Odours: Sampling of odorous air from extracted duct was achieved in order to determine odour concentrations. Air samples were analysed by CERTECH Laboratory (Seneffe – Belgium) for the determination of the odour threshold which is defined as the dilution factor at which 50 % of an odour panel can just detect an odour (CEN, 1999). Equipments and procedures were conformed to current recommendations (French Standard,NF X43-101, NF X 43-104 – AFNOR, 1986 and 1990 – European project standard prEN 13725 – CEN, 1999). Odour concentration is expressed as odour unit (OU). The rate of emission from an odour source is the product of the concentration and the volumetric flow rate (m^3/h) of the emissions and is expressed in $OU.h^{-1}$. Values were converted in odour emission per day per animal integrated difference between sows, piglets and growing-finishing pigs.

Ambient physical parameters: Outside and ambient temperatures and ventilation rates were recorded during measurements. Measurements were always achieved during the same period of the day (morning). The cold period was from October to April with average outside temperature of 7±3°C and the hot period from May to September with average outside temperature of 18±3°C.

Results and discussion

Dust concentration : Average dust presentated in the following table were calculated with 34 values obtained in 10 FW rooms, 52 in 11 PW rooms and 98 in 27 GF rooms.

Table1: Average dust concentrations (mg/m^3)

	FW	PW	GF
Hot period	1.8 ± 1.1	4.1 ± 2.0	2.4 ± 1.3
Cold period	2.5 ± 1.2	6.3 ± 3.3	3.6 ± 1.8
Mean value	2.0 ± 1.1	5.4 ± 3.0	2.9 ± 1.6

In PW rooms, dust levels are twice higher than levels measured in FW and GF rooms. This fact can be explained by the greatest activity of young pigs in PW rooms compared to sows and GF pigs. The link between pig activity and dust concentration in pig housing was clearly illustrated by results of Pedersen (1993)

The effect of the season is clearly identified on dust concentrations. Whatever physiological stages, dust levels measured during the hot period were always smaller than levels measured during the cold period. The effect of the ventilation rate is partially responsible of this difference. In fact, the rise of temperature during the hot period act on the ventilation rate by increasing it. Dust concentrations are reduced by dilution of the atmosphere

inside the building. During the hot period, the reduction of dust could also be explained by the reduction of pig activity.

Ammonia concentrations; Average ammonia concentration in the ambiance presented in the following table were calculated with 55 values obtained in 10 FW rooms, 65 in 7 PW rooms and 98 in 16 GF rooms.

Table 2: average ambient ammonia concentrations (in ppm)

	FW	PW	GF
Hot period	5.4 ± 2.9	4.4 ± 2.7	8.3 ± 5.4
Cold period	11.5 ± 5.2	7.8 ± 2.9	12.7 ± 7.2
Mean value	8.9 ± 5.3	6.3 ± 2.9	10.2 ± 6.6

Values presented in table 2 are in accord with previous values published in literature (Wathes, 1998 – Nicks *et al.*, 1989). As for dust, ammonia concentrations were always smaller during the hot period. Also, increasing ventilation rate and the effect of dilution on the atmosphere were responsible of the reduction of ammonia inside rooms (Hoff and Bundy, 1992).

Ammonia emissions: Average ammonia emissions presented in the following table were calculated with 16 values obtained in 5 FW rooms, 35 in 7 PW rooms and 68 in 13 GF rooms.

Table 3: Average ammonia emission (in g day per animal)

	FW	PW	GF
Hot period	28.6 ± 10.6	3.8 ± 3.1	10.1 ± 4.4
Cold period	23.1 ± 7.3	3.1 ± 2.8	8.8 ± 4.2
Mean value	25.6 ± 9.1	3.5 ± 2.9	9.5 ± 4.3

Average quantities of ammonia emitted per animal were calculated including average ammonia emission of table 3 and the average time of presence per animal. The average value slightly lower than 1 kg of ammonia emitted by the building per GF pig is very close to previous values obtained in the same conditions (Guarino *et al.*, 2003 – CORPEN, 2001).

Table 4 : Average ammonia quantities emitted per animal

	FW	PW	GF
Average time of presence (days)	365	50	105
Average quantity of ammonia emitted per animal (grammes)	9300	175	998
% of emission by stage in case of a 100 sows closed farm	28.9	6.3	64.8

Effect of the season on ammonia emission was already observed by Gustafsson (1987). The increasing air flow inside room may increase air speeds above emitting surface (slatted floor) and enhance emissions.

Odour emissions: Average odour emissions presented in table 5 were calculated with 14 values obtained in 4 FW rooms, 23 in 5 PW rooms and 63 values in 13 GF rooms. In the same approach that this applied for ammonia emission, odour emission per animal and percentage for both physiological stage were presented in table 6.

Table 5 : Average odour emission (in odour units per day per animal)

	FW	PW	GF
Hot perod	$1.6\ 10^7 \pm 4.8\ 10^6$	$3.0\ 10^6 \pm 2.4\ 10^6$	$4.1\ 10^6 \pm 1.8\ 10^6$
Cold period	$1.3\ 10^7 \pm 5.6\ 10^6$	$1.6\ 10^6 \pm 1.1\ 10^6$	$2.4\ 10^6 \pm 1.9\ 10^6$
Mean value	$1.5\ 10^7 \pm 4.8\ 10^6$	$2.4\ 10^6 \pm 2.0\ 10^6$	$3.3\ 10^6 \pm 2.0\ 10^6$

Table 6 : Odour emitted per animal

	FW	PW	GF
Average time of presence (days)	365	50	105
Average quantity of odour per animal (odour unit x 10^6)	5475	120	346.5
% of emission by stage in case of a 100 sows closed farm	35	15	50

Around 50 % of odours are emitted by GF rooms in our example of 100 sows closed farms. The high level of odour emission of GF rooms and the time of presence explained the weight of this stage.

Conclusions

Data obtained in this study show the diversity of air quality in relation with physiological stages. Except for dust concentrations, GF rooms appear to have the worst air quality in comparison to FW and PW rooms. The effect of the season on dust, ammonia and odours was clearly noticed. For dust and ammonia in the atmosphere, concentrations were lower during the cold period. At the opposite, ammonia and odour emission were higher during the hot period. In both cases, the effect of the ventilation rate in relation with ambient temperature was the main explanation. In France, the increasing number of conflicts with neighborhood about odours and the eventual tax linked to ammonia emission should encourage pig farmers to invest in air treatments especially adapted to GF rooms.

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IS THERE AN INFLUENCE OF ANIMAL BEHAVIOUR ON INDOOR GAS CONCENTRATION ?

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Introduction

Indoor air quality results first from indoor production of gases, dust, and pathogens, and second, on dilution by fresh air input. Ventilation depends mostly on inside air temperature. As a consequence, indoor air quality should depend on climate, building thermal insulation, animals, and emitting surfaces. It is of common sense in animal production that emitting surfaces, animals, management practices and ventilation interact, and that these interactions can lead to various irreversible changes in the emitting surfaces. Therefore, the influence of animal behaviour on indoor air quality can be obviously assumed.

Observations confirmed this assumption in the case of feeding behaviour, and ammonia, carbon dioxide, or dust (Hinz & Linke, 1998b; Jeppsson, 2002; Groenestein *et al*, 2003), while diurnal variations of nitrous oxide and methane emissions were not observed (Nicks *et al,* 2003). Ammonia emission has received many attention since decades. However, animal behaviour is not accounted for explicitly in modelling concentrations and emissions from livestock buildings (Aarnink & Elzing, 1998; Ni, 1999; Pinder *et al*, 2004). Filling this knowledge gap can help to improve management practices, building conception, and mitigation strategies through a finer tuning to climate, farmer, and animals.

The pig-on-litter system is a suitable example for this objective because the pigs choose a defecating area, they explore the litter, they adapt their behaviour to the air temperature (Ducreux *et al*, 2002), while the litter evolution is affected by this behaviour (manure surface and manure amount in the litter: Jeppsson, 2002; the exploring behaviour also influences the gas exchanges within the litter and the microbial transformations), the methods of air or behaviour monitoring have been already discussed (Hinz & Linke, 1998a; Phillips *et al*, 1998; Jensen *et al*, 1986). Therefore, short-term as well as long-term changes can be observed. We focused here on short-term relationships. We chose warm conditions, assuming the ammonia concentration to rise when the pigs stand on. During warm periods they lie on the dirty part of the litter and the warm emitting surface increases as soon as they stand up. The number of standing animals can be a key variable to link the behaviour to air concentrations (Groenestein *et al*, 2003).

Material and Methods

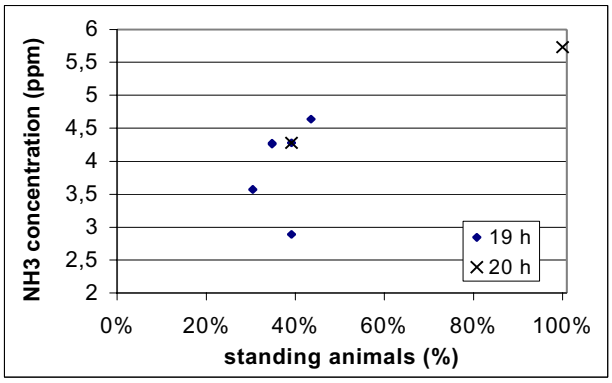
We chose a building with low animal density (2,6 m^2/pig) in order to have contrasted lying and excreting areas. The room contained 23 pigs between 80 and 120 kg. We recorded the animal behaviour with a camera and 24h-video recorder between 6h and 23h. We measured continuously the NH_3, CH_4, and N_2O concentrations with a multigas photo-acoustic analyser connected to a sampler (INNOVA, 1312+1303) and controlled by a computer. We monitored four different sampling points inside and two outside a commercial building with natural ventilation during two weeks in july 2003. We also monitored the air flow rate with the SF_6 dosing-tracer method, and inside and outside air temperature and humidity (all details are given in Robin *et al*, 2003). We studied time sequences of some minutes where the air flow rate and the outside and inside climates were stable, the animal and litter metabolisms were assumed stable, so that the animal behaviour was assumed to be the main variable influencing the inside gas concentrations. We chose sequences where the gas concentration changed and all animals could be counted. We measured the number of standing and digging animals on the video records for each concentration measurement during those sequences.

Results

The final number of moments where the gas concentration changed, all the animals could be counted, and the overall conditions were stable was very low because of the data rejected due to difficulties with the material or uncertainties with the synchronisation of the video-recorder and the gas equipment.

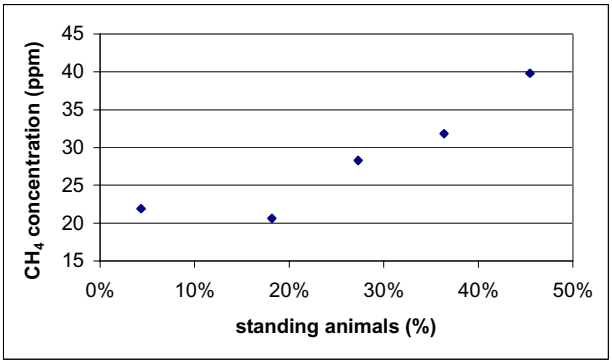
NH_3 concentrations increased above the excreting area but not above the lying area. On the contrary, concentrations of N_2O and CH_4 increased above the lying area and not above the excreting area.

Figure 1: variation of NH_3 concentration above excreting area when the proportion of standing animals increases.



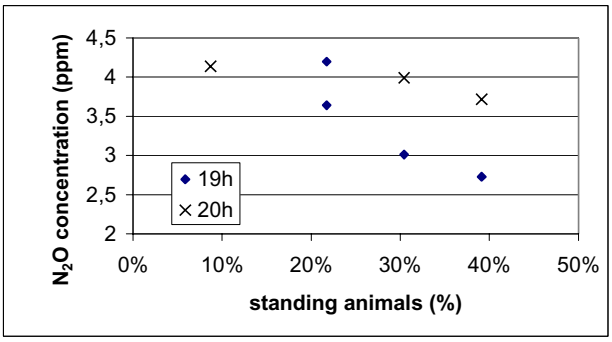
NH_3 concentration increase was not related to the number of standing animals when it varied slightly but a relationship was observed when the number varied strongly (Fig. 1).

Figure 2: variation of CH_4 concentration above lying area when the proportion of standing animals increases.



The CH_4 concentration increased with the number of standing pigs when both increased (Fig. 2) but the points were scattered during the decrease of the concentration. The N_2O concentration increased when the number of standing animals decreased, i.e. when the pigs began to lie and dig the litter around them (Fig. 3). As for CH_4 no relationship was observed during the decrease of the concentration.

Figure 3: variation of N_2O concentration above lying area when the proportion of standing animals decreases.



Discussion

We assumed from previous work that relationships could be observed for ammonia and not for methane or nitrous oxide (Groenestein *et al*, 2003; Hinz & Linke, 1998; Jeppson, 2002; Nicks *et al,* 2003). In our experiment, relationships were observed for all gases, while the relationship for ammonia was not as clear as assumed initially. In the case of ammonia, this result can be explained by a lower ammonia concentration in the excreting area and a relative lower surface of the animals. As a matter of fact, the animal density was low in our case. It can have induced a higher organisation or adsorption of excreted nitrogen in the litter, and a higher excreting area not covered by lying animals regarding the surface variation that occurred when a small proportion of animals stood up. It shows that modelling this relationship should account for the type of breeding system.

The increase in methane concentration can be explained by the emission of gas from the porosity closed by the lying pigs. The lack of relationship above the excreting area can be explained by a to small free air space in this area. The lack of relationship during the concentration decrease can be explained by new processes such as some emitting sites becoming aerobic.

In the case of nitrous oxide the same increase as methane is not observed because the redox conditions where methane accumulates in the free air space are not favourable to nitrous oxide accumulation. When more pigs lie and dig around themselves, increasing sites with enough oxygen for nitrification and too much for complete denitrification can release the produced nitrous oxide. It can explain the increase in concentration of nitrous oxide.

Conclusion

The existence of relationships between gas concentration and animal behaviour at the scale of the livestock room can be obviously assumed though they are absent of most models of gas emission developed since decades.

We looked for short-term relationships between ammonia, methane and nitrous oxide, and the number of standing animals in the case of the pig-on-litter system. Relationships were observed when the gas concentration increased but not during the decrease.

The gas concentrations varied very little and could be explained by local interactions between the pigs and the litter porosity. It shows that acting on short term behaviour will not change the level of the gas concentrations and can be neglected in models. However, the long term interaction between pig behaviour and litter management practices can change the biological transformations within the litter and have a much stronger influence on the gas concentrations and emissions from the building. Modelling this process to improve management practices requires to characterise the feed-back between behaviour and surface heterogeneity within the breeding room. This modelling needs a coupled description of the nitrogen, carbon and water cycles within the system, more complete than it is generally done in modelling livestock buildings.

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THE EFFECTS OF AERIAL AMMONIA AND STREPTOCOCCAL ORGANISMS ON THE FEED INTAKE, IMMUNE FUNCTION AND PHYSIOLOGY OF THE PIG

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Introduction

Growth rates and feed :gain efficiency of pigs raised under commercial conditions in Australia are well below their genetic potential, and the values that could be achieved if the animals were housed under 'ideal' conditions (1). This difference has a significant impact on the potential profitability of a pig enterprise. There are many factors within a commercial piggery environment that can contribute to the depression in feed intake, growth rate and efficiency of feed use and act to increase the stress level of the pigs. The stressors can be divided broadly into three categories; social, climatic and hygiene. In most commercial environments several stressors are acting simultaneously. Hygiene and air quality in intensive animal housing is a major concern to producers, employees, housing and farming specialists, and veterinarians involved in the intensive livestock farming industries. In recent years a number of reports have highlighted the negative effects of sub-optimal air quality and hygiene on the health and production of animals, as well as the health of workers (3,7).

Material and Methods

A series of experiments were conducted to quanitify the effects of varying concentrations of aerial ammonia and viable *Streptococcal* organisms on the feed intake, immune function and physiology of 16 week old gilts.

The experiments were conducted in the Research Facility of the PPPI Roseworthy Piggery, University of Adelaide. The experimental room (24^{o}C, 55% RH), was cleaned and disinfected between each trial, and hosed three times daily during the trial. Pigs were removed from the room during cleaning and the pit was flushed every second day. Pigs with faecal matter on their skin were washed and dried and returned to their stall. Other stressors, such as stocking rate, background ammonia and bacterial levels were minimised.

Large White x Landrace respiratory disease-free gilts, housed individually on a partially slatted floor, were used in each experiment. Pigs were weighed daily and given access to water at all times. They were fed 2.5 kg of a commercial diet daily, divided into equal portions (morning and afternoon). Twenty pigs were used in each experiment with 5 pigs per treatment, replicated 4 times.

Ammonia gas in nitrogen was pumped into the feed bin at a rate of 12L/min for 20 minutes while pigs were eating, and each pig was observed to ensure maximum exposure. Concentrations of ammonia used were 0, 10, 25 and 50ppm. After feeding, pigs were dosed intranasally with a solution of *Viridans streptococcus* suspended in buffered saline at a concentration of $2x10^5$ cfu/ml. The organisms used were collected from the airspace of a shed housing growing pigs, using a 6-stage Andersen Sampler loaded with Columbia Horse Blood Agar plates.

Pigs were weighed daily and any uneaten food was collected and weighed.

Pigs were bled prior to pollutant exposure and again 14 days later, and lymphocyte proliferation, phagocytosis (as a measure of neutrophil function), and surface markers CD4, CD8, CD21 measured. Blood analysis included T cell proliferation assays - expressed as CCPM measuring the incorporation of 3HT into actively dividing cells in response to a mitogen and Phagocytosis assay - expressed as % granulocytes (eosinophils and neutrophils) with phagocytic potential. Pigs were slaughtered at the end of each experiment and lungs examined grossly for lesions.

Data were analysed by ANOVA (Statistix 7.1)

Results

No lesions were observed in lungs examined macroscopically after slaughter.

There was a significant reduction in the growth rate of pigs exposed to ammonia compared with untreated controls (0 ppm) and the reduction increased as levels increased from 10 to 50ppm (Table 1). Growth rate suppression was further increased when pigs were exposed to bacteria as well. Similar reductions were also recorded in feed efficiency (Table 1).

Table 1. Mean growth rate (ADG) and feed efficiency (FCR) pre- and post- exposure with NH_3 or NH_3 and bacteria ($2x10^5$ CFU/ml) (NH_3 + B).

NH_3	ADG	FCR	NH_3 + B	ADG	FCR
0	796 ± 25.8^{a}	3.12 ± 0.11^{f}	0	691 ± 24.5^{b}	3.61 ± 0.14^{f}
10	754 ± 27.4^{a,b}	3.41 ± 0.13^{f}	10	555 ± 20.8^{c}	4.24 ± 0.17^{f}
25	713 ± 27.2^{b}	4.01 ± 0.14^{f}	25	464 ± 23.6^{d}	5.76 ± 0.16^{g}
50	590 ± 40.3^{c}	5.85 ± 0.45^{g}	50	264 ± 32.8^{e}	10.97 ± 1.27^{h}

a→h.– ADG/FCR different superscripts significantly different (P<0.05)

Although lymphocyte proliferation (as measured by the stimulation index) was not consistently increased in pigs exposed to ammonia, a significant increase was recorded in pigs exposed to both ammonia and bacteria (Table 2). In the latter groups, the stimulation index increased as concentrations of ammonia increased.

Table 2. Mean lymphocyte proliferation (SI) pre- and post- exposure with ammonia (NH3) or ammonia and bacteria ($2x10^5$ CFU/ml) (NH3 + B).

NH3	Before (SI)	After (SI)	NH3 + B	Before (SI)	After (SI)
0	38.90 ± 4.66^{a}	46.40 ± 5.19^{a}	0	45.20 ± 2.71^{a}	65.50 ± 4.09^{b}
10	47.40 ± 5.31^{a}	56.3 ± 6.96^{a}	10	47.00 ± 3.48^{a}	92.1 ± 7.15^{c}
25	38.80 ± 5.34^{a}	46.60 ±6.23^{a}	25	50.60 ±3.03^{a}	152.10 ± 8.23^{d}
50	35.50 ± 4.97^{a}	47.4 ± 5.89^{a}	50	42.80 ± 2.99^{a}	178 ± 13.37^{e}

a→e – Rows with different superscripts significantly different (P<0.05)

While phagocytic activity (expressed as % granulocytes - eosinophils and neutrophils with phagocytic potential) in pigs exposed to ammonia tended to increase, a significant increase was recorded in pigs exposed to both ammonia and bacteria (Table 3). In the latter groups, the neutrophil phagocytic activity increased as concentrations of ammonia increased.

Table 3. Mean phagocytosis activity pre- and post-exposure to NH3 and NH3 and bacteria (NH3 + B).

NH3	Before	After	NH3 + B	Before	After
0	9.30 ± 1.03 [a]	11.25 ± 1.22 [a]	0	15.25 ± 1.30 [a]	28.25 ± 2.28 [c]
10	10.85 ± 0.81 [a]	13.10 ± 0.99 [a]	10	13.30 ± 0.80 [a]	33.20 ± 2.34 [d]
25	10.10 ± 1.07 [a]	14.65 ± 1.62 [a]	25	14.55 ± 0.94 [a]	51.00 ± 3.24 [e]
50	11.75 ± 1.04 [a]	17.63 ± 1.55 [b]	50	14.65 ± 0.96 [a]	66.50 ± 4.47 [f]

a→e – Rows with different superscripts significantly different (P<0.05)

Discussion

While neither overt clinical signs, nor macroscopic lesions suggestive of respiratory disease, were observed in pigs throughout the experiments, significant production effects and immune changes were recorded. This finding is consistent with previous reports where immune responses and growth rate suppression was demonstrated in disease free pigs exposed to poor air quality and hygiene (5).

While exposure to bacteria appeared to have a greater effect than ammonia on growth rate and feed efficiency, as well as aspects of immune function, the most significant effects were observed in pigs exposed to high levels of ammonia followed by bacteria. This agrees with previous studies (8) where pleurisy prevalence was higher in sheds with both high levels of ammonia and bacteria, compared with sheds with concentrations of ammonia below 5ppm and levels of bacteria above 1.5 $x10^5$. Hence, we hypothesise that ammonia damages the integrity of the mucosa allowing bacteria, or their toxins, better access to the animal's immune tissues.

The bacteria used in the study are part of the normal flora of the airspace in pig sheds and commonly referred to as faecal Streps. They are non-pathogenic but do contain chemicals such as alpha-glucans and peptydoglycans in their cell walls, both of which are known to be immunogenic. While the proportion of live and dead or decaying bacteria was not assessed, dead bacteria, which have released the cell wall toxins, could be expected to be more harmful than living organisms (2). In previous studies (8) the concentration of Streptococcal organisms in the airpsace of a pig shed was identified as a major risk factor for high pleurisy prevalence in growing pigs. Murphy (7) also reported a significant negative correlation between the concentration of bacteria in the airspace and growth rate and a similar correlation between stocking density (m^3/pig) and bacterial concentration. The immune response and production effects recorded after 14 days expsoure, without overt clinical signs, suggests an acute response. Whether the reaction would become muted over time was not studied. However pigs need not show clinical signs of disease to have reduction in performance (9). Pigs exposed to pathogenic organisms at a dose lower than that which causes overt clinical disease may develop immunity to the organisms but with a resulting depression in performance. In this study, pigs with high stimulation of the immune system ate 5.5% less feed and grew 17% more slowly than pigs with low immune stimulation.

Conclusion

There is strong evidence that many of the factors (social, climatic and hygiene) which reduce the performance of pigs raised in commercial environments act to increase the stress level of the pigs. This suggests that the removal of one stressor should have a positive effect on performance (4).

The results indicate a close relationship between growth rate and air quality, in particular aerial ammonia and *Streptococcus* sp. bacteria. Building hygiene has also been shown to be one of the most important factors associated with post-weaning enteric diseases in pigs (6).

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POTENTIAL OF EMITABLE AIRBORNE ENDOTOXIN CONCENTRATIONS IN AN AVIARY AND A CAGED HUSBANDRY SYSTEM FOR LAYING HENS

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Introduction

Gaseous and particulate pollutants in livestock buildings are potential health hazards for animals and humans. Due to this knowledge environment-related threats are also expected, because the ventilation system transports loads of pollutants into the surrounding air [1]. Especially for bioaerosols little is known about the environmental effects. To clarify such deficiencies a first step is the assessment of emission amounts for bioaerosols [2]. Poultry houses are generally causing the biggest particulate emissions, but different husbandry techniques can significantly influence the bioaerosol burdens. So far, husbandry systems for laying hens equipped with cages are increasingly criticised due to poor animal welfare. For that reason alternative animal keeping systems such as aviaries are promoted which fulfil requirements for adequate animal behaviour. Aviaries allow the birds to move freely, to scratch in litter and to fly. On the other hand sustainable livestock building designs have also to consider occupational and environmental aspects. Therefore, pro-inflammatory effective airborne endotoxins (as important part of bioaerosols) were simultaneously measured in a caged husbandry system and an aviary of an experimental laying hen house. The aim of the study was to show the dynamic of emission rates of endotoxins over a housing period of the two herds.

Material and Methods

Investigations were conducted in an experimental laying hen house with completely separated barns one equipped with conventional cages and one with structures typical for aviaries. Each barn was forced ventilated and housed 1,500 brown hens (Lohmann, Cuxhaven, Germany) with a mean body weight of approximately 1.75 kg. Within the compartments two sampling positions were installed in the longitudinal axis of the barn. For endotoxin analysis (LAL-Test) airborne inhalable and respirable dust were sampled at two and one position, respectively, with IOM samplers and cyclones (SKC Inc., USA). A central pump guaranteed the necessary flow rates. Ventilation rates were estimated with the aid of the carbon dioxide balance method using an infrared spectrometer for gas measurements. The 24 hour surveys were conducted 16 times over the housing period of the flocks and additionally accompanied by indoor and outdoor temperature measurements to get insight in seasonal variations, which possibly may cause none standardised monitoring conditions. Differences of husbandry-related data were statistically checked by the Mann-Whitney U-Test and associations between variables were calculated via Spearman rank correlation (r_S).

Results

The median indoor temperature did not differ significantly over the year (18.0 °C vs. 17.1 °C), although outdoor temperatures showed a clear seasonal trend. As expected from spring to summer increasing outdoor temperatures were observed (max. 19.5 °C), which declined down to a minimum value of 1.7 °C in winter time (Fig. 1).

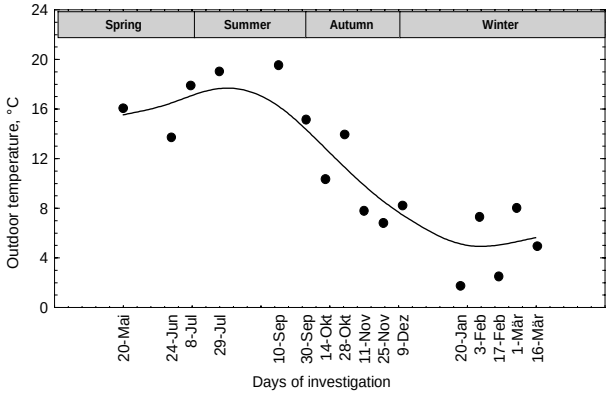

Fig. 1 Trend of seasonal depending outdoor temperatures during the investigation period from May (Mai) to March (Mär) in the following year. Dots represent the measured average temperature over 24 hours.

According to the ventilation needs during the seasons and the magnitude of endotoxin generation the emissions rates are quite variable as seen in Fig. 2.

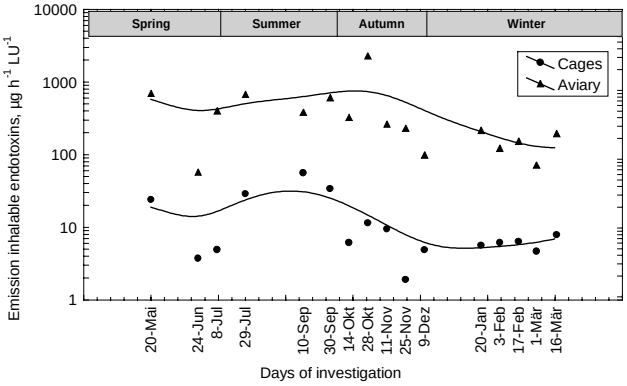

Fig. 2 Scatter plot of determined emission rates of inhalable endotoxins for hens in the caged system and in the aviary. Trend line indicate seasonal influences. LU =Livestock unit (500 kg body weight).

The emission pattern over the season is generally continued for the respirable endotoxin fraction as shown in Figure 3. However there is an exception during winter time. In the caged system a considerable and temporarily decline was observed in comparison to the aviary, which showed a constant decrease in the emission strength of respirable endotoxins. Therefore a significant correlation was just missed (r_S = 0.39; $p < 0.14$). A significant correlation could only be confirmed between cages and aviary in terms of inhalable endotoxin emissions (r_S = 0.69; $p < 0.003$).

Nevertheless, over the whole survey the median emission rates for inhalable endotoxins in the caged husbandry system and in the aviary were 6.3 µg h^{-1} LU^{-1} and 244.8 µg h^{-1} LU^{-1}, respectively. These values correspond to 0.7 µg h^{-1} LU^{-1} and 20.5 µg h^{-1} LU^{-1} in case of released respirable endotoxins. The differences were highly significant ($p < 0.001$).

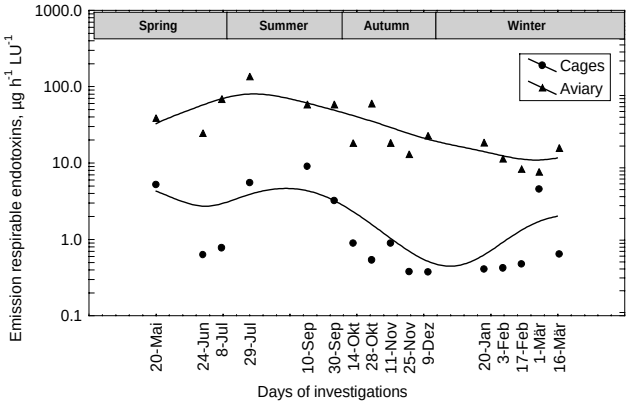

Fig. 3 Scatter plot of determined emission rates of respirable endotoxins for hens in the caged system and in the aviary. Trend line indicate seasonal influences. LU =Livestock unit (500 kg body weight).

Discussion

In the public the evaluation of husbandry systems is mainly dependent on animal health and animal welfare. But since the EU directive about the integrated pollution prevention and control has to be converted in national laws and regulations policy makers are forced to define so called best available techniques also in agriculture, which should cause a minimum pollution for the environment. In front of this background both the conventional husbandry systems have and the so-called alternative housing systems have to be evaluated. A comparison in respect to airborne endotoxins is given in this paper.

The emission potency of the caged system was significantly lower than the emissions from the aviary. The calculated mass flows for endotoxins was based on the multiplication of the ventilation rate and the airborne concentration of sampled endotoxins. Therefore, differences in emission quantities can be caused either by changes in air flow or by changes in endotoxin concentration. Indoor temperatures were rather equal in both barns indicating similar ventilation rates. As expected, no significant difference in the average ventilation rates was observed, but the determined inhalable and respirable endotoxin yields in the two investigated laying hen barns differed significantly ($p < 0.001$). In the aviary both endotoxin fractions were about 28 times higher in comparison to the conventional cages. A result which is confirmed in recent studies, where aviaries had also the highest endotoxin burdens (Seedorf et al. 1998). It is probable, that the availability of bedding material (scratching, sand bath) and a considerable higher animal's activity in the three-dimensional aviary (flying) has generated more airborne endotoxin containing dust. The results show that it is necessary to discuss the future development of animal friendly keeping systems for laying hens which are at the same time low in emissions. This is especially important in view of the occupational health of the workers in laying hen systems, because biohazards like endotoxins are causative agents for respiratory disorders.

Conclusion

In an experimental laying hen house an aviary and a battery cages system were compared in terms of their emission potency for inhalable and respirable endotoxins. The aviary showed a significant greater emission potency than the cage system. Potential confounders such as indoor temperature or ventilation rates could be ruled out as bias, because no significant differences between both husbandry systems were observed based on calculations over one housing period. Therefore, the differences of the emission loads were mainly caused by varying endotoxin concentrations within the barns. Feces loaded bedding material and the scratching and moving activities of the animals are main factors, which are causing considerable endotoxin releases dispersed in the environment.

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INHALABLE AND RESPIRABLE DUST IN WORK PLACE ATMOSPHERES OF LAYING HEN HOUSES

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Introduction

The air in modern livestock buildings contains varying amounts of aerial pollutants such as gases, dust and micro-organisms, also addressed as bio-aerosols, which can be harmful for the health of the animals and people working in this atmosphere (RADON et al., 2002). Particularly high amounts of dust (TAKAI et al., 1998) and micro-organisms (SALEH et al., 2003) are found in laying hen house air. It is assumed that nearly 15 % of all farmers working in laying hen houses complain about respiratory health disorders such as asthma, asthma like syndrome, chronic bronchitis, mucous membrane irritation and organic dust toxic syndrome (ODTS) (NOWAK 1998). Little is known about the concentrations of airborne dust, which can carry micro-organisms and gases such as ammonia, in the recently introduced new animal-friendly (alternative) aviary and enriched cage systems.

This paper compares the concentrations of the inhalable dust and respirable dust fraction in the air of a conventional cage system, an enriched cage and an aviary system and relates the dust levels to German occupational health limits (OHL), the so called MAK values (maximum concentrations at the work place).

Material and Methods

The investigations were carried out in three different types of laying hen houses of the laying hen research centre on the Experimental Farm Ruthe of the University. 1533 birds were kept in groups of ten to thirty animals in a three tier system of so called enriched cages (AK), type Aviplus (Fa. Big Dutchman, Vechta). Each cage was equipped with perches, a separate laying area and a dust bath. A floor area of 750 cm² per bird was provided. The second animal house was equipped with conventional four tired battery cages for 1345 animals providing 600 cm² per bird. The third animal house was built as an aviary where the 2304 birds can roam freely on three tiers and reach food and water in each of the three levels. The alleys are covered with litter for scratching. Along the walls nest boxes are installed. The birds have access to an outdoor scratching area which is littered with straw. This area was open for the animals daily after laying period. For more details see BRIESE et al. (2001). All birds were of a special layer breed called ,,Silver,, delivered by Fa. Lohmann, Cuxhaven from the same parent flock at the same day.

Measurements were carried out during the course of one year every second Monday each month over a period of 24 h between 6.00 h a.m. and 6.00 h a.m. the next day. In each of the three buildings two sampling positions were defined for the sampling heads of inhalable and respirable dust. These places were draught free, easy to reach for installation, out of the reach of the animals and as far as possible representative for air composition. The seventh sampling point was outside the building in a distance of about 20 m to take samples in the ambient air. The eight sampling place was set up in the outdoor scratching area. All sampling heads were installed about 1.5 to 1.6 m above the floor which represents approximately the breathing height of adult humans. The 24 h sampling period was chosen to eliminate short term variations of the dust concentration and give a broader base in the direct comparison of the three animals houses. In this paper average and minimum/maximum values are given only.

Inhalable dust was sampled by IOM heads (Institute of Occupational Medicine, Edinburgh), respirable dust by SKC cyclones (Blandfort Forum, UK) on glass fibre filters (Whatman, UK). The cyclones have a 50% cut off effectiveness for particles < 5 µm. The air was sucked through the sampling heads by sufficiently strong pumps. The flow rates were adjusted by help of critical jets to 2 l/min (IOM) and 1,9 l/min (SKC). The filters were conditioned in a climate chamber at 23 °C± 2 and 45± 5 % relative humidity before sampling and before weighing. In the same room the weighing took place (balance Sartorius AG, Göttingen). The dust concentration was calculated by air volume and the weight difference before and after sampling and is given in mg/m³.

Results

Table 1 shows the average concentrations of the inhalable dust in the three animal houses, in the scratching area and in the ambient air obtained by 12 measurements at each place. The highest concentrations were found in the aviary followed by the battery cages and the enriched cage system. Distinctly lower concentrations are reached in the outside scratching area and at the sampling position which should represent ambient air quality. and with peak concentrations of about 0.1 mg/m³. In rural regions total dust concentrations of about 40 µg/m³ are common. This corresponds to the average value at this sampling place.

Tab. 1: Mean inhalable dust concentrations in mg/m³ and minimum and maximum values at different sampling places inside and outside the laying hen houses. No. of measurements n = 12. n.m. = not detectable.

ANIMAL HOUSE	MEAN VALUES	MINIMUM	MAXIMUM
Enriched cage	**0,8**	**0,44**	**1,32**
Battery cage	**1,**	**0,24**	**2,05**
Aviary	**3,8**	**1,20**	**9,50**
Scratching outside	**0,3**	**0,01**	**1,09**
Ambient air	**0,04**	**n. d.**	**0,10**

Highest concentrations were measured in the aviary system. The total dust can reach at certain times average concentrations of 9.5 mg/m³ over 24 h periods which is more than double than the occupational health threshold

for an eight hour working day. The 24 h average total dust concentrations in the caged systems are distinctly lower. The variations in concentration are particularly high in the scratching area which is caused by the different activity phases of the animals.

Figure 1 explains the differences between the animals houses and to the ambient air in greater detail and shows the large standard deviations which are associated with such measurements. With 3.8 mg/m³ of total dust as an average value all over the year, the pollution of atmosphere in aviaries is permanently close to the occupational health threshold of 4.0 mg/m³ (MAK, 2003). The results show that there is a need to improve air quality in aviary systems in order to protect the health farmers.

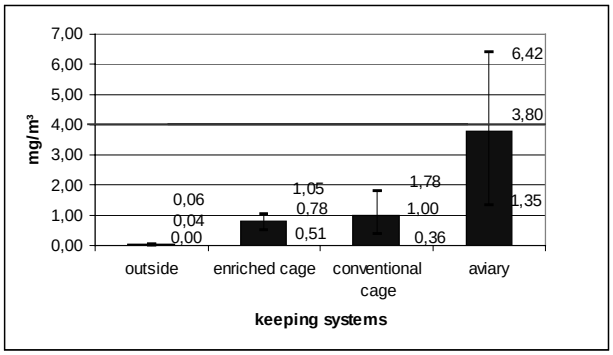

Fig. 1: Mean inhalable dust concentrations (incl. s.d.) in three different laying hen housing systems and in the ambient air close to the building. n = 12.

In **Table 2** the figures for the respirable dust are given. The highest concentrations of total dust are again observed in the aviary system. Concentrations of more than 4 mg/m³ are found during some 24 h measurements. The ratio between the highest and the lowest concentrations is about 10. Distinctly lower concentrations were found in the battery cages and the enriched cage system. The minimum values are similar whereas the maximum values differ considerably.

Tab. 2: Mean respirable dust concentrations in mg/m³ and minimum and maximum values at different sampling places inside and outside the laying hen houses. No. of measurements n = 12. n.m. = not detectable.

ANIMAL HOUSE	MEAN VALUE	MINIMUM	MAXIMUM
Enriched cage	**0,33**	**0,23**	**0,62**
Battery cage	**0,66**	**0,21**	**1,09**
Aviary	**1,93**	**0,40**	**4,40**

The extent of the standard deviations can be seen in **Figure 2** where the concentrations of respirable dust found in the three laying houses is compared to the occupational health threshold. It is remarkable that the average value across all 12 measurement campaigns (1,93 mg/m³) is distinctly above the occupational health threshold of 1.5 mg/m³ (MAK, 2003).

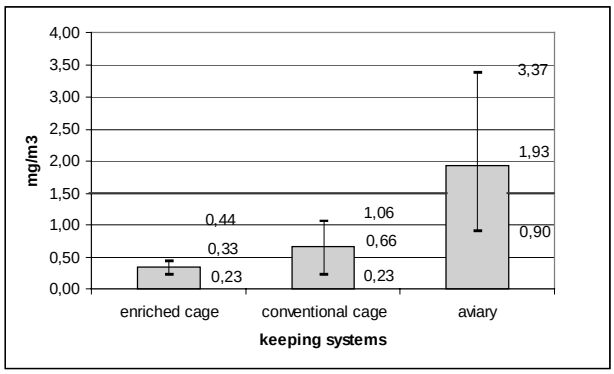

Fig. 2: Mean respirable dust concentrations (incl. s.d.) in three different laying hen housing systems. n = 12.

Diskussion

The results of this survey show that the concentration of airborne dust in laying hen houses decisively depends on the type of the keeping system. The investigations were carried out under management practices which were typical and common for these types of laying hen houses. Dust concentrations (inhalable and respirable) above occupational health thresholds were found in the aviary system where the animals can move freely and have permanent access to litter on the floor. The high average values indicate that these thresholds are exceeded regularly. Considering that the presented results are means over 24 h measuring periods which included the resting times at night, it can be assumed that the concentrations during the day when the staff is working in the animal house, the atmosphere is even higher polluted by dust particles. There is an urgent need for more investigations how to reduce the air pollution particularly in alternative laying hen housing systems for the protection of the health of the farmer, for the animals which live permanently in this atmosphere and also for the outside environment in which the pollutants are emitted and distributed by the exhaust ventilation system. As a first measure, farmers should be advised to carry filter masks in aviary and similarly constructed laying hen housing systems.

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Air quality in animal houses

Posters

INSTRUMENTATION KIT FOR MEASURING AIRBORNE POLLUTANTS IN LIVESTOCK BUILDINGS

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Introduction

Different airborne pollutants, sometimes in high concentrations can be found in the airspace of intensive piggery buildings (Cargill *et al.* 2002). High airborne pollutant concentrations could potentially affect the environment, production efficiency of animals and the health/welfare of both humans and livestock. A coordinated national project was undertaken in Australia to identify key factors affecting the concentrations of airborne pollutants in piggery buildings in order to predict and control the concentrations and emissions of these pollutants. Other researchers who conducted similar surveys usually used sophisticated and relatively complicated instrumentation (Phillips *et al.* 1998). However, this survey purposely used a more simplified instrumentation kit in order to ensure that the results of the survey and more importantly the equipment used during the survey can be applied routinely on farms, after the study concluded. The selection of the components of the measurement kit used during the study was based on reliability, accuracy, practicality and cost effectiveness of the individual items. Fulfilling all of these, sometimes opposing requirements for the many components used was difficult. Therefore the selection of the components was based on a healthy balance between different requirements and involved compromises. This paper describes the methodology and the instrumentation kit used during the field survey of the project and reports on operational experiences.

Farm selection and monitoring procedures

A total of 160 piggery buildings were surveyed between the autumn of 1997 and the autumn of 1999. The standardised instrumentation kit, data collection forms and associated graphing and reporting softwares were developed in South Australia (SARDI) and distributed to participating research organisations in other states. Training programs were implemented for the collaborators to standardise data collection procedures. The study sheds included a wide range of design and management options to provide a representative sample of industry practice in Australia. Five working days were allocated to individual buildings to complete all tasks associated with the measurements. Equipment was set up usually on either Monday or Tuesday and collected on Thursday or Friday from the buildings. The remaining day of the week was used to implement a very thorough cleaning and disinfection procedure to avoid any cross contamination between farms and/or buildings. On each farm, dry sow, weaner, grower/finisher buildings, farrowing rooms and on some farms, deep bedded shelters (DBS), were surveyed during the study. Continuous data was collected over a 3.5 day period, but the data was truncated to 60 hours to provide a balanced data set. The level of hygiene was assessed visually by estimating the percentage of manure-covered solid area in the pen and classifying into three distinct classes (poor=more than 50% manure cover on pen floors, fair=between 10 and 50% manure cover on pen floors, good=less than 10% manure cover on pen floors) at the time of data recording (Banhazi *et al.* 2002). Seasons were arbitrarily defined as summer from November to April and winter from May to October. A pro-forma was developed to collect data relating to the management and engineering characteristics of the buildings (Table 1). In Figure 1 the usual layout of the monitoring instrumentation used in the study buildings is shown.

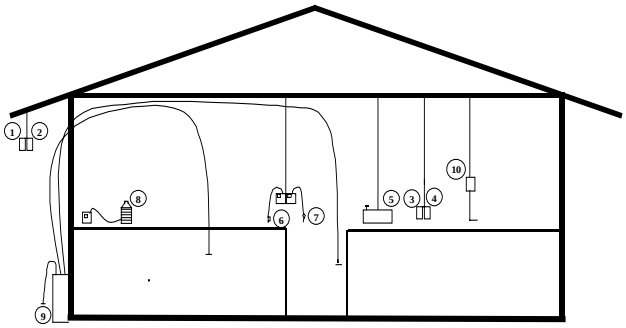

Figure 1: Instrumentation kit used for the survey (1-external temperature, 2-external humidity, 3-internal temperature, 4-internal humidity, 5-continuous dust monitoring, 6-inhalable dust, 7-respirable dust, 8-Andersen samplers, 9-MGM machine, 10-internal wind-speed measurements).

Table 1. Information collected about the study buildings.

Questions	Comments
Farm identification	Unique ID
Shed identification	Unique ID
Management system	CF vs AIAO
Class of pigs	Weaner, grower/finisher, dry sow, farrowing, deep-bedded
Age of pigs	Weeks
Age of buildings	Years
Farm size	Number of sows
Pen size	Length, width and area
No of pens/building	#
No of pigs/building	#
Volume of building	Length, width, height and volume
Average no. of pigs/pen	#
Drain/pit area	Percentage of pen area
Slat material	Concrete, steel, plastic etc
Flushing frequency	Eg. Twice a week
Cleaning method used	Pressure cleaning, hosing etc
Level of hygiene	Good, fair, poor
Solid or open pen walls	Concrete vs tubes
Feeder type	Wet/dry; single/multi-space etc
Feed presentation	Mash vs pelleted
Feeding regime	Ad-lb vs restricted
Ventilation type	Natural vs mechanical
Ventilation control	Manual vs automatic
Air inlets	Height of shutters/blinds
Ridge vent size	Width, height
Forced ventilation	Negative vs positive pressure
Climate control	Heaters or cooling devices
Roof/wall insulation	Eg. Sandwich panels, asbestos etc
Insulation thickness	Centimetres

Particle measurements
The concentration of airborne particles was determined gravimetrically using cyclone and "seven-hole" samplers for respirable (< 5 µm) and inhalable particles, respectively (SKC Inc., Pennsylvania). The sampling rate was controlled at 1.90 L/min for respirable and at 2.00 L/min for inhalable particles. The samplers were connected to GilAir (Gilian® Instrument Corp., USA) air pumps and were placed in a standardised position, usually above the walkways. An eight-hour sampling time was standardised throughout the project, starting at 09.00 h. The equipment was placed in the buildings the day before the actual measurement to allow animals to settle. A built-in timer automatically started the sampling routine. The sampling time was selected in the light of previous publications and aimed at sampling during times when the particle concentrations were likely to peak (Pedersen and Takai 1999).

Endotoxin measurements
For the estimation of endotoxins in airborne dusts, the exposed dust filters were extracted with sterile and pyrogen-free water at room temperature. The commercially available test kit used was based on the Limulus Amoebocyte Lysate (LAL) test. This test utilises the initial part of the LAL endotoxin reaction to activate an enzyme that in turn releases p-nitroaniline from a synthetic substrate, producing a yellow colour. The measurement of endotoxin concentration was performed using a microplate method, which involved reading the absorbency of each microplate well at 405nm, with distilled water used to adjust the photometer to zero. All disposable products used were pyrogen-free (SARSTEDT, Germany). Each filter was diluted with pyrogen-free water (Delta West Pty Ltd, Australia) at 25mL per filter. The optimum pH was 7 and was adjusted by the addition of NaOH or HCl. The water/dust suspension was vortexed for 20 seconds, shaken for 2 h at room temperature and centrifuged at 2000rpm for 10 minutes. A 50 µL aliquot was taken for subsequent analysis. For calibration, six standard solutions were made with the endotoxin of *E. coli* (BioWhittaker Inc., USA) with a control standard endotoxin (CSE) potency equivalent to 10 EU/ng. A multipoint calibration with 50 µL solutions with concentrations of 20, 5, 1, 0.5, 0.25 and 0.10 EU/mL was used and the magnitude of colour intensity measured by photometry. All data were analysed by linear regression and compared with a standard curve from the reference endotoxin and a coefficient of correlation of 0.97 or higher was accepted. Solutions of endotoxin-free water and lysate served as controls. The measurements were made using a QCL-1000® Chromogenic LAL test kit (BioWhittaker Inc., USA) with a Kinetic-QCL™ Reader (BioWhittaker Inc., USA).

Bacteria measurement
Sampling of airborne microorganisms was carried out using a standard Anderson sampler or six-stage bacterial impactor (Jones *et al.* 1985). Horse-blood-Agar (HBA) was used (Medvet Science, Australia) for the determination of the total amount of microorganisms. Samples were taken around mid-day between 11.00 h and at 15.00 h, usually in the centre of the animal house and above the pens. The flow rate during sampling was 1.9 L/min and the sampling time was 5 minutes. The exposed HBA plates were incubated at 37 ^{o}C under aerobic conditions and bacteria colonies were counted after 24h.

Temperature and humidity measurements
Self-contained and battery operated data loggers with built-in sensors (Tinytalk®-2, Hasting Dataloggers, Australia) were used to measure temperature and relative humidity in all buildings at each visit and outside temperature and humidity data were logged simultaneously. Sensors were placed as close to pig level as practically possible, without allowing the pigs to interfere with the instruments. Most loggers were attached to the ceiling or a beam, using wire cable and were lowered to pig level above a selected pen, representing the average condition of the shed.

Measurement of gas concentrations
Carbon dioxide and ammonia concentrations were measured continuously inside and outside the buildings using a Multi Gas Monitoring (MGM) machine (Banhazi and Cargill 2000) with built-in sensors. An electrochemical monitoring head (Bionics TX-FM/FN, Bionics Instruments Co., Japan) was used to detect the internal concentrations of ammonia and an infrared monitoring head was used to detect carbon dioxide (CO_2) (GMM12, Vaisala Oy, Finland). The MGM machine incorporated an air sampling system, which delivered air samples from the sampling points within and outside of the buildings to the actual enclosure containing the gas monitoring heads. Air was drawn at a nominal rate of 0.5-0.8 l/min from the sampling points and between the sampling points the system was purged using fresh air drawn from outside of the buildings. After each sampling point was monitored for 15 minutes, the system was purged for 15 minutes to flush out the sampling lines and zero the ammonia monitoring head. An electronic (voltage) tag was logged corresponding to the internal and external sampling sites, which enabled the automatic separation of the data. A computer program was built in MS Excel® to facilitate the automatic separation and graphing of the data. The program also contained the algorithms to calculate the amount of time spent above and below the relevant recommended levels. At the end of each data collection period the raw data was assessed by the data collectors. If drift had occurred in the raw data set (i.e. the data did not return to zero in the case of ammonia or to expected ambient levels in the case of carbon dioxide during the purge periods), the data was discarded. The MGM machine was frequently calibrated using a custom-made 2,500 ppm carbon dioxide mixture and a standard 50 ppm ammonia calibration gas mixture. All intake tubes had a filter attached to the end of the line to prevent deposition of particles in the sampling line.

Data storage and statistical analysis
Survey data collected in all states were transferred to a central location in South Australia for storage and analysis. A detailed model was developed to test various interactions based on fixed effects and covariates. This

was done using a general linear model procedure (PROC GLM) (SAS 1989). The effects treated as fixed effects were building type, assessment of hygiene level, management type and season. The effects treated as covariates were weight of pigs per building (kg), building size (m^3), ventilation air flow (m^3 air/hour), internal temperature (^oC), humidity (%), and farm size (number of sows). Due to model size restrictions only first order interactions could be tested. The models were developed from the maximum model tested by sequentially removing non-significant interactions and effects ($P<0.05$, based on type III estimable functions) until only significant effects and interactions remained. The results from this analysis presented in subsequent papers are based on LS means of predicted building values and the best-fit slopes, where this is relevant.

Assessment of methodology and instrumentation

The instrumentation kit used during the survey proved to be relatively easy to install/remove under field conditions. The large number of buildings included in the study and the varied nature of the buildings selected ensured that the study population of the buildings was representative. Particle measurements were done using a simplified instrumentation, compared to previous studies (Phillips *et al.* 1998). The commercial kit used for endotoxin measurements required considerable additional work and fine-tuning. However, as one operator conducted all analysis, the comparative concentrations of samples were consistent ensuring reliable interpretation of the statistical analysis. The self-contained temperature and humidity loggers proved to be useful and reliable instruments. The measurement accuracy of humidity sensors was occasionally questionable. However as large amount of data was collected and averaged over a period of time the overall results were considered to be reliable. The MGM machine performed well, but the ammonia sensors required frequent calibration. The transportation of the enclosure was sometimes problematic, due to the size and weight of the equipment. The statistical analysis applied proved to be sophisticated, sufficiently complex to ensure interpretable results.

The instrumentation kit is currently under development to further simplify the operation of the various components and therefore make it routinely available for the farming community for building assessment purposes (Banhazi 2003).

Conclusion

1. A large number of buildings were included in the survey to ensure a good representation of industry practices.
2. Particle, temperature, bacteria and carbon dioxide measurements proved to be relatively simple and reliable. Ammonia sensors needed frequent calibration and endotoxin measurements required extra care and fine-tuning.
3. The statistical modelling was sophisticated but necessary to enable main effects and interactions to be tested simultaneously. Simultaneous comparisons are important when many factors affect the variable being studied.
4. The kit is currently undergoing further development to be used routinely in livestock buildings by the farming community.

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Animal nutrition and health

Oral Communications

THE RISK ASSESSMENT OF CONTAMINANTS OR RESIDUES IN ANIMAL FEED USING TRANSFER FACTORS

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In recent years increasing attention has been paid to the risk posed to animal feed by chemical contamination. Cases concerning the contamination of milk, eggs and other animal products by potentially hazardous chemicals have sparked massive interest. Assessments of the risk to consumers posed by chemical contaminants or residues in animal feed have often been hampered by a lack of knowledge about how contaminants and residues behave when consumed by livestock.

Transfer database
In assessing consumer risk, the transfer of contaminants or chemical residues from animal feed to animal products is predominantly an unknown factor. To gain a better understanding of this transfer we performed a meta-analysis of public literature. The relevant data on the transfer of various groups of chemicals from animal feed to products of animal origin were gathered and recorded in a database. In total, over 200 records were entered into the database, each of which held information on the transfer of contaminants and residues. In our database, the quantitative transfer of a given chemical was recorded as its 'transfer factor'. This was defined as the ratio of the concentration of a chemical in animal products to the concentration of the chemical in animal feed. In total, more than 2,500 individual transfer factors were entered in the database.

Database-derived transfer factors
Besides the use of chemical specific transfer factors, the database was also used to assess the relative vulnerability of animal matrices to contamination by chemical compounds. In addition, statistical analyses were performed for various classes of chemicals. In the absence of compound-specific information, this provided the basis for a probabilistic assessment of the carry-over of substances.

The overall results of these analyses are given per matrix in the table below. As shown, fat and edible offal appear to have the highest transfer factors, whereas eggs, milk and meat tend to have lower transfer factors. Separate, similar statistical sub-analyses have been performed for various classes of chemicals.

The risk assessment of contaminants in animal feed: case study on nickel
To illustrate the value of the database, we envisaged a case study in which a raw material in animal feed may have been contaminated by nickel. The raw material in question was fat and its inclusion rate in dairy cattle feed was 6%.

As we had no actual information about nickel transfer from feed to edible products, or about the relative susceptibility of animal tissues to nickel retention, a worst-case scenario was used. We assumed complete absorption and retention of nickel by livestock animals and complete transfer to one of the edible commodities. To give the worst-case approach a little nuance, we assumed a steady state after feeding for about one week (> five times the plasma half-life). This would mean that only the cumulative nickel intake for one week would add to the ultimate residue levels in edible commodities.
Using these assumptions, the following presumed worst-case transfer factors were derived for dairy-cattle products: milk: 0.8; meat: 0.6; liver: 20; kidney: 74; fat: 20.

We used these figures as the basis for the risk assessment. In our case the feed's contamination level was expected to be at most 1.5 mg/kg feed (dry weight).

For a human-health risk assessment, a Tolerable Daily Intake (TDI) for nickel of 0.05 mg nickel per kg body weight per day (or 3 mg/person/day, assuming a body weight of 60 kg) can be used, as proposed by the Dutch National Institute of Public Health and the Environment (RIVM, 2000).

Finally, we used the consumption pattern as assumed in the health-risk assessment for the residues of veterinary medicinal products (i.e. daily consumption of 1.5 kg of milk and milk products, 100 g eggs and egg products, 300 g meat, 50 g fat, 100 g liver and 50 g kidney).

On the basis of these figures and the presumed worst-case assumptions, consumption of the commodities would lead to intakes amounting to the following percentages of the TDI:

milk: 60%; meat: 9%; liver: 100%; kidney: 185%; fat: 50% of the TDI.

These results indicate that in our worst-case approach it is possible that intakes (whether or not incidental) in excess of the TDI may occur. To verify this conclusion, information from the database on the nickel transfer factors was used to refine the risk assessment. This resulted in the following worst-case percentages:

milk: 2%; meat: 89%; liver: 55%; kidney: 28% of the TDI; insufficient data were available for fat.

Unexpected risk areas
Compared to the results of the worst-case approach, the database-derived information indicates that meat rather than edible offal may be the major source of nickel residue intake by consumers. In fact, database-derived information indicates that the consumer intake via meat would be about 10 times higher than that anticipated in

the worst-case assessment. By contrast, it appears that the intake via milk would be much lower than that assumed by the worst-case assessment. Even the combined intake of milk and tissue commodities is unlikely to cause an exposure in excess of the TDI.

When the worst-case scenario was compared to the database-derived transfer, we found that the database-derived factors offer a more accurate risk assessment. Where no information on the specific contaminant is available in the database, transfer factors may be calculated using data on compounds within the same chemical class or with comparable physico-chemical properties. A more accurate risk assessment may be possible using, for instance, the overall 90 percentile of the transfer factors for the representative chemical class for each of the animal products. Using the transfer database enables a better understanding of the transfer of feed contaminants and residues to animal products and results in a more realistic risk assessment.

Added value of the database

- The use of database-derived transfer factors enables more realistic risk assessment
- Even if little information is available, scientifically founded transfer factors can be derived using the data for comparable chemicals (in terms of either chemical group or physico-chemical properties)
- In cases where contaminated feed products are found, those products of animal origin most susceptible to contamination can be identified
- Rapid risk management decision-making and/or intervention is possible using the transfer database

Animal Product	**ɔr**						
	Average	**SD**	**Median**	**P90**	**P95**	**Maximum**	**Minimum**
Eggs	0.29	0.61	0.037	0.98	1.6	5.5	0.000030
Milk	0.12	0.22	0.020	0.38	0.56	2.2	0.00000011
Meat	0.20	1.4	0.013	0.26	0.50	31	0.0000025
Offal	1.4	6.0	0.12	3.0	5.0	126	0.000038
Fat	4.7	14	0.16	14	18	180	0.000000

Transfer of chemicals from feed to animal products: data for all chemicals together

MYCOTOXINS IN FEEDSTUFFS PRODUCED OR IMPORTED IN EUROPE FOR ANIMAL FEEDS

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Introduction

Works on mycotoxins are numerous in the world and have been reviewed recently (CAST, 2003). However, it is important to point out the subject in focusing on the European commercial feedstuffs and feeds because the European context is changing. Consumers are more and more educated and are interested in the sanitary quality of foods and feeds. The European Commission has the will to manage a high quality sanitary policy for human beings as well as for animals. Finally, the feed industry and farmers who produce home feeds are responsible for the quality of their feed production.

The main mycotoxins

Mycotoxins are non proteic molecules produced by some microscopic fungi belonging to different species. They are not necessary for the life of the fungi. All the fungi species are not genetically able to produce mycotoxins and in a species, all the varieties are not genetically able to produce them. Moreover, in a genetically able variety, production depends on environmental factors which are not always understood.

Mycotoxins are very numerous in the world (more than 400) but European feedstuffs and feeds are concerned by only a few of these toxins.
Aflatoxins B1, B2, G1 and G2 are mainly produced by *Aspergillus* outside Europe. Ochratoxin A is produced by different *Penicillium* (mainly *P. verrucosum*) in feedstuffs during their storage. Fusariotoxins are produced by different *Fusarium* species on cereals during their growth in the field because of a high aw needed both for the growth of fungi but also for the production of toxins. Fusariotoxins are very numerous and may be classified into the trichothecens group, the zearalenone group and the fumonisines group. Trichothecens are themselves classified in groups A (comprising T2 and HT2 toxins) and B (comprising deoxynivalenol and its acetyl forms, and nivalenol). Deoxynivalenol (DON) and its acetyl forms, nivalenol and zearalenone (ZEN) are mainly produced by *F. graminearum* and *F. culmorum*, whereas fumonisines are mainly produced by *F. moniliforme, F. verticilloides and F. subglutinans.*

Other mycotoxins are evoked from time to time. Among them, the alkaloids from ergot (*Claviceps purpurea*) are not completely eliminated from cereal production even the risk of finding them is very low. Similarly, *Alternaria* toxins have been identified in different feedstuffs and especially in rapeseed, but it is not known if they can be deleterious for animals in the practical conditions.

The main effects of mycotoxins in animal feeding

The effects of mycotoxins are often studied in a short time period with high concentrations in feeds but in practice, mycotoxin effects concern low doses consumed over a long time.

Mycotoxins may cause a great diversity of effects. A single mycotoxin may have different effects on an animal. Some mycotoxins have more pronounced effects in one animal species and not in other ones. Some mycotoxins concern an organ and not another one (and not always the same in all the species). The nature of the pathology is varied: aflatoxins and ochratoxin mainly induce cancer, DON mainly reduces feed intake (especially in the pig) and slightly modulates the immune system, ZEN has estrogenic effects especially in the pig, fumonisins reduce immunity defence and efficiency of vaccination, T2 and HT2 toxins reduce immunity and lead to tissue necrosis, alkaloids from ergot have effects on nervous system and constrict blood vessels.
The effects of association of mycotoxins are questioned, especially when mycotoxins contents in the feed are low.

Mycotoxins consumed by animals may only concern the productivity, the health and the welfare of the animals but in some cases, they may concern meat, milk and eggs which are consumed by humans. The main problems concern aflatoxin M1 in the milk (coming mainly from the metabolism of aflatoxin B1), and deposition of ochratoxin in the kidneys, liver and muscle.

How to evaluate the mycotoxin contamination

Mycotoxin contents are characterized by heterogeneous distributions. In a wheat field, DON content may range from 1 to 15. After harvest, the range of variation is less (because the most contaminated grains are small and remain in the field, and because the harvest mixes grains) but is still important. In a silo, cereal contamination by ochratoxin is also heterogeneous because filling the silo may be done with different cereal batches with different moisture content, or due to the migration of moist air among grains.
So it is important before measuring a mycotoxin content to ensure that the sample on which the measurement will be done is representative. The European Commission has published directives concerning sampling before aflatoxins or ochratoxin measurement but these directives are not well adapted to huge batches for the feed industry.

After isolation of a sample, toxins must be extracted from it. Some of the tasks realized for this purpose as size of particles obtained by grinding or saturation of immunoaffinity columns in the case of purification of fumonisins play an important role (Raimbault et al, 2004).

Measurement of mycotoxins may be done by different techniques. Rapid methods using immunoenzymatic reactions are proposed by different commercial firms. An immunoenzymatic test concerns one mycotoxin and only in a specific range of content. So such tests may be useful to sort batches, but are not precise. Moreover they cannot be used by non-specialized persons. Precise measurements are based on chromatography, especially

high performance liquid chromatography (HPLC) or gas chromatography (GC), with or without mass spectroscopy. GC allows the measurement of different trichothecens in a simple operation.
New ways are investigated such as the infra red (Petterson, 2000) or indirect measurement as the evaluation of gene Tri5.
Interlaboratory tests are carried out to improve the quality of analyses, but important differences between laboratories remain.

Occurrence of mycotoxins
In spite of these differences, the European Commission has organized surveys on contamination of food for human beings. These surveys, called scientific cooperative tasks (SCOOP tasks) concerned aflatoxins (2000), ochratoxin (2002) and fusariotoxins (2003). They can be used for evaluating some feedstuffs (grains). Other surveys have been carried out but few are published.
DON is the most prevalent mycotoxin in European cereals. In French wheat, about 3% of the batches have more than 1000 µg/kg on average; but regional effects may be seen each year. DON content in maize is higher than in durum, than in wheat, than in barley in France.
ZEN may be found in all cereal species but more often in maize than in other grains.
Fumonisines are quite specific for maize, and especially in batches grown in the south of Europe.
T2 and HT2 toxins are less prevalent.
OTA have been found in a few cereal batches, in low amounts.
Aflatoxins are mainly found in imported feedstuffs coming from intertropical areas but they have also been founded in small amounts in maize samples coming from the south of Europe. It is not understood if this situation is occasional or if it reflects the greenhouse warming of the Earth.

Factors influencing the production of mycotoxins
Factors influencing the production of mycotoxins by fungi have begun to be estimated. DON content in wheat and durum depends principally on the climate between flowering and harvest of the cereal (Barrier-Guillot, 2004). But some other factors can modulate this important factor. The quantity of contaminated residues coming from the previous crop increases the level of DON. This occurs when wheat is grown after maize, especially with maize grown for grain comparatively to the whole plant for silage (Obst, 2000); this occurs also when no tillage is applied, and there is an interaction between these two factors. The level of resistance of the variety to Fusarium attack influences the DON content, but this role is limited; moreover, the level of the resistance of all the varieties to *Fusarium* attacks is not well known. At last, the phytosanitary protection of wheat may play a role. Only triazoles are efficient against *Fusarium* and lead to reduced DON content in wheat; strobilurines, which are efficient against *Microdochium* - a fungus responsible for the same symptoms on wheat as *Fusarium* - are unable to limit wheat DON content when wheat is concerned with Fusarium head blight.
DON content in maize depends on the same factors as for wheat, but may also be influenced by a late harvest or by delays between harvest and drying, or by storage in cribs.
Factors of variation of ZEN content have not been studied so well as for DON, and are considered as the same ones.
Fumonisines contents are thought to be influenced by hot summers and wounds on grains caused by insects, especially the European corn borer (*Ostrinia nubilalis*). The combat against this insect may be conducted by classical ways but it could be done by using Bt varieties (Bakan et al, 2002).
Ochratoxin A content depends on grain moisture, and more precisely on the aw of the part of the batch where *Penicillium* development is possible.

Means of decontamination
Means of decontamination of contaminated batches by mycotoxins are numerous. Unfortunately, the practical means are not numerous (for example, washing grains may be used to eliminate a part of the fusariotoxines which are soluble, but this technique is not appropriate in industry). Sorting is based on the fact that small grains and residues of crops contain more fusariotoxins than medium sized grains, but is this technique economically interesting? Different clays have been tested to adsorb mycotoxins but success depends on the considered mycotoxin: percentage of mycotoxin adsorbed is higher for aflatoxin than for OTA than for ZEN than for DON (Devegowda et al, 2000). Studies are in progress to degrade mycotoxins by enzymes but commercial applications are still disappointing for DON (Dänicke et al, 2004). Ammoniation is currently used to inactivate aflatoxins in tropical feedstuffs such as groundnut meal.

Legislation concerning mycotoxins
European legislation concerning mycotoxins in feedstuffs and feeds is limited. Aflatoxins and ergot are ruled by directives 2003/100 and 2002/32.
European legislation dealing with mycotoxins for food is useful to know because it has consequences on the quality of batches which are designed to animal feeds. This legislation is made of regulations concerning aflatoxins, ochratoxin A and patuline; it also concerns ergot for intervention in the case of wheat and durum.
Projects concerning fusariotoxins in foods and different mycotoxins for feeds are in progress.

Conclusion
The effects of mycotoxins on man and animals must be considered case by case, in considering couples ‘a mycotoxin/a target which is an organ or a function of a given animal with its age and its sanitary conditions’. They must not be taken globally: all the mycotoxins are not as dangerous as aflatoxins.
Mycotoxins do not generally present a sanitary risk for animals in the European conditions, but represent a decreased performance of animals and therefore economical losses.
The fight against mycotoxins must be supported by a global strategy. Decontamination of feedstuffs by the feed industry is limited. So the device must be: prevention of contamination is better than cure.

PRESENCE AND RISKS BY MYCOTOXINS IN ANIMAL NUTRITION

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A variety of crops are susceptible to fungal invasion and might be contaminated with mycotoxins, originating from the secondary metabolism of moulds. It is estimated that 25% of the world's crop production is contaminated to some extend with these mycotoxins. Contamination of food and feed commodities with mycotoxins can affect human and animal health. The following overview is mainly based on the result of two desk studies, initiated by the Dutch Product Board Animal Feed with the aim to implement control measurements for mycotoxins in the entire animal production chain (Dutch Product Board Animal Feed, 2003).

Presence of moulds and mycotoxins in feed components

The most frequently found toxigenic mould species in feed components belong to the genera *Aspergillus, Fusarium, Penicillium* and *Claviceps.* These moulds are able to grow over a wide temperature range, varying from 0°C for *Fusarium* species up to 48°C for *Aspergilli*. According to their prevalence, the above-mentioned fungal genera can be allocated to two categories. These categories are field moulds (*Fusarium* and *Claviceps spp*) invading plants prior to harvest resulting in pre-harvest mycotoxin contamination and storage moulds (*Aspergillus* and *Penicillium spp*), invading stored feed commodities, resulting in post-harvest mycotoxin formation.

The allocation into two groups indicates also the possibilities to apply preventive measures to avoid or reduce mycotoxin contamination. Under field conditions, mycotoxin formation occurs in response to stress situations such as temperature challenges, draught or water stress, reduced plant resistance due to phytopathogens, improper use of fertilisers, pesticides or fungicidal agents, time point of harvest and structural damage during harvest. This indicates the reasons why the formation of mycotoxins at the pre-harvest stage is variable and difficult to predict depending on local agricultural practice and geographical circumstances.

In contrast, proper crop management, including drying, cooling and facilitation of proper storage conditions, can largely avoid post-harvest contamination. Post-harvest storage of feed commodities and subsequent processing can be subjected to control measures following the general principles of a HACCP-protocol.

The design of a control programme for mycotoxins, should be based on the expected prevalence of toxinogenic moulds and mycotoxins in individual feed commodities.

Aflatoxins are present in tropical and subtropical products such as oil seeds, trichothecenes and zearalenone in (mainly) cereals and maize silage, fumonisins in maize and products thereof from mainly only EU imported commodities. *Penicillium roqueforti* toxins can be found in ensilaged feeds due to the preference of this mould for an acidic environment. However, P. roqueforti toxin production in naturally contaminated material has found to be time dependent (Müller and Amend, 1997). This production pattern hinders not only a successful P. roqueforti mycotoxin monitoring in ensilaged commodities, but also affects the evaluation of actual adverse health effects in animals. Ochratoxins (particularly ochratoxin A), can be expected in Northern Europe in grains, nuts, peas and sometimes in grass pellets. *Claviceps* sclerotia, which can be found in rye and grass, contain ergot alkaloids. Ergot alkaloids are also produced by endophytic moulds which are mainly found in grasses in tropical arid areas such as *Lolium perenne* L. (Perennial Ryegrass) and *Fescue arundinacea* Shreb. (Tall Fescue grass). Grass cultivars used in Europe for grasslands for cattle are endophyte-free.

Taking into consideration that all above-mentioned toxins are able to induce clinical signs of intoxication at high levels and are known to impair health and productivity of animals at lower levels, a monitoring programme for these most relevant mycotoxins (in Europe) should be considered.

Mycotoxin exposure assessment of farm animals

An indication of the exposure to mycotoxins for the different categories of farm animals can be made on the basis of the average and the maximum concentration of mycotoxins in raw feed materials. For ruminants, the exposure assessment is more complex as the diet may consist of three types of feed: concentrate, by-products and forages; the latter generally represent 50-80% of the diet. Therefore, in table 1, the average and maximum calculated mycotoxin concentrations in a regular feed for only poultry and pigs are given.

The outcome of these calculations allow the conclusions that the observed zearalenone concentrations in feeds are high enough to adversely affect health and reproduction of gilts and sows. The maximum safe limit (ML)-value for zearalenone in diets for sows, as formulated by the German authorities (Dänicke et al., 2001) is 250 µg/kg and for gilts even 50 µg/kg. Poultry is not very sensitive for zearalenone (> 25,000 µg/kg); even the maximum zearalenone concentrations in poultry feeds are far below the safe ML-value. The assessed deoxynivalenol concentrations are likely to reduce performance in pigs. The mean concentration is below the safe ML-value of 1,000 µg/kg for pig feeds and 5,000 µg/kg for poultry feeds (Dänicke et al., 2001), but the calculated maximum concentration is clearly higher, indicating that frequently negative health and performance effects may occur in pigs. Poultry and pigs are both sensitive to ochratoxin A. The Dutch Product Board Animal Feed has set a safe ML-value for ochratoxin A in feed of 50 µg/kg and 200 µg/kg for respectively pigs and poultry (pers. com.).

The mycotoxins aflatoxins, fumonisins and ergot alkaloids occur less frequently in feed commodities or at low concentrations (av. and max. conc. not shown) that adverse effects induced by these toxins seem to be less significant in poultry and pig feeds in Western Europe.

Table 1 Average and maximum calculated mycotoxin concentrations (µg/kg feed) in an average regular feed for poultry and pigs

Animal category	Deoxynivalenol		Zearalenone		Ochratoxin A	
	Average	Maximum	Average	Maximum	Average	Maximum
Broiler	18	198	5	50	0.2	4.6
Layer	30	404	7	32	0.1	5.0
Turkey	55	805	16	122	0.5	12.5
Fattening pig	335	5793	84	1150	3.4	91.4
Piglet	65	917	11	181	1.4	28.4
Gestating sow	182	3390	208	1346	2.9	65.6
Lactating sow	285	5834	84	1160	4.6	113.9

Forages generally represent 50-80% of the diet of dairy cattle, meaning that they have a significant impact on daily mycotoxin intake by dairy cattle. Grass and maize are the main forage crops in Europe. The average and maximum daily intake of deoxynivalenol and zearalenone by dairy cows showed that maize silage contributed approximately 80% of the daily deoxynivalenol intake and approximately 50% of the daily zearalenone intake, indicating that maize silage is a major source of mycotoxins in the diet of dairy cows (Driehuis and Te Giffel, 2003). However, there is no evidence that mycotoxins in rations for dairy cattle, *i.e*. concentrate with forage constitute a serious risk factor with respect to animal health.

Carry-over of mycotoxins into animal derived products

Farm animals can be substantially exposed to mycotoxins via the diet, but the transfer to animal products is usually much less. For dairy cows, the rumen act as an effective "filter" against mycotoxins, so the transfer of mycotoxins to milk is extremely low (0.03% or lower) except for the carry-over of aflatoxin B_1 to aflatoxin M_1 which varies between 1-6%. In order to prevent too high a concentration of aflatoxin M_1 in milk (> 0.05 µg/kg milk), European Community countries regulate the content of aflatoxin B_1 in feed: max. 5 µg/kg feed for dairy cows.

For pigs and poultry the carry-over of fumonisins, trichothecenes and zearalenone in edible tissues and eggs is very low due to poor absorption (fumonisins), rapid metabolisation (zearalenone) and rapid metabolisation plus excretion (trichothecenes). Ochratoxin A is an exception with carry-over rates of 5-20%, as it binds to plasma-proteins once absorbed, resulting in accumulation in the animal body. Accumulation of ochratoxin A in kidneys and to a lesser extend in liver and muscles has been reported which resulted in regulations for pig organs and meat in some EU member states. For the consumer however, the contribution from animal derived products to the total intake of ochratoxin A is small compared to plant derived products.

Conclusion

Feed components are often contaminated with mycotoxins, mostly at lower levels but the degree of contamination may change from year to year. A monitoring programme for the most relevant mycotoxins should be considered. Evaluation of mycotoxin exposure to farm animals revealed that negative health and performance effects of pigs may occur with deoxynivalenol or zearalenone contaminated feed. The risk for animal production losses is low for poultry and dairy cattle. Carry-over of mycotoxins to animal products should under modern agricultural practice not pose a human health hazard if proper control measures are in place, preferably based on HACCP principles.

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THE CHALLENGE OF FINDING ALTERNATIVES TO ANTIBIOTICS GROWTH PROMOTERS

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Introduction–The ban of antibiotic growth promoters

The animal feed industry worldwide has been using antibiotics for over 50 years. To date antibiotics are used in farm animals at therapeutical levels to control actual disease and at subtherapeutical levels to promote growth and feed efficiency. However, more recently, the use and apparent over-use of antibiotics in animal feed as been widely discussed in the scientific literature, at scientific meetings and in the general press. The main concern is the emergence of a so-call superbug, an antibiotic resistant human pathogens, after the prolonged use of antibiotics in animal feed (22). The first step toward controlling the use of antibiotics as growth promoters was made by the Swann Committee in 1969 (29). This committee initiated restriction of the use of AGPs without veterinary prescriptions. As a result of increased pressure from consumer groups to further reduce AGP in animal feed, Sweden was the first country to implement a partial ban on the use of AGPs in farm animals in 1986. Sweden was joined by the European Union (EU), which placed a partial ban on the use of AGP in 1997 which will be replaced in 2006 by the general ban of all AGP (including ionophore anti-coccidials) in all animal feed. In the US one of the largest purchaser of meat, McDonalds Corp. has adopted a police that prohibits its supplier from using medically important antibiotics as growth promotants (18). Some group heavily criticize the total ban of AGP arguing that such a ban follows "precautionary principles" rather than scientific facts (28). Despite this, it appears to be inevitable that we will face a global ban on the use of antibiotics as growth promotants in the not too distant future. The animal industry will be forced to develop alternate strategies to maintain current standards of animal production, animal health and welfare. The objective of this paper is to discuss some of the challenges faced by the industry without AGP as well as some of the strategies which can replace AGP now and in the future.

The use of AGPs–Advantages and disadvantages

The use of antibiotics in animal feed has a wide range of benefits. Undoubtedly, AGP are an effective tool to improve growth performance in farm animals. In a review of over 12'000 studies Rosen, 1995 (25) concluded that antibiotics will improve growth and FCR in 72% of the time. However, the use of AGP has wider implications than just improving performance. AGP selectively modify the gut flora, suppress bacterial catabolism, reduce bacterial fermentation and reduce the intestinal wall thickness, all of which lead to increased health, increased nutrient availability for the animal and subsequently increased growth performance (3). Improved feed utilisation means that feed resources will last longer. This is of particular relevance when feed ingredients are limited due to extreme weather conditions and poor crop yield. The more efficient use of nutrients by the use of AGP results in a significant reduction of nutrients that are excreted into the environment (6). Furthermore, the selective use of AGP has a major impact on overall animal health and welfare. One of the main reasons AGP are still used at present is to protect animals against subclinical infections of such as clostridial infections (Necrotic enteritis), E. coli infections (post-weaning diarrhoea in piglets) or coccidial infections.

The biggest concern on the use of AGP is the occurrence of resistance to these AGP as well as the occurrence of resistance to antibiotics used in human medicine. There is considerable controversy between leading scientists as to whether the ban of AGP in feed is justified on the basis of increasing resistance. Veterinarians defend the use of AGP on the basis that there is no link between the use of AGP in feed and any resistance pattern in human medicine (7, 28). In addition, the ban of AGP, antibiotics that are generally not used to treat human diseases, has led to an increase in the use of therapeutical antibiotics that are also used to treat human disease. As a result there is now a trend of increased resistance to antibiotics used in hospitals of human pathogens such as *S. typhimurium*, *E. coli* or *C. jejuni* (8). There is also considerable doubt over whether a simple ban of AGP will reduce or eliminate resistance. A study at the University of Kentucky showed that even after the complete withdrawal of all antibiotics, population of antibiotic resistant bacteria can survive in a pig herd for decades (20). This is in direct contrast to the reports from Denmark. The Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) has reported that after the ban of AGP in Denmark the occurrence of resistance to *E. faecium* has drop significantly (8). Possible the most direct link between the use of AGP and the increase resistance in a human pathogen is the occurrence of vancomycin resistance enterococci (VRE) in hospitals and in the general population (5, 24).

Despite this apparent controversy on the benefits of a total ban of AGP in animal feed it seems to be very unlikely that any banned antibiotic will be reintroduced. The challenge to the animal industry is therefore to find acceptable and effective alternative additives.

Manipulation of the intestinal microflora without AGPs

Antibiotic growth promoters work primarily by reducing the microbial load in the intestine. In the absence of a microflora the demands on nutrients to maintain intestinal tissue and the immune system is reduced, hence more nutrients are available for growth and production. It is known that germ-free animals have increased performance parameters compared to 'conventional' animals (19, 31). The key to a successful animal production without AGP is clearly controlling and maintaining a healthy and diverse gut microflora. Reports in the literature emphasis the fact that the incidence of clostridial infections is significantly higher in birds fed diets based on wheat, barley, oats or rye containing high levels of indigestible soluble NSP which

leads to increased digesta viscosity and decreased digesta passage rate and nutrient digestibility (4). A highly viscous intestinal environment will increase the proliferation of facultative anaerobes like gram-positive cocci and enterobacteria (30). Larger amounts of undigested material in the small intestine together with a slower flow of digesta increases the chances of rapid bacterial colonisation. Pluske, 2001 (23) showed that the incidence of Porcine intestinal spirochaetosis (PIS), swine dysentery (SD) and post weaning diarrhoea is closely related to the amount of indigestible starch and non-starch polysaccharides in the diet and the proliferation of pathogenic bacteria in the intestine. Similarly the use of poorly digestible protein sources alters the microflora and creates favorable condition in the intestine for the proliferation of pathogens. The microflora population depends very much on the balance between communities of organisms and the diet composition as the source of available substrates for microorganisms. The colonisation of potential pathogen is greatly reduced in animals fed highly digestible and balanced diet according to their nutrient needs.

Knowing that specific feed ingredients can influence the intestinal microflora is a powerful tool to formulate feed rations without AGP. In additions, a number of possible feed supplements have been identified that specifically alter the intestinal microflora and eliminate potential pathogens. It has to be pointed out that most of these supplements possess a distinctly different mode of action to AGP, hence these become clear alternatives to AGP rather than merely replacement of the currently used AGP.

Alternative additives

The numbers of publications on the efficacy of possible alternatives has been steadily growing over the last years. The basic mode of action of these supplements can be divided into four basic groups with distinct strategies: 1) improvement of nutrient utilisation by the host (exogenous feed enzymes); 2) stimulation/modulation of the immune system (cytokins, vaccines, gluco-and mannanoligosaccharides (MOS); 3) stimulation or introduction of beneficial bacteria (probiotics or direct fed microbials, fructooligosaccharides (FOS)) and 4) direct reduction of pathogens (MOS, organic acids, botanicals and herbs, bacteriocins, antimicrobial peptides, bacteriophages). Within these general categories there are hundreds of commercial products available claiming to be as effective to improve growth performance and animal health. Rosen, 2004 (26) proposed a seven question test by which producers can assess the potential value of a potential alternative. Two of the central questions in this test are the number of feeding test conducted and the frequency of positive response. Many replacement products have only been recently developed and therefore have not been tested under a wide range of condition. Two of the potential replacements, which have been extensively tested under scientifically controlled feeding tests, include exogenous feed enzymes and mannan oligosaccharides (MOS).

Enzymes: Today the use of exogenous enzyme supplementation is almost standard in all pig and poultry feed. The efficacy of these enzymes to improve animal growth performance as been established in over 2500 publications (27). Inclusion of gylanases and phytases significantly improves nutrient availability by depolymerising indigestible feed ingredients such as phytate and soluble NSP. As a result nutrient digestibility by the host is significantly increased and bacterial population in the small intestine is reduced (2). Apajalahti, J, 2000 (1) suggested further that the depolymerisation of larger arabinoxylans in wheat with xylanase produced xylo-oligomers and xylose which could only be partially utilised by the microflora. Subsequently the total number of bacteria in the ileum was reduced by 60%. However, it as to be mentioned that the inclusion of exogenous enzymes are only useful if the diets contain the specific substrate for the enzyme to work on.

Oligosaccharides: Unlike exogenous enzymes mannan-oligosaccharides (MOS) have little influence on nutrient utilisation. The growth promoting effect of MOS is primarily based on inhibiting colonisation of pathogenic bacteria by blocking type-1 fimbriae on the bacteria surface (10) and the improvement in overall intestinal health by improving gut integrity and modulating the immune system (9, 11, 15). It has also been reported that MOS has a direct influence on nutrient utilisation in the intestine. Addition of MOS can improve specific population of microbes with enhance fibre fermentation capacity and reduce the population of microbes using starches and sugars (12, 16). The relationship between the specific modes of action of MOS (Bio-Mos®, Alltech Inc.) and the effects of animal growth performance and health under a range of conditions have clearly been establish in over 300 research trials and scientific publications and (13, 14, 17, 21).

Conclusions

Consumer demands and legislative pressure will dictate the future use of AGP worldwide. The challenge for producers is to find suitable, reliable and most importantly cost effective replacements for AGP for a sustainable and successful animal production in the future.

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MANIPULATING INTESTINAL MICROFLORA THROUGH NUTRITION

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Introduction

Scientific, political and market developments are forcing animal production to reduce the use of antibiotics. However, in order not to impact or at least to minimize negative effects on animal well-being, food safety and production efficiency alternative measures have to be taken to maximize animal health when reducing the use of antibiotics.

A healthy digestive system is crucial for optimal animal performance. However, due to its large surface area and the heavy microbial load the gut is a vulnerable site for pathogen entry into the body. The large surface is necessary to optimize nutrient absorption. To allow an efficient transfer of nutrients to the blood the gut is only protected with one layer of epithelial cells. Unfortunately, this thin layer does not only facilitate nutrient transfer, but also weakens the gastro intestinal (GI) tract in keeping pathogens from entering the body.

Therefore, an array of additional protection systems is in place to minimize the risk of intestinal disease. Mucins and glycoproteins associated with the intestinal brush border serve as important barriers protecting the delicate absorptive surface from the abrasive action of feedstuffs, bacteria colonization, and toxins. Endogenous acids, digestive enzymes and bile reduce bacterial growth. Digestive flow and peristaltic movements transport the digesta through the digestive tract, and with it bacteria, thus limiting bacterial development. To further optimize gut protection the animal has devoted more than half of its immune cells to protecting the digestive tract. In addition, the GI microflora plays a crucial role in gut defense. Through different complex mechanisms beneficial bacteria limit the growth of pathogens, trying to exclude them from the system (Rolfe, 1991).

Profound knowledge of the development and composition of the GI microflora and its regulatory forces is essential to understand the dynamic of the GI microflora as well as interactions with feedstuffs and feed additives.

Competitive Exclusion

Competitive exclusion (CE) implies the prevention of entry or establishment of one bacterial population into the GI tract because a competing bacterial population already occupies potential attachment sites. To be able to succeed, the latter population must be better suited to establish or maintain itself in that environment or must produce compounds inhibitory to its competition (Bailey, 1987). Nurmi and Rantala (1973) were the first to apply the CE concept to domestic animals. The mechanisms, which are involved in CE, are very complex. The mechanisms can be grouped in direct and indirect mechanisms. Indirect mechanisms are the result of the normal microbial flora altering the physiologic response of the host, which in turn affects the interaction between the host and microorganism (Rolfe, 1991). Direct mechanisms are exerted by different bacterial populations on each other.

Two basic nutritional approaches can be applied to promote the beneficial GI microflora. First, by feeding beneficial bacteria (probiotics) we can support and complement the endogenous microflora. The effect of probiotics is well documented in the literature. Hollister *et al.* (1999) reduced salmonella colonization in chicks by feeding a live cecal culture from salmonella-free poultry. Fedorka-Cray *et al.* (1999) has shown similar response to microbial cultures in young pigs. Gram-positive bacteria, including *Lactobacillus, Enterococcus, Pediococcus, Bacillus,* and *Bifidobacteria*, and fungi of the *Saccharomyces* (yeast) genus are often fed after antibiotic therapy as a means of re-introducing a beneficial flora to the gut of affected animals.

A second possibility to influence the outcome of bacterial competition comes through influencing specific mechanisms, which have an impact on CE, such as:

- GI environment
- Substrate / nutrient supply
- Substances with antimicrobial properties
- Inhibiting of bacterial adhesion
- Stimulation of gut peristalsis
- Modulation of the immune response

All those mechanisms can be influenced through specific modifications of the diets.

Substrate availability controls proliferation of the GI microflora

Gut health and enteric disease resistance is often dependent upon the digestibility of feed components and feed formulation. Poorly digested protein meals cause the proliferation of putrefying bacteria in the hindgut, which increases toxic metabolites (ammonia and biogenic amines) that compromise gut health. In general, antibiotics are most effective in birds fed diets containing high levels of indigestible protein (Smulders *et al.*, 2000). Similarly, poultry fed diets containing high levels of poorly digested non-starch polysaccharides from wheat, barley or rye are more susceptible to enteric disease, such as necrotic enteritis (Riddell and Kong, 1992; Kaldhusdal and Skjerve, 1996). Langhout *et al.* (1999) observed that dietary NSP significantly increases gut populations of pathogenic bacteria at the expense of beneficial bacteria. However, the digestibility of wheat, barley, rye, triticale and even corn-based diets can be significantly improved through use of exogenous enzymes including xylanases, phytases and ß-glucanases (Rosen, 2001). Because supplemental enzymes mediate their beneficial effects primarily by enhancing feed digestibility and nutrient availability to the host, they also influence the gut microbial ecosystem. The use of enzymes has been shown to alter the gut microflora populations in the small intestine and caeca (Choct *et al.*, 1996; Hock *et al.*, 1997; Bedford, 2000a) and reduce mortality rates (Rosen, 2001). Such a benefit is brought about by a more rapid digestion and absorption of starch, protein and fat from

the small intestine, which effectively limits available substrate for the resident flora.

Beside modifying nutrient availability from raw ingredients for bacterial fermentation, the addition of specific carbohydrates such as lactose or FOS, which are preferably fermented by beneficial microorganisms, can positively influence the composition of the GI microflora. The effects of dietary FOS on the intestinal microflora are well documented (Mitsuoka *et al.,* 1987; Hidaka *et al.*, 1991; Patterson *et al.* 1997; Hidaka and Hirayama, 1991). Hidaka *et al.* found that consumption of 8 g FOS/day increased numbers of bifidobacteria, improved blood lipid profiles and suppressed putrefactive substances in the intestine of humans. However, Waldroup and coworkers (1993) found that supplementing broilers with 0.375% FOS had few consistent effects on production parameters or carcass *Salmonella* concentrations. These authors also caution of possible antagonism between FOS and BMD.

Recent evidence suggests that novel oligosaccharides with improved anti-pathogen effects in probiotic microorganisms can be synthesized (Rastall, personal communication). When the relationship between sugar structure and those effects are better understood, it should be possible to design novel prebiotics to maximize the protective effect. Another dimension to influence animal health through carbohydrates is to exploit the involvement of carbohydrates in cell-to-cell interactions. Carbohydrates are important surface entities of animal and bacterial cells that function in a variety of ways to influence cell-to-cell communication, impact the immune system and allow bacterial attachment to the host. The science of understanding the sugars, which make up the cells and their structures is known as glycomics.

Carbohydrates, cell-to-cell communications and defense against pathogens

Carbohydrates project from the cell surface and form the antigenic determinants of certain cell types. One of the classical examples of this antigenicity is blood type in humans. The ABO blood group antigens are glycoproteins on red blood cells. Small differences in the terminal sugar residues distinguish the A and B blood-group antigens (Kuby, 1994) Mannose binding protein (MBP) is an integral part of the immune system. MBP in the serum can bind to terminal mannose groups on the surface of bacteria and interact with two serine proteases (MASP and MASP2), which ultimately lead to antibody independent activation of the classical pathway of the immune system (Roitt *et al.,* 1998). Bacterial infection is due in many cases to the ability of the bacteria to recognize host cell surface sugars and use specific receptors that allow them to attach, colonize, and in the case of pathogens, cause disease in the animal. Mannose-specific adhesins (the binding entity on the surface of bacterial cells) are utilized by many gastrointestinal pathogens as a means of attachment to the gut epithelium. One way to prevent pathogens from causing disease is to prevent them from attaching to the epithelial cells in the gut. Early studies using mannose in the drinking water of broiler chicks demonstrated that this therapy could reduce colonization rate of *Salmonella typhimurium*.

Purified mannose and a complex sugar called mannan oligosaccharide (MOS) have been successfully used to prevent bacterial attachment to the host animal by providing the bacteria a mannose-rich receptor that serves to occupy the binding sites on the bacteria and prevent colonization in the animal.

Several studies have been conducted examining the role of mannans and their derivatives on binding of pathogens to epithelial cells in the gastrointestinal tract. *E. coli* with mannose-specific lectins did not attach to mammalian cells when mannose was present (Salit and Gotschlich, 1977). Spring and coworkers (2000) used a chick model to demonstrate that MOS could significantly reduce the colonization of *Salmonella* and *E. coli*. Animal trials in other species show similar benefits in reducing pathogen concentrations. In dogs, as well as in poultry, reductions in fecal clostridial concentrations have also been noted with MOS supplementation (Finucane *et al.,* 1999; Strickling, 1999). Different researchers also found improved performance with MOS (Miguel *et al.,* 2002, Hooge, 2004a,b). It has been suggested that improvements in the GI microflora are a main factor leading to improved performance. While inhibition of mannose receptors are commercially exploited, adhesions which are specific for other sugars are currently being investigated.

Glycomics also plays a vital role in viral diseases. The influenza virus infects by first attaching to a cell surface carbohydrate called sialic acid. This attachment 'opens the door' of the cell and allows the virus to replicate within. The commercial drugs Tamiflu and Relenza shorten the duration of the flu by binding to the active site of an enzyme produced by the virus that frees the virus from the sialic acid. By tying up this enzyme, the virus cannot easily spread and infect other cells (Schmidt, 2002). There are also data examining a novel anti-human immunodeficiency virus (HIV) protein. This protein, called actinohivin, binds to a glycoprotein on various HIV strains and simian immunodeficiency virus (SIV) inhibiting viral entry into cells by binding to this envelope glycoprotein. Further investigation showed that only yeast mannan can inhibit the binding of actinohivin to these viruses. These results demonstrate that the mannose saccharide chains of the virus glycoprotein are the molecular targets of the anti-HIV activity of actinohivin (Chiba *et al.,* 2004). Sulfated galactomannans also demonstrate *in vitro* and *in vivo* activity against the flaviviruses, yellow fever virus and dengue virus (Ono *et al.,* 2003)..

Conclusions

The intestinal microflora itself is a unique protection system, as beneficial bacteria are continuously competing with pathogens through competitive exclusion (CE). Nutrition offers an array of approaches to influence different bacterial control mechanisms that play a role in CE. While mannan oligosaccharide is currently being used to improve health and production of animals, there are enormous possibilities to use other sugars as possible agents against pathogen infections.

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RECENT DATA ON THE PHYSIOLOGY, MICROBIOLOGY AND IMMUNOLOGY OF THE GUT OF PIGLETS AROUND WEANING. IMPLICATIONS FOR ALTERNATIVES TO IN-FEED ANTIBIOTICS

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Introduction

Weaning is a critical phase for pigs. It is characterised by a drastic but transient reduction in voluntary feed intake, a growth check and an increased susceptibility to gastrointestinal disorders, pathogens and diarrhoea. The EU ban on in-feed antibiotics and severe restrictions in the use of metals including copper and zinc, makes this period especially difficult to manage. Changes in the architecture and functions of the small intestine have been well documented (1), and restoration of intestinal integrity occurs within one to two weeks.

In terms of microbiology, the gut is colonised at birth and the microflora develops from a simple and unstable to a complex and more stable community. At weaning, major changes happen in the microflora composition as influenced by the diet and environment (2, 3). Developments in this field have been important over recent years, especially towards the non-cultivable bacteria, thanks to molecular techniques. At birth, the piglet is immunologically naïve and is dependent on the passive transfer of immunity from the sow to the piglet through colostral immunoglobulins. By contrast, the development of active immunity, that is the capacity to become tolerant to dietary antigens and commensal bacterial together with the ability to mount appropriate immune responses against pathogens, is still poorly understood (4).

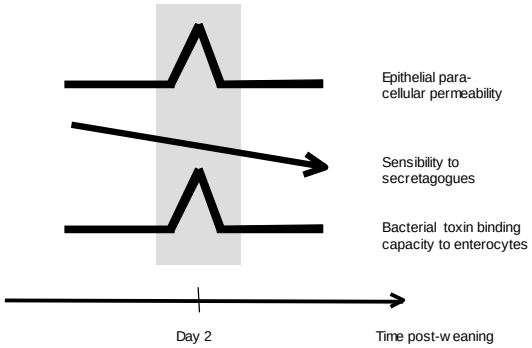
Here we report recent data obtained in intestinal physiology, microbiology and immunology in young pigs and some implications in the dietary management of weaning without antibiotics.

Intestinal physiology

The intestine executes various functions including digestion and absorption of nutrients, absorption-secretion of minerals and water, secretion of mucus, and epithelial barrier against pathogens and noxious substances. Villous atrophy and crypt hyperplasia of the proximal small intestine together with longitudinal alterations in digestive enzyme activity profiles have long been recognised as the hallmark of weaning (1). Several years ago it was hypothesised that an allergy to dietary proteins was the cause of these changes but more recent data suggest that post-weaning anorexia may also play a role (5).

Weaning has marked effects on the expression of cell protection systems such as heat shock proteins (HSP), with regional and temporal specific patterns (6). For example, the over-expression of HSP27 and HSP70 was precocious and more pronounced in the proximal segments of the gut. By contrast, HSP90 expression was unchanged or reduced, this happening later and more distally along the gut. *In vitro* studies with Ussing chambers recently revealed a hyper-secretory state of the mucosa of the proximal small intestine and colon 2 to 4 d post-weaning (7, 8). This was also associated with a transient increase in small intestinal paracellular (tight junction) permeability. By contrast, the intestinal secretory capacity to secretagogues, as stimulated with various bacterial toxins, was found to decrease with time throughout weaning (8) (figure 1).

Figure 1. Changes in intestinal physiology over time post-weaning in pigs (G. Boudry, unpublished).



Collectively, these data highlight the deep disorders in intestinal architecture and functions immediately post-weaning. They are probably related to the transient anorexia since many of them occur following fasting, as shown in various animal species. This acute period is then followed by the acquisition of a gastro-intestinal tract of adult-type anatomy and functions.

Intestinal microbiology

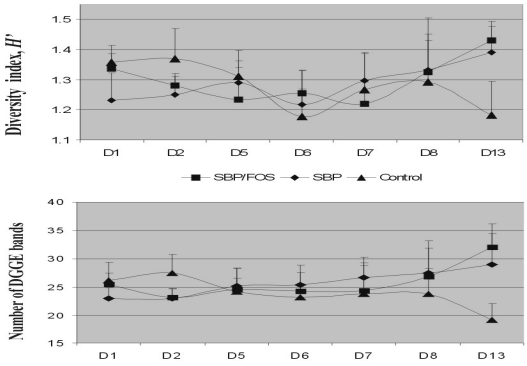
In pigs, the development of the intestinal microflora follows a rapid succession between birth and weaning, but it is relatively stable and dominated by lactic acid bacteria as long as piglets are kept suckling. However, marked qualitative and quantitative changes appear with solid feed consumption. For example, strict anaerobes such as *Bacteroides* establish in the large intestine, at a time when the number of facultative anaerobes is declining (9). In connection with health, some intestinal bacteria are known for their pathogenicity (*Clostridia*, *Salmonella*, etc.), others for their protective effects (*Lactobacilli*, *Bifidobacteria*), and some having variable influences depending on local conditions (*Escherichia coli*, *Bacteroides*, etc.). Modern molecular microbiology techniques have been applied recently to study the ecology of the porcine gastrointestinal tract. They have provided new insights into bacterial colonisation and its temporal changes, especially as far as the non-cultivable bacteria are concerned (10).

The effect of diets with two levels of fibre and with or without antibiotics was studied throughout weaning (11). Combining classical and molecular techniques revealed intestinal dominance of *Lactobacilli*. Irrespective of the diet, they were transiently depressed after weaning. Most of these *Lactobacilli* corresponded to *L. amylovorus* previously detected in Danish pigs (12).

The influence of dietary carbohydrate fermentability on the gut microflora was also investigated. Piglets consuming a diet supplemented with sugar beet pulp alone or mixed with fructo-oligosaccharides (FOS) showed a faecal bacterial community that was more diverse and more stable than the controls fed the non-supplemented diet (13) (figure 2).

Numerous bacterial species identified using molecular techniques did not correspond to those obtained by cultivation. For example, 16 rRNA gene sequences related to *Ruminococcus spp.* were detected in the colon of all the supplemented piglets but not in the controls (13). Thus, these non-cultivable bacteria would probably play an important role in the fermentation of dietary fibres in weaned piglets. In a similar manner, the responses of the gut bacterial communities upon the addition of inulin, lactulose, wheat starch and sugar beet pulp were analysed. The results indicated that the dietary addition of these fermentable carbohydrates support the growth of certain lactobacilli in the upper part of the intestine and lead to a higher bacterial diversity in the colon (14). Specifically, it was found that *L. amylovorus*-like populations were prevalent in the gut of the piglets fed with these fermentable carbohydrates. Such stimulation of the *Lactobacillus* community within the gastrointestinal tract of piglets may be of specific importance because of their possible antagonistic activities towards intestinal pathogens, a process termed "colonization resistance". Since a stable and complex commensal bacterial community is a prerequisite of a healthy gut ecosystem (15), the promotion of colonization resistance through the addition of fermentable carbohydrates (prebiotics) may be a comparatively easy way to improve enteric health at stressful times, such as early and abrupt weaning as experienced by piglets within a production environment (16).

Figure 2. Influence of supplementation of the diet with fermentable carbohydrates on bacterial diversity over time post-weaning in the faeces of piglets (13).



Collectively these data illustrate rapid succession at the time of weaning, and that some dietary formulas may improve the microbial balance, ensuring normal animal growth and nutrition of weaning piglets.

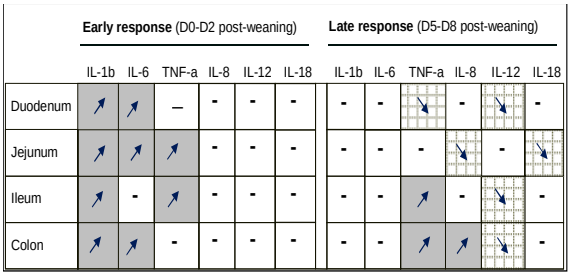
Intestinal immunology

The first immune line of gut protection after birth is passively derived IgA, because a passive transfer of maternal IgG during gestation is not possible as the pig has a six layered placenta. However, more complex immune pathways are required for the development of appropriate active immune responses and for the maintenance of gut epithelial integrity.

The immune system of the young pig is immature at birth, probably reflecting the lack of exposure to antigens. Then the structural development of the gut immune system depends on antigen exposure (17) for while at birth, the intestine is virtually devoid of lymphoid structures, the Peyer's patches develop during the first two weeks and the intestine is rapidly colonised with immune cells. Between weeks 2 and 4, CD4+ T cells appear in the lamina propria together with B cells expressing IgM. Cytotoxic CD8+ T lymphocytes in the epithelium and IgA+ B lymphocytes in the lamina propria appear from week 4 of age onward (18). Thus an adult-type intestinal immune system is reached by approximately 7 weeks of age. The piglet is able to mount active immune responses against viruses and dietary antigens from week 3 onward (4) but it acquires oral tolerance to dietary antigens only after week 8 (19). This inability to regulate immune responses to harmless antigens might contribute to post-weaning diarrhoea (20). Functional aspects, including intra-epithelial lymphocyte responses to mitogens and ability of T lymphocytes to secrete IL-2, have been shown to be transiently reduced at weaning (4). Also atypical (CD2+) cell types accumulate in the lamina propria, probably indicating a relocalisation of circulating immune cells to the gut (17). The dendritic cell network in the lamina propria and the Peyer's patches provide the basis for adequate antigen presentation. So far the development and function of these cells at the period of weaning are not known yet.

Recent investigations showed that after weaning, piglets express mRNA for both inflammatory (IL-2, IFNɔ, IL-12p40) and anti-inflammatory (IL-4, IL-5, IL-10) cytokines in all intestinal segments (E Sowa & HJ Rothkötter, unpublished). Inflammatory cytokines play a key role in inflammatory processes of the intestine, as shown in various laboratory animal species. In piglets, weaning is associated with intestinal inflammation (5). We studied in detail this period from the cytokine gene expression perspective (21).

Figure 3. Influence of weaning on intestinal cytokine gene expression in piglets (21).



During the first 2 days post-weaning, we observed increased levels of mRNA expression for IL-1ɓ, IL-6 and TNF-Ŭ along the small intestine and proximal colon (fig 3). This inflammation was contemporary with intestinal villous atrophy and reduced digestive enzyme activities of the mucosa (21). Then, expressions of IL-1ɓ and IL-6 returned to pre-weaning levels while that of TNF-Ŭ was still high in the ileum and colon. This might be related to post-weaning development of fermentations in the large intestine. Finally, mRNA expressions of IL-12 and IL-18 were not changed up to 2 days post-weaning but they were reduced thereafter, reaching probably adult-type profiles (21).

Feeding fermentable carbohydrates to piglets post-weaning favoured the reduction of IL-6 and IL-12p40 mRNA expression but it had limited impact on that of IL-1ɓ (IP Oswald *et al*, unpublished).

Therefore, beside the ontogenetic development of the local immune system of the pig, the gastro-intestinal tract displays

important changes in the gene expression of cytokines throughout weaning. The role of immune effectors in intestinal tissue alteration and restitution remains to be investigated.

Some dietary implications

Whilst post-weaning anorexia is often considered to be a primary factor in the aetiology of gut disorders in piglets (5), other factors stimulating early voluntary feed intake must contribute to enhancing gut health through physiology, microbiology and/or immunology pathways. Many alternative substances to in-feed antibiotics have been studied thus far. Here we highlight some dietary factors of interest only.

Different studies have shown that feeding liquid, fresh or fermented, diets stimulates appetite and improves gut condition (22). This helps to maintain gastric pH below 4 with high levels of lactic acid, thus inhibiting the outgrowth of pathogens (*E. coli*, *Salmonella*) and promoting the development of yeasts (3). However, growth is usually lower with fermented feed, probably due to degradation of supplied free amino acids.

Another example is spray-dried plasma (SDP). Although being an animal protein source non-authorized in the EU, this product has been demonstrated to stimulate feed intake (+25%) and growth performance (+27%) (23). Incidence and severity of post-weaning diarrhoea together with intestinal alterations are usually reduced. Beside improved feed palatability, other factors including plasma growth factors (IGF-1), non-immunoglobulin glycoproteins and total and *E. coli* specific antibodies are probably implicated (23). SDP reduced the tissue expression of inflammatory cytokine genes and density of immune cells in the intestinal mucosa (24, 25). However, piglets supplemented with SDP displayed surprisingly elevated immune responses and increased intestinal tissue alterations following stimulation with bacterial lipo-polysaccharide (25).

Feed composition per se appears to have contrasting effects on gut health criteria immediately post-weaning (5, 7, 26). For energy, supplying either glucose, lactose or starch to weaner pigs did not make any difference on post-weaning intestinal alterations (26). The impact of dietary fibre supplementation is contrasted and may depend on the type of fibre. Danish investigations reported a reduction in intestinal coliform populations (3) while Australian researchers noted an increased colibacillosis (27) post-weaning. As mentioned above, association of carbohydrates with different fermentability properties are thought to be protective to the gut of young pigs (13-16).

Supplying protein in either a native or a partially hydrolysed form did not make any difference (26). By contrast, various nitrogenous compounds have proven to be of interest. Supplementing diets with glycine and alanine was earlier shown to reduce post-weaning diarrhoea through the stimulation of the production of the so-called anti-secretory factor (28). Glutamine, glutamate and arginine were also shown to have positive effects on growth and intestinal tissue alterations (29, 30).

Thus, various dietary solutions are possible for improving gut health status post-weaning in pigs. However, further research is warranted to determine the optimal doses and possible associations between compounds.

Conclusion and perspectives

Numerous factors interact with developmental patterns for generating spatial-temporal changes in the intestinal physiology, microbiology and immunology of the pig, often leading to transient disorders at weaning. Recent data has allowed the definition of a first phase of acute responses including intestinal hyper-secretion and increased tight junction permeability together with cell protection system (HSP) and pro-inflammatory cytokine gene over-expression. This is followed by a chronic phase of restoration of intestinal architecture and functions leading to maturation towards more adult-type profiles. Weaning also appears to favour a transient instability and reduced diversity of the intestinal microflora. Its stabilisation could be stimulated by subtle manipulation of dietary carbohydrate fermentability. Recent data highlighted the fact that some, but not all, dietary factors could contribute to alleviate post-weaning intestinal disorders.

The cross-talk between the intestinal epithelium, the microflora and the local immune system are still not clearly understood and, therefore, awaits further investigation. Efforts should also be aimed at elucidating the modes of actions of in-feed antibiotic alternatives with known positive properties and at developing new solutions, more natural, and acceptable by the consumers.

Acknowledgements

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THE IMPORTANCE OF DIETARY TRYPTOPHAN FOR PRESERVING GROWTH AND CONTROLING INFLAMMATORY RESPONSE OF WEANED PIGS SUBMITTED TO IMMUNE STRESS

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Introduction

Immune system stimulation prevents the pigs to express their growth performance. This is explained by a decrease in feed consumption but also by the existence of a competition between immune system and growth process for nutrient utilization. Preliminary results showed that pigs suffering from a chronic lung inflammation had lower plasma tryptophan (Trp) concentrations than healthy pair-fed pigs suggesting an increased Trp utilization for other metabolic purposes than for body protein deposition [3]. Moreover, inflammation caused an induction of indoleamine 2,3 dioxygenase (IDO) activity an enzyme involved in Trp catabolism through the kynurenine pathway [4]. In this article we present experimental data suggesting that Trp availability for growth may be decreased by immune system activation and that Trp metabolism is involved in the control of inflammatory response.

Material and Methods

Experiment 1: this experiment was conducted to assess the limiting character of Trp for post weaning growth performance in pigs submitted to a moderate immune system stimulation obtained by depressing the quality of environment where pigs were kept [2]. To do that, 20 blocks of four littermate piglets from INRA-UMRVP herd and weaned at 28 days of age were constituted according to their body weight (7.8 kg). Within each block, one piglet was affected to one among the four treatments resulting from a 2 x 2 factorial design: two levels of dietary Trp (adequate and deficient diets) and two levels of sanitary status (clean, with dietary antibiotics, *vs* unclean environment, without supplement). The experimental period, from weaning to 50 d post weaning, was divided into 3 periods : the first 20 days with a phase I type diet, the following 20 days with a phase II type diet then a 10 d period following transfer to the growing unit also with phase II diet. Phase I and II Trp adequate diets (Trp-adeq) were formulated to meet nutritional requirements of post weaning piglets [8] whereas Trp supply was decreased by 20% in the deficient diets (Trp-def). Pigs were fed according to body weight at restricted feeding level in order to suppress or limit feed refusals. Pigs were weighed weekly and blood was taken 12, 33 and 47 days post weaning after an overnight fast for analysis of plasma concentrations of amino acids and haptoglobin, a major acute phase protein (APP) in pigs.

Experiment 2: a second experiment was designed to study Trp metabolism and its interaction with pig inflammatory response. Ten blocks of three littermate pigs (40 d of age, 11.8 kg) were constituted on their body weight basis. Pig's genotype and experimental diets (only phase II diet was used) were the same as those used in the previous experiment. Within a block, pigs were affected to one of the three following experimental groups: 1) healthy control pigs fed the Trp-def diet, 2) pigs suffering from lung inflammation induced by an intravenous injection of a single dose of complete Freund's adjuvant (CFA) and fed the Trp-def diet or 3) the Trp-adeq diet. Within a block pigs were fed the same amount of feed. Blood samples were taken every two days and pigs were slaughtered 9 days after the induction of lung inflammation for tissue sampling.

Results

Experiment 1: there were no significant interactions between dietary Trp and sanitary conditions on growth performance, plasma Trp and haptoglobin concentrations. Despite restricted feeding and contrary to what was observed in a previous experiment [2], pigs kept in an unclean environment had lower feed intake (AFI) than control pigs. From weaning to 19 d post weaning then from 40 d to 50 d post weaning (table 1) daily gain (ADG) and feed per gain (F/G) were significantly altered by depressing the quality of the environment.

Table 1. Effect of sanitary status and dietary Trp level on growth performance.

	Trp-def		Trp-adeq		Main
	unclean	clean	unclean	clean	effects
AFI, g/d					
0-19d	256	278	261	285	S
20-40d	744	754	758	765	T
41-50d	859	943	926	977	S, T
ADG, g/d					
0-19d	174	207	194	227	S, T
20-40d	483	476	492	488	
41-50d	322	459	399	508	S, T
F/G					
0-19d	1.71	1.38	1.36	1.26	T
20-40d	1.57	1.57	1.54	1.57	
41-50d	3.62	2.06	2.45	1.94	S

Main effects correspond to a significant effect ($P < 0.05$) of sanitary status (S) and dietary level of Trp (T).

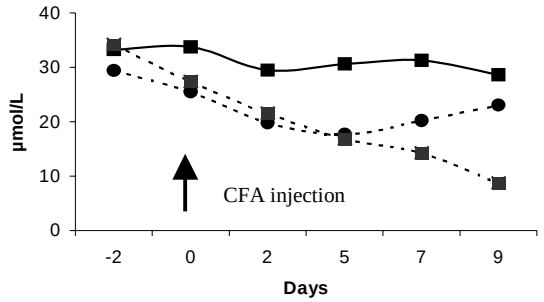
At 12 and 47 d after weaning pigs kept in the unclean environment had lower plasma Trp and higher haptoglobin concentrations (table 2). The effect of sanitary status on Trp concentrations remained significant when values were adjusted to constant feed intake by covariance. Trp deficiency induced lower growth performance and plasma Trp concentrations but did not affect haptoglobin concentrations.

Table 2. Effect of sanitary status and dietary Trp level on haptoglobin and Trp plasma concentrations.

	Trp-def		Trp-adeq		Main
	Unclean	clean	unclean	clean	effects
Haptoglobin, mg/ml					
W+12d	2.11	1.46	2.55	1.16	S
W+33d	0.74	0.87	0.63	0.34	
W+47d	2.12	1.45	2.58	0.93	S
Trp, nmol/ml					
W+12d	18.6	24.7	21.1	29.6	S, T
W+33d	22.9	23.9	27.6	30.3	T
W+47d	14.0	22.8	21.9	28.9	S, T

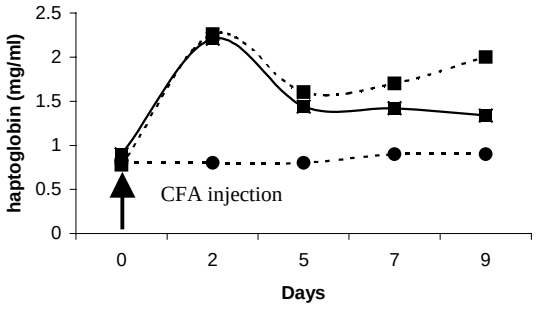
Experiment 2: pigs suffering from chronic lung inflammation exhibited a strong decrease in their plasma Trp concentrations when they were fed a Trp-def diet (figure 1) whereas they were able to maintain plasma Trp concentration when they were fed a Trp-adeq diet.

Figure 1. Plasma Trp concentrations in healthy control pigs fed a Trp-def diet (--●--), CFA fed a Trp-def diet (--■--) or a Trp-adeq (—■—).



In pigs fed the Trp- def diet, IDO activity measured in the lungs was induced by inflammation (1 *vs* 31 nmol of kynurenine produced from Trp/mg of protein/h) but IDO induction caused by inflammation tended to be less pronounced in pigs fed the Trp-adeq diet (12 vs 31 nmol of kynurenine produced from Trp/mg of protein/h). In addition, pigs suffering from chronic lung inflammation had lower signs of inflammation (haptoglobin response presented on figure 2, rectal temperatures and lung lesions observed post mortem) when they were fed the Trp-adeq diet.

Figure 2. Plasma haptoglobin concentrations in healthy control pigs fed a Trp deficient diet (--●--), CFA fed a Trp deficient diet (--■--) or a Trp adequate diet (—■—).



Discussion

Our studies showed increased levels of plasma haptoglobin in pigs suffering from lung inflammation and in pigs kept in unclean conditions. Haptoglobin is an acute phase protein synthesised and released by the liver in response to activation by pro-inflammatory cytokines such as IL-6 and IL-1. This protein is considered as a sensitive and relevant indicator of acute or chronic infectious or non-infectious diseases [6]. In experiment 1, the increase in haptoglobin concentration noticed at 12 then at 47 days post weaning was related to decreased growth performance probably due to a moderate immune response.

Pigs submitted to an immune challenge were not able to maintain their plasma Trp concentration when they were fed a low Trp diet (exp. 1 and 2) but also a Trp-adeq diet (exp.1). Because the decrease in plasma Trp could be related neither to a decrease in food consumption nor to an increase in Trp utilisation for body protein accretion, we hypothesize that immune system stimulation modifies Trp metabolism and may lead to a reduction of Trp availability for growth and other metabolic purposes. In exp. 1, we showed that growth depression caused by immune system activation is limited by increasing the dietary Trp. However, since decreased Trp concentration was also noticed for pigs fed the Trp-adeq diet and submitted to a moderate immune system stimulation, we can speculate that, in these pigs, Trp may be still limiting for growth.

During immune system activation, the decrease in plasma Trp can be explained by the synthesis of APP which are Trp rich proteins [7] and by the degradation of Trp into kynurenine through the induction of IDO activity. This enzyme, located in several tissues but the liver, and immune cells such as macrophages and dendritic cells, is induced by interferon gamma. IDO induction and consecutive Trp depletion might be a T cell proliferation modulator mechanism involved in immune tolerance process [5] but it has been also proposed that the production of some Trp metabolites along the kynurenine pathway could act as free-radical protector [1]. An adequate Trp dietary supply helps pigs submitted to an immune challenge to maintain their plasma Trp concentration and to reduce inflammatory response (experiment 2). The benefit of Trp as a moderator of the inflammatory response may be ascribed to a restoration of inflammation control by T-cells or by the antioxidant properties of Trp and its metabolites but remains to be demonstrated.

Conclusion

In conclusion, these two experiments showed that stimulation of the immune system by an inflammatory challenge or through depressing the quality of the environment where pigs are kept modifies Trp metabolism and probably limits Trp availability for growth performance. Moreover our results showed the importance of maintaining an adequate Trp supply in order to preserve health and growth performance of pigs submitted to immune system stimulation.

Acknowledgements

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EFFECTS OF DEOXYNIVALENOL ON GROWTH PERFORMANCE OF PIGLETS AND GROWING TURKEYS

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Introduction

Deoxynivalenol (DON) is a fusariotoxin produced mainly by *Fusarium graminearum* and *F. culmorum* and may be found on wheat which is an important feedstuff for European feeds. Numerous references of the literature report that DON reduced feed consumption of piglets (D'Mello et al, 1999 ; Dänicke et al, 2000 ; Grosjean et al ; 2003), it has no adverse effect on feed intake and growth of broiler chickens up to 5000 µg/kg (Dänicke et al ; 2002) whereas its effects on the growth performance of turkeys are not well known. In order to validate the ideas that DON is more problematic in pig than in poultry and that turkey reacts to DON as for broiler, two trials were carried out on piglets (trial 1) and turkeys (trial 2) with wheat-based diets.

Material and methods

The same batch of contaminated wheat was used in both trials. It was highly contaminated since it contained near 6000 µg/kg; it also contained 100 µg ZEN /kg.

Trial 1. Crossbred piglets P76 x Naïma were weaned at day (d) 27. After weaning, 24 castrated males and 24 females from a first band and 24 castrated males and 24 females from a second band (3 months later) were used to compare four diets between d41 and d69. They were housed in individual stalls. They were weighted at d41, d55 and d69. At d41, they weighted 11.5 kg on average.

The four diets have different DON content (0, 720, 1440 and 2880 µg DON /kg). Diets were formulated to have the same protein content (CP = 180 g/kg diet) and the same net energy level (NE=9.6 MJ/kg diet). They were given pelleted and *ad libitum*. Feeders were weighted every 2 or 3 days. Water was available *ad libitum*.

Trial 2. Male turkeys BUT9 were individually housed (with 24 replicates per treatment). They were used to compare three treatments – each of them composed of a wheat-based diet for a first period coming from d21 to d42 and another wheat-based diet for a second period coming from d42 to d63. They were weighted at d21, d42 and d63.

In diets used in period 1, DON contents were respectively 0, 2412 and 4885 µg/kg; in diets used in period 2, DON contents were 0, 2436 and 5208 µg/kg. Diets were formulated to have the same protein content (CP = 255 for the first period and 220 g/kg for the second period) and to have the same apparent métabolisable energy (AMEn=11.9 MJ/kg for the first period and 12.3 MJ/kg for the second period). They were given pelleted and *ad libitum*. Feeders were weighted at d21, d23, d42 and d63. Water was available *ad libitum*.

Résults

Feed was the less consumed as its DON content was high, and differences between diets appeared very soon after the beginning of the trial (2 days) – results not presented here).

Growth performance of piglets is presented in table 1. From d41 to d55, feed intake (FI) and body weight gain (BWG) were significantly reduced with diets containing 1440 and 2880 µg DON/kg, but not with the diet containing 720µg DON/kg. Feed conversion ratio (FCR) did not significantly differ between diets.
From d55 to d69, FI was reduced with the contaminated diets, but the difference with the control diet was significant only with the more contaminated diet. BWG reflect intake levels. FCR did not significantly differ between diets.

During the whole period (from d41 to d69), FI and BWG were reduced with the contaminated diets, but the differences with the control diet were significant only with the more contaminated diet. FCR was not affected by the diet DON content**.**

Growth performance of turkeys is presented in table 2, excepted comparison of feed intakes during the first 2 days because no difference was observed on this period. From d21 to d42, there was no significant difference in feed intake, body weight gain (BWG) and feed conversion ratio (FCR) between the three treatments.

From d42 to d63, there was no significant difference of feed intake between the three diets; BWG of turkeys receiving diets with contaminated wheat were higher than the BWG obtained with the control diet ($p<0.01$). FCR obtained with diets containing contaminated wheat were better than FCR obtained with the control diet ($p<0.05$).
During the whole period (d21 to d63), FI did not vary significantly between treatments. BWG of turkeys receiving diets containing contaminated wheat were higher than those obtained with control diet ($P<0.05$). FCR obtained with diets containing contaminated wheat was slightly better than that obtained with the control diets.

Discussion - Conclusion

In piglet feeding, DON reduces feed intake. The magnitude of the feed intake decrease agrees with the literature data (Dänicke et al, 2001). The response is significant with high DON content in feed and a trend is shown with low content. The heterogeneous distribution in the wheat and therefore in the feed probably explains why the response is not significant with low DON content in the feed. It is careful to consider that the effect of DON on feed intake is linear. Growth rate is reduced

with DON as a consequence of feed intake decrease and feed conversion ratio is not affected in the range of DON contents which were studied here.

In turkey feeding, DON has no adverse effect with contents which may be considered as high (5000 µg/kg diet). This result agrees with previous results (Morris et al, 1999; Hamilton et al, 1985. Manley et al, 1988). It can be concluded that turkeys are no more sensitive to DON than broiler chickens.

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Table 1 : Growth performance of piglets (trial 1)

Estimated DON content (µg/kg)	0	720	1440	2880	P	RSD
From d41 to d55						
Feed intake (g/d)	989 ^{a}	983 ^{a}	919 ^{ab}	820 ^{bc}	***	128
Body weight gain (g/d)	723 ^{a}	748 ^{a}	689 ^{ab}	627 ^{b}	***	104
Feed conversion ratio	1.37	1.31	1.34	1.31	NS	0.07
From d55 to d69						
Feed intake (g/d)	1425 ^{a}	1347 ^{a}	1356 ^{a}	1150 ^{b}	**	175
Body weight gain (g/d)	845	768	819	686	$	
Feed conversion ratio	1.69	1.76	1.66	1.69	NS	0.12
From d41 to d69						
Feed intake (g/d)	1207 ^{a}	1165 ^{a}	1137 ^{a}	985 ^{b}	**	139
Body weight gain (g/d)	784 ^{a}	758 ^{a}	754 ^{a}	657 ^{b}	*	85
Feed conversion ratio	1.54	1.53	1.51	1.50	NS	0.06

$^{a\ b}$ Values with different superscripts differ significantly (P<0.05), RSD: residual standard deviation
$: interaction band x diet

Table 2 : Growth performance of turkeys (trial 2)

Estimated DON content (µg/kg)	0	2412/2436	4885/5208	P	RSD
From d21 to d42					
Feed intake (g/d)	136	134	130	NS	14
Body weight gain (g/d)	82	83	80	NS	10
Feed conversion ratio	1.65	1.61	1.63	NS	0.10
From d42 to d63					
Feed intake (g/d)	252	247	248	NS	20
Body weight gain (g/d)	121 ^{b}	132 ^{a}	125 ^{ab}	<0.01	11
Feed conversion ratio	2.10 ^{a}	1.96 ^{b}	2.00 ^{b}	<0.05	0.16
From d21 to d63					
Feed intake (g/d)	194	195	189	NS	15
Body weight gain (g/d)	102 ^{b}	107 ^{a}	103 ^{ab}	<0.05	8
Feed conversion ratio	1.92 ^{a}	1.82 ^{b}	1.85 ^{ab}	<0.05	0.11

$^{a\ b}$ Values with different superscripts differ significantly (P<0.05), RSD: residual standard deviation

Animal nutrition and health

Posters

USE OF THE BACTERIA *Pediococcus acidilacti* MA18/5M AS A PROBIOTIC FEED ADDITIVE IN POSTLARVAE AND JUVENILES OF BLACK TIGER SHRIMP *Penaeus monodon*

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Introduction

In Vietnam intensive shrimp farming has become a break-through business recently, with black tiger shrimp (*Penaeus monodon*) being the major culture species. However, the trend in global shrimp farming was not able to rise as expected since the shrimp aquaculture industry is beset by diseases, mostly due to bacteria (especially the luminous *Vibrio harveyi*) and viruses (Moriarty, 1999). A whole range of antibiotics and other chemotherapeutants have been used widely to control bacterial and viral diseases in shrimp hatcheries and farms. Particularly, the prophylactic use of antibiotics gave rise to multiple antibiotic resistance among bacterial pathogens (Moriarty, 1999). Moreover, there is a growing concern in transfer of antibiotic resistance to human pathogens, and the antibiotic accumulation in shrimp has been found to be health hazards to human consumers (Primavera, 1994).

The application of probiotics, appearing as a solution to these problems, has launched into aquaculture quite recently, since the 1980s. Recently there has been a growing number of studies reporting the implication of lactic acid bacteria to improve health and quality of fish and shrimp larvae, although this group of bacteria is not dominant in the normal intestinal microbiota of fish (Ringo and Gatesoupe, 1998). However, no attempt of using lactic acid bacteria as probiotics in shrimp has been reported so far.

The present study aimed to evaluate the possible effects of a commercial probiotic feed additive bacteria *Pediococcus acidilactici*, on growth, and survival of black tiger shrimp juveniles *Penaeus monodon*.

Material and Methods

Black tiger shrimp (*Penaeus monodon*) PL15 were reared in 1-m³ tanks with conical bottom. Initial stocking density was 200 larvae per tank. The probiotic preparation tested was *Pediococcus acidilactici* CNCM MA 18/5M. The three treatments were dispatched at random in triplicates among 6 larval rearing tanks: 3 control tanks & 3 treated tanks with a probiotic dose of 10^6 CFU/g of feed. The probiotic was mixed with the feed prior to feeding. The experiment was conducted for two months. Survival was determined at the end of each month by counting all the shrimps in each tank. Growth rate was determined every two weeks by measuring weight and length for 10 shrimp individuals from each tank. Every week 10 shrimps were sampled from each tank for determining *Pediococcus* counts in the gut. The treated group was not supplemented with antibiotics.

Results &Discussion

In treated group, the molting and feeding activities of shrimps under trial were more pronounced compared to shrimps in the control treatment. Moreover Pediococcus counts in the gut has shown the gut colonization of probiotics (population of $10^4 – 10^5$ CFU/g gut) during the trial. The survival of treated group was increased by 30% compared to the control group in the 1st and 2nd month of experiment. The addition of *Pediococcus* into the feed has shown a significant effect on the shrimp growth in both weight and length. In the second month, mean weight (+52%) and length (+15%) of shrimp in treatment were significantly higher ($p<0.05$) than those in the control group.

Conclusion

The introduction of *Pediococcus acidilactici* (BACTOCELL®) gave optimistic results in improving survival and growth of the shrimp larvae, thus improving the resistance to stress and disease. There's a need to further work to assess how the *Pediococcus acidilactici* can interact with pathogenic bacteria species.

ORAL FEEDING WITH LIVE YEAST: IMPACT ON SOME GALT (GUT-ASSOCIATED LYMPHOID TISSUE) PARAMETERS AND CELL PROLIFERATION IN WEANING PIGLETS.

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Introduction

Live yeast dietary supplementation has been reported to produce a variety of beneficial responses in growth rate, feed intake, feed efficiency, milk composition, egg production (Kornegay *et al.,* 1995). It is well known that probiotics display a lot of beneficial effects on gastrointestinal tract : production of essential nutrients at colonic mucosa level, beneficial effects on intestinal immunity, recovery in case of disturbed gut mucosal barrier, prevention of microbial translocation, and competition with microbial pathogens.

The aim of this study was to investigate the effects of a live yeast on piglets growth and selected histometrical and morpho-functional aspects of the gut during the first month after weaning under field practical conditions.

Material and Methods

352 weaned piglets of average 6.5 kg L.W. were allotted into four groups : two groups coming from control (C) sows, the other two coming from treated (T) sows (i.e. 10^6 cfu[1]/g of feed of Levucell® SB – CNCM I-1079, Lallemand, France). Treated sows were fed with the live yeast from 85 d of gestation throughout lactation. Piglets were respectively fed a starter diet supplemented with 0% yeast (C) and 0.01% of the same yeast (i.e. 2.10^6 cfu/g) (T), so that the following experimental groups resulted : CC, TC, CT and TT. Individual body weights and feed intakes were recorded at 0, 15, and 30 d. post-weaning (PW). After 30 d. post-weaning, 5 female piglets per group were slaughtered (n=20). Serial microtome sections (4 µm-thick) were examined to determine the depth of intestinal crypts (C), the height of intestinal villis (V), the V:C ratio, the mitosis index, the mucosal cells in S-phase of the cell cycle, the mucous glycoconjugate profile, the thickness of the adherent mucous gel and the mucosal macrophages. Cells were expressed as the percentage of the total number of counted cells. The data were analyzed by ANOVA using the GLM procedure of the SAS Institute, Inc. (1985). Cells counts were co-variated for the number of recorded cells.

Results

At 30 days PW, the treated piglets (TT, CT) were heavier than control even though the difference was not significant (20.00 kg *vs* 19.63 kg). In addition, average daily gain (ADG) of treated (TT, CT) piglets resulted significantly higher than control piglets (0.43 kg/d *vs* 0.46 kg/d; P<0.001). TT and CT piglets showed higher ADG than CC and TC animals during the post-weaning period. Histometric analysis (Table 1) in the ileum of CT and TT animals resulted in an increase in villus (V) height (P<.01) and crypt (C) depth (P<.01), as well as in a decrease in V:C ratio (p<.01) compared with controls. The counts of proliferating epithelial cells resulted in an increase of mitosis in CT/TT piglets compared with CC/TC animals (P<.05). CC/YC animals showed a thicker adherent mucous gel in the ileum than CY/YY piglets (P<.01), whereas CY/YY groups were associated with a higher mucous cells count The mucosal macrophages were appreciably more numerous in animals supplied with live yeast (CT/TT) than in piglets without any supply (CC/TC) (P<.01).

Table 1 : Piglets ileum histo-morphological parameters

	CC (n=5)	YC (n=5)	CY (n=5)	YY (n=5)	SEM
Villous µm	195.13^{A}	193.46^{A}	242.97^{B}	242.99^{B}	3.19
Crypts µm	129.93^{A}	136.40^{A}	177.70^{B}	176.64^{B}	2.14
V:C ratio	1.53^{Aa}	1.42^{Bb}	1.39^{B}	1.39^{B}	0.02
Mitotic cells %	41.97^{a}	43.50^{a}	49.18^{b}	48.87^{b}	2.05
Macrophage TLD %	4.00^{A}	4.02^{A}	4.82^{B}	4.93^{B}	0.07
Mucous layer µm	2.89^{A}	2.70^{A}	1.83^{B}	1.70^{B}	0.09
Goblet cells					
200µm-villis	9.6^{A}	11.7^{AC}	12.5^{BC}	18.8^{B}	0.78
100µm crypts	9.7^{A}	10.9^{A}	14.6^{B}	18.4^{B}	0.47
Liver kg	0.48	0.61	0.53	0.54	0.02
Intestine kg	1.99	2.06	2.04	2.00	0.09

A, B, C different : p<0.01 ; a, b different : p<0.05

Discussion

The higher number of intestinal proliferating cells in CT and TT groups may not prelude to possible hypertrophic aspects due to equal weight of the intestine in both groups. The higher mitotic index found in the treated piglets likely supports a good intestinal capability of restoring the mucosal thinning which frequently occurs at weaning (Isolauri et al., 1998). This is in accordance with the producing parameters : good conditions of the intestinal mucosa likely allow better ADG and growth performances (Jurgens *et al.,* 1997). The increase of macrophages may support a good defensive capacity of ileal mucosa in the treated piglets, above all against viral pathologies.

Conclusion

Inclusion of 0.01 % of live yeast (CNCM I-1079) to post-weaning diet had beneficial effects on piglets growth performance and likely promoted a proper intestinal efficiency by a fast restoration of the mucosal thinning after weaning. Thus, live yeast administration may possibly assist animals in intestinal disorders by the gut trophic action and the positive effects upon mucosal macrophages, as well as inhibiting the colonisation of pathogens by blocking their attachment to the intestinal mucosa.

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IMPACT OF A PROBIOTIC YEAST *SACCHAROMYCES CEREVISIAE BOULARDII* ON *CLOSTRIDIUM DIFFICILE* NEONATAL DIARRHEA IN PIGLETS

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Introduction

Clostridium difficile is one of the main bacteria responsible for diarrhea in human at the hospital. It is found in number of animal species, including piglet, where it is more and more frequently identified as a cause of neonatal diarrhea (1, 2).

The live yeast *Saccharomyces cerevisiae boulardii* is widely prescribed in humans to prevent *Clostridium difficile* diarrhea.

Several pathways of this specific yeast strain have already been documented in rodents and humans (3):

- production of a 54-kDa protease, which digests toxins A, and B.
- reinforcement of the intestinal epithelial barrier.
- induction of non-toxinogenic *C. difficile* clones.
- enhancing of the mucosal immune response.
- direct action on the toxin synthesis pathway through the release of a vitamin.

In piglets, birth is a critical period. Its digestive tract, sterile at farrowing, is quickly and gradually colonized by a complex microbial population. The impact of *Saccharomyces cerevisiae boulardii* against toxins of *Clostridium difficile* and on clinical signs of neonatal diarrhea in piglets have been assessed in a field trial.

Materials and methods

Description of the products:

The probiotic yeast *Saccharomyces cerevisiae boulardii* (CNCM I-1079), Levucell SB®, is a microbial feed additive authorized by CE n°1436/98.

Tiamuline is used in the prevention of *Clostridium difficile* diarrhea: preventive single injection IM of 0,25cc at birth and repetition as soon as clinical signs were observed.

The post-weaning feed (from day 21) is supplemented with colistine (120 ppm), to prevent digestive disorders.

Experiments and animals:

78 Dalland sows and their 934 piglets were used in the study. The piglets were followed from birth until 42 days of age. Sows were randomly distributed between 3 treatments : Control, Levucell SB® and Antibiotic, for 5 successive groups, through 3 experiments (A, B and C). In experiment A (groups 1 and 2), Levucell SB® was given in a single-dose, *per os* at farrowing by a dosing syringe system. In experiments B (groups 3 and 4) and C (group 5), Levucell SB® was added in sow feed 4 days prior to and 4 days after farrowing. Only group 5 did not receive any Antibiotic. The piglet feed then contained the probiotic yeast from 4 to 42 days of age.

For treatments Control and Levucell SB®, post-weaning feed did not contain any antibiotic.

Measured parameters:

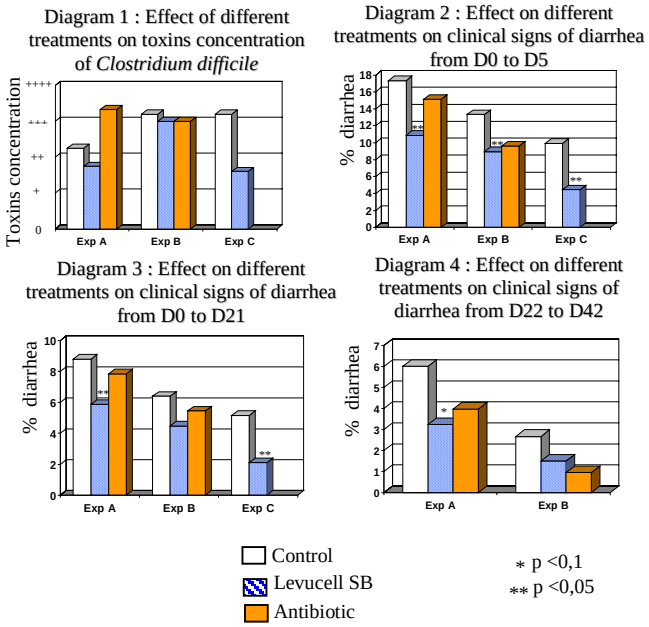
Toxins of *C. difficile*:

Piglet fecal toxin concentration of *C. difficile* was evaluated by an immuno-enzymatic test on day 4: Kit Toxin A/B (Meridian), expressed according to a qualitative scale from 1 to 4.

Diarrhea scores: The animals were observed individually from birth to 42 days of age. Three periods of observation were defined: 0 to 5 days of age (two daily diarrhea scores), 6 to 21 days of age (a daily diarrhea score) and 22 to 42 days of age (a diarrhea score per week on each piglet).

The statistical data were analyzed by SYSTAT® for Microsoft Windows® (Analysis of variance in GLM and Chi-square).

Results and Discussion



Addition of *S. c boulardii* made it possible to appreciably reduce toxin A and B concentrations of *C. difficile* in piglet feces (Diagram 1). This may result from the *S. boulardii* specific protease inhibiting *C. difficile* toxins (3).

In parallel, the clinical signs of diarrhea were less important during time D0-D5 (Diagram 2), D0-D21 (Diagram 3) and in the post weaning period between D22 and D42 (Diagram 4) compared to Control.

Less clinical expression of neonatal diarrhea is reflected at weaning and post-weaning. The rate of relapse in the post-weaning period decreased and future performance was better (4).

The benefit of *S. c. boulardii* on digestive disorder reduction in the suckling period can also be due to a reinforcement of the digestive mucosa and local immunity (5).

This study falls under an exploratory step by highlighting interesting effects on digestive hygiene in piglets. The use of *S. c. boulardii* earlier in the animal's life contributes to protection from intestinal disorders, for example due to *C. difficile*, while preserving beneficial effects of growth in the animals.

Additional microbiological and histological research will provide more evidence for a better comprehension of the phenomena.

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EFFECTS ON INTAKE OF CONCENTRATE WITH INCREASING LEVELS OF FLAVOUR AGENT IN THE DIET OF DAIRY CALVES

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Introduction

The utilization of additives in calves nutrition to accelerate their development are a new advantage as possibility to reduce cost and time of nursing in these category. Among additives used are flavour agents, which increase the intake of solid feeds, proportioning a fast ruminal development and anticipating weaned period. Several flavour agents reproduce the preferred taste, as milk and citric flavour, to ruminants, and principally calves. This work evaluated increasing levels of citric flavour agent in the diet of dairy calves and his relation with intake of concentrate.

Material and Methods

The trial was conducted in Department of Animal Science, Universidade Federal de Santa Maria, Brazil, in June, 2003. It were utilised 8 Holstein male calves, weaned with three months of age housed in individual cages and all animals were submitted at the four treatments: T1-without addition of flavour agent, T2-150 gr. of flavour agent/ton. of concentrate, T3-300 gr. of flavour agent/ton. of concentrate and T4-600 gr. of flavour agent/ton. of concentrate. The flavour agent utilised was Euroarom®RW-47, which reproduce citric smell and taste. The trial occurred during three days, where the calves has ration *ad libitum*, and intake was calculated by difference between ration offered and ration rested. The ration was a commercial concentrate and nutritional value found in Table 1. During the trial, the animals also received alfalfa hay as fibre source on the diet and had water supply *ad libitum*. The experimental design utilized was entirely randomized and data were submitted at ANOVA and Tukey's Test.

Table 1- Nutritional value of concentrate offered

DM(%)	CP (%DM)	CF (%DM)	Fat (%DM)	Ash(%)	TDN(%)
86.65	19.18	4.61	9.96	6.41	78.41

Results

The results found in Table 2. Average daily intake of concentrate observed during three days of trial was 347.92 gr. to the level without flavour agent and 83.88 gr., 83.92 gr., and 367.08 gr. of concentrate to the levels 150, 300 and 600 gr. of citric flavour agent/ton. of concentrate, respectively. Levels 0 and 600 gr. of flavour agent were significantly different ($P<0.0001$) at the levels 150 and 300 gr. of flavour agent/ton. of concentrate.

Discussion

The data observed for intake agree with Albright (1993) who said there is a tendency to increase of intake with the use of flavour agent. Lucci (1989) and Montardo (1988) obtained increase in the intake using apple flavour agent and milk flavour agent, respectively. Cheeke (1991) observed that flavour agent induce a major intake in foodstuffs with poor quality, but our data are more similar at the obtained by Morril &Dayton (1974), Lucci (1989) and Nombekela et al. (1994) which utilized foodstuffs with good quality and obtained increases when added flavour agents in concentrate.

Table 2 – Average daily intake with increase of levels of addition of flavour agent:

	T1	T2	T3	T4
Average intake (gr./day)	347.92	83.88	83.92	367.08

Conclusion

The authors conclude that high levels of flavour agent in the diet of dairy calves have a positive influence on intake of concentrate.

Acknowledgements

We acknowledge Eurotec Nutrition™ of Brazil by support of trial and supply of citric flavour agent.

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USE OF CONVENTIONAL MEDICATION (clorobutanol) AND HOMEOPATHY (*Thuya occidentalis*) ON CONTROL OF THE BOVINE PAPILOMMATOSIS: A STUDY OF CASE

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Introduction

Papilommatosis is a disease caused by a virus of the family Papovaviridae and it is characterized, in the bovine ones, for the disordered proliferation of epitelials cells, forming warts, mainly, in skin level (Fraser, 1996). The damages caused by this pathology are related the trauma, local bleeding cutaneous and appearance of secondary lesions (infectious and parasitic). For being of origin viral it is of difficult control with specific conventional medications and the use of autogenous vaccines is, usually, of difficult access to most of the producers. For those reasons, the use of medications homeopathic has if turned frequent in the control of the bovine papilommatosis.

Material and Methods

The experiment was driven in the Department of Animal Science, Federal University of Santa Maria (UFSM), Brazil, using six Holstein calves with ages between 11 and 19 months, between 198 and 386 Kg body weight. For two animals it was administered clorobutanol by subcutaneous way in the dose of 12.5 mg/Kg of body weight in three applications with three days of interval. For four calves it was administered the medication *Thuya occidentalis*, CH12 (centesimal hahnemaniana) potency, prepared in agreement with the Brazilian homeopathy pharmacopehy, in the form of sucrose globules, for five serial days. As there was not visible evolution of the clinical picture, 30 days after the first administration it made himself the repetition of the medication, being used the CH30 potency, also in the form of sucrose globules and for five serial days. The globules were supplied with the concentrate. Made himself a count before treatment to establish the comparison of effectiveness of the medications in the reduction of the papilommas number in the animals. After the supply of the medications, the counts of the papilommas was made at the 30, 60, 90, 120 and 150 days, being observed, also, the reduction in the size of the papilommas. The used experimental design was it entirely at random with unequal number of repetitions for treatment. The treated animals with clorobutanol served as witness to the reverse (group testifies that received comparative treatment).

Results

In clorobutanol treatment the medium number of papilommas/animal it was of 62.5; 86; 97.5; 47.5 and 13.5 at the 30, 60, 90, 120 and 150 days powder-treatment, respectively. This characterized an increase of 29.75%; 54.70% and 77.25% to the 30, 60 and 90 days and reduction of 10.55% and 50.14% just to the 120 and 150 days after the use of the clorobutanol. In *Thuya occidentalis* treatment the medium counts they were of 46.25; 33.25; 10.5; 0.75 and 0,25 papilommas/animal at the 30, 60, 90, 120 and 150 days, respectively. An increase of 30.07% was observed in the average of the papilommas/animal number in *Thuya occidentalis* treatment to the 30 days, and reduction of 2.1%; 53.33%; 77.01% and 99.54% at the 60, 90, 120 and 150 days post-treatment, respectively. It was observed, also, reduction in the size of the papilommas, more precocious and evident in *Thuya occidentalis* treatment.

Discussion

Even the before experimental averages having been similar, that is to say, 36 and 29.25 papilommas/animal for the clorobutanol and *Thuya occidentalis*, respectively, the answer of each treatment was differentiated. The evolution of the clinical picture in *Thuya occidentalis* treatment happened as it describes the homeopathy, that is to say, an initial fast aggravation and improvement gradual and effective (Kent, 1996). To the 30 days the aggravation was evidenced. Starting from the 60 days after treatment the improvement gradual of the case was observed, and to the 150 days it arrived the medium reduction of 99.54% of the papilommas, just staying a calf with a wart that disappeared few days after. That evolution demonstrated that the medication used for the case was of good election. The beginning of the only medication was used, in agreement with the basis of the homeopathy (Hahnemann, 1996). The *Thuya occidentalis* was chosen the symptoms of the picture observed in the animals being taken: production of pathological vegetations, warts, split warts, similar the cauliflower and that tend to bleed (Araújo Filho, 2000) (Boericke, 1997) (Tiefenthaler, 1996). As progressive evolution of the case was observed, the need was not considered treatment again. In the clorobutanol treatment the reduction happened only after 120 days, and it just presented 50% of reduction in the medium count of papilommas for animal when the treated animals with *Thuya occidentalis* no longer they possessed more papilommas.

Conclusion

The authors conclude that *Thuya occidentalis*, used sequencelly in the CH12 and CH30 potencies was effective on control of the papilommatosis bovine, on bovines and conditions tested.

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UTILIZATION OF PHYTOTHERAPIC PRODUCTS ONTHE CONTROL OF *Haematobia irritans* IN DAIRY COWS CONTAMINATED NATURALLY

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Introduction

Haematobia irritans is an haematophag fly that attack, principally, the cattle (Faria, 1998). His disturbs at cattle herd are relationed with intake decrease (Silva, 2002). Chemical products utilized in his combat lead need of milk rejected, causing economic troubles to farmers. The use of phytotherapic products, reduce costs of exploration and decrease levels of contamination for animals, environment and human.

Material and Methods

The trial was developed of November, 2nd to 14th, 2003, in Department of Animal Science, Federal University of Santa Maria, Brazil, utilizing 30 animals of Holstein breed, naturally contaminate. As treatment was used *Azadirachta indica* extract at 1%, conform recommended by Martinez (2002), and a phytotherapic compound formulated with 50g of *Eucaliptus globulus*, 50g of roots and rhizomes of *Cymbopogon citratus*, 10g of bulbs of *Allium sativum* and 300g of animal fat, this item recommended by Garcia & Lunardi (2001). A group of animals did not receive any treatment, had been considered control group. *Azadirachta indica* extract was applied in aspersion with manual machine, utilizing four liters of solution/animal in cows and heifers and three liters/animal in calves, with precaution that aspersion was uniform for whole animal. The phytotherapic compound was made triturating dry leaves of *Eucaliptus globulus*, roots and rhizomes of *Cymbopogon citratus* and bulbs of *Allium sativum*, additioning, gradually, animal fat in this mixture. Was applied the compound in dorsal line of animals, in all extension. The count of fly were realized daily, at 08 a.m. for two observers, during six days, before application of products. After application, counts were realized until return and fixation of infestation at anterior levels at treatments, with caution in differentiation of other fly species. Experimental design utilized was randomized blocks with unequal number of repetitions to treatment.

Results

In calves, the reduction in count of *Haematobia irritans* in treated group with phytotherapic group was 33.00 %, 75.00 % and 62.24 %, in first, second and third days after treatment, respectively, when compared with average count of pre-treatment period. *Azadirachta indica* extract presented reduction of 35.17 and 10.40 % in count of *Haematobia irritans* in first and second days, respectively. Later, both treatments, number of fly return to be superior at pre-treatment period. In heifers and dry cows, the effect of products was similar with 37.80; 48.00 and 38.20 % of reduction in infestation of fly in first, second and third days after treatment and *Azadirachta indica* extract with 10.44 and 52.42 % of reduction in first and second days after treatment, respectively. In lactation cows treated with compound, reduction of count was 39.60 % only first day after treatment and in group treated with *Azadirachta indica* extract did not observe any reduction of number of *Haematobia irritans*.

Discussion

In all categories was observed an increase in number of fly of control group, later application of treatments. Also, was verified an oscillation in infestation of animals of control group, with high concentration coincided with period of high efficiency in treated animals. With loss of efficiency, was observed a reduction of number of fly in non-treated animals. This result may be explained by migration of fly of treated group to non-treated group. The results demonstrated that *Azadirachta indica* extract utilized in concentration of 1 % presented a low efficiency in repellence of *Haematobia irritans*. So, do not confirm the orientations of Martinez (2002), where *Azadirachta indica* extract utilized in concentrations minor at 2 % had effect of repellence about *Haematobia irritans*. Phytotherapic compound, as recommended by Garcia & Lunardi (2002) also presented low efficiency, because showed low reduction in total number of fly and, still, a short period of reduction (one to three days). The results obtained demonstrate need to study levels more higher of *Azadirachta indica* extract and components of phytotherapic compound, associated at repetitions of application.

Conclusion

The authors conclude that *Azadirachta indica* extract at 1% applied by aspersion and a phytotherapic compound (*Eucaliptus globulus*, *Allium sativum* and *Cymbopogon citratus*) applied in dorsal line of animals presented low efficiency on control of *Haematobia irritans* in dairy cows naturally contaminate.

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EXPERIMENT ON ɓ-CAROTENE EFFECT ON SOW FERTILITY

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Introduction

In order to see if ɓ-carotene, in any form, influences sow fertility, the present experiment was carried out. The experiment hypothesis considered that consistent doses of ɓ-carotene added to the diets containing recommended doses of vitamin A can increase sow fertility if the pigment is transformed into vitamin A or acts by itself in this direction.

Material and methods

To have as high as possible homogeneity in the experimental and control groups, sows of the same breed (Large White) and with only one former farrow were used. Also in the experiment were included sows giving birth to an equal number of piglets,the latter being similar to the herd average. One group of sows was formed out of 30 heads to have a better accuracy of the statistical data.

The sows received 4 kg of feed per day containing 10000 IU of vitamin A per kg of feed. After weaning the sows were fed 2,5 kg of feed per day containing 7500 IU vitamin A per kg of feed. Thus the sows in control group received per head and per day 40000 IU vitamin A when lactating and 18 750 IU after weaning. The first experimental group received the same diet and the same amount of feed as the control group. In addition the sows of this group received 400 mg of ɓ-carotene per day 5 days before weaning and 10 days after. The second experimental group was submitted to the same treatment but in addition the sows of this group received 200 mg ɓ-carotene daily for the other 18 next days. All the sows were fed individually along all the experiment in order to control the quantity of the ingested feed and ɓ-carotene. On the day of weaning the sows were not fed. Restrictions concerning the age and the prolificacy of sows made necessary to form the groups step. The first phenomenon registered in the experiment was coming in heat after estrus. First heat after weaning appeared within 6-7 days. However we have to notice a high variation. It was necessary to have 9 extractions of sows till the experimental and the control groups were formed. Six (6) sows were excluded or lost during the experiment.

Some sows were not detected in heat at the first cycle. Adding ɓ-carotene in the diet didn't help estrus to appear. If we deduce 6.4 days, the mean interval for estrus appearance of the first cycle from 25.7 days, the mean interval for estrus appearance of the second cycle, we obtain 19.3 days, a close value to the mean length of estrus cycle in swine (21 days). Since 100% of sows exhibited heat in the first 35 days after weaning, feeding is not involved in delayed mating. In case we accept that feeble heat intensity caused the delayed mating, it is very clear that ɓ-carotene didn't help heat intensity.

The conception rate (pregnant sows) after the first mating including both estrus cycles, but no return in heat sows, is very high. We may say the vitamin A content of the diet used in control group feeding is satisfactory for a good fertilization of sows. Data concerning prolificacy are presented and statistically analyzed in table1. As it can be seen in the table, ɓ-carotene supplement didn't influence the sow prolificacy or sows can't use it, as vitamin A precursor. At the same time the coefficient of variation was rather high in all three groups of sows denoting the presence of some uncontrolled factors action. The number of live born piglets after the first mating of sows is presented in table 2.

Conclusion

Under our experimental conditions, neither short term nor long term ɓ-carotene administration had effect in improving littersize of sows or variability of the piglets born. At the end of this experiment it is seen that no reproduction index was significantly modified. And the second conclusion is that pigs, seem to be unable to transform ɓ-carotene into vitamin A.

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Table1. - Prolificacy at the first mating after weaning in experimental and control groups

Statistics	Groups in experiment			Total sows
	1st	2nd	Control	
Number of sows (n)	29	28	26	83
Total number of born piglets (Ex)	338	321	302	961
Mean number of born piglets ($\bar{x}$)	11.66	11.46	11.62	11.58
Difference of means Significance of differences	$\bar{x}_1 - \bar{x}_c = o.o4$ *t =0.05 no significance*	$\bar{x}_2 - \bar{x}_c = -o.12$ *t=o.16 no significance*		

Table 2. - Number of live born piglets after the first mating of sows in experiment

`Statistics	Groups in experiment			Total sows
	1st	2nd	Control	
Number of parturient sows {n)	29	28	26	83
Number of live piglets born (Ex)	305	286	272	863
Mean number of live piglets ($\bar{x}$)	10.52	10.21	10.46	10.40
Differences of means Significance of differences	$\bar{x}_1 - \bar{x}_c = 0.06$ *t=0.092 no significance*	$\bar{x}_2 - \bar{x}_c = -0.25$ *t=0.397 no significance*		

EFFECTS OF DIETARY MANNAN OLIGOSACCHARIDE ON FIBER DIGESTIBILITY IN MONOGASTRIC ANIMALS

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Introduction

Fiber digestibility in monogastric species is generally limited (except in species such as horses, ostriches, and rabbits) but may be improved by adaptation to fibrous feedstuffs or by altering intestinal fermentative and cellulytic microfloral activity with dietary supplements. Although one of the least digestible oligosaccharides, mannan oligosaccharide (MOS) can affect the intestinal digestibilities of organic components of the diet in monogastric animals. This brief review considers fiber digestibility studies using monogastric animals fed diets supplemented with MOS (Bio-Mos®, Alltech, Inc., Nicholasville, Kentucky, USA) or no additive (negative control, nCON).

Materials and Methods

A literature review was conducted on the topic of fiber digestibility in monogastric animals fed diets containing MOS or no supplement. The research was reported from 1997 to present.

Results

Table 1. Body weight (42 d), feed conversion ratio (FCR, 42 d), and crude fiber digestibility (22-42 d) of caged Ross broilers fed nCON or MOS diets (Kumprecht et al., 1997).

	Dietary MOS, %					
	0	0.05	0.10	0.15	0.20	0.25
Body wt, g	1809[b]	1889[a]	1858[a]	1893[a]	1888[a]	1901[a]
FCR, g/g	2.16[a]	2.11[ab]	2.09[ab]	2.07[b]	2.09[ab]	2.07[b]
Fiber dig., %	6.23[b]	10.1[ab]	13.3[ab]	13.6[ab]	15.4[a]	11.4[ab]

[a-b] $P < 0.05$.

Table 2. Digestibility (%) of diet components in adult dogs fed 0 or 0.11% MOS with various fiber sources (none, beet pulp, cellulose, soy hulls) (Kappel, 1998)

MOS, %	Dry Matter	Organic Matter	Total Fiber	Insoluble Fiber	Soluble Fiber
0	82.0	83.6	25.6	15.7	61.5[b]
0.11	81.8	83.1	25.7	15.2	72.9[a]

[a-b] $P < 0.01$ (main effects)

Table 3. Crude fiber digestibility and in vitro volative fatty acid production using fecal suspensions from dogs fed diets with MOS 0 or 0.5% (Zentek et al., 2002).

Item	Basal before	MOS 0.5%	Basal after
CF dig., %	61.8[ab]	69.1[a]	57.7[b]
VFA, µmol/mL	26.8[b]	41.3[a]	26.2[b]

[a-b] $P < 0.05$.

Discussion

Kumprecht et al. (1997) demonstrated that Ross broilers in cages had improved ($P < 0.05$) crude fiber digestibility with MOS 0.20% diet (15.43%) compared to control (6.23%), with MOS 0.05, 0.10, 0.15, and 0.25% results intermediate (range 10.06-13.64%). In adult dogs, Kappel (1998; Research Report, Louisiana State University, Baton Rouge) found that MOS 0.11% increased soluble fiber digestibility compared to no supplement (72.9 vs 61.5%; $P < 0.01$) consistently across four diets differing in added fiber source (none, beet pulp, cellulose, or ground soy hulls). Zentek et al. (2002; J. Nutr. 132:1682S-1684S) determined using adult female Beagle dogs that crude fiber digestibility was greater ($P < 0.05$) during an experimental period feeding MOS 0.50% diet (69.1%) than in the control period after (57.7%), with the control period before being intermediate (61.8%). In vitro incubation of fecal suspensions from the MOS period produced more ($P < 0.05$) volative fatty acids (VFA) (41.3 µmol/mL) than those from the control before or after periods (26.8 and 26.2 µmol/mL). In rabbits, Fonseca (1999; Master's thesis, Technical University, Lisbon, Portugal) discovered that MOS 0.2% diet increased ($P < 0.05$) acid detergent lignin digestibility compared to control diet (30.9 vs 22.5%).

Conclusion

Dietary MOS improved crude fiber, soluble fiber, and acid detergent lignin digestibilities and in vitro VFA production from fecal suspensions in monogastric animals. The mode of action remains to be determined but may involve altering the microbial population (by pathogen adsorption or immune stimulation) to favor proliferation of cellulytic and/or fermentative microbes.

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SUMMARY ANALYSIS OF POST-WEANED RABBIT TRIALS WITH DIETARY MANNAN OLIGOSACCHARIDE

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Introduction

In commercial rabbitries, enteric disorders after weaning are a continuing problem, and the decline in use of antibiotics had led to interest in alternative growth promoters. This summary article presents data from several growing (weaned) rabbit experi-ments comparing diets supplemented with either MOS (Bio-Mos®, Alltech, Inc., Nicholasville, Kentucky, USA), an antibiotic (positive control, pCON), or no additive (negative control, nCON).

Materials and Methods

Caged rabbit feeding trials were conducted in Brazil (Scapinello et al., 2001), France (Girard et al., 1997; Guillou and Arveux, 2000), Hungary (Bersenyi and Gippert, 1995; Tibor, 1995), Portugal (Fonseca, 1999; Medeiros Mourão and Carvalho Pinheiro, 2003), and the United States (Reed, 1994). New Zealand White (Bersenyi and Gippert, 1995; Reed, 1994; Scapinello et al., 2001; Tibor, 1995), Hybrid Hyla (Fonseca, 1999), Vitaline (Guillou and Arveux, 2000), and an unidentified strain of broiler rabbits (Medeiros Mourão and Carvalho Pinheiro, 2003) were utilized. Primarily mixed sexes were used, but one report (Gillou and Arveux, 2000) had males only and one report (Tibor, 1995) listed male and female results separately. Dietary antibiotics used in the pCON diets were oxytetracycline (Fonseca, 1999) or zinc bacitracin (Medeiros Mourão and Carvalho Pinheiro, 2003). Data were analyzed by Paired t-test.

Results

Table 1. Body weight gain, feed conversion ratio, and mortality of rabbits fed nCON or MOS diets.

Days on Test	Wtd Avg MOS, %	Diet		MOS Relative Change Versus nCON, %
		nCON	MOS	
Body weight, kg (n = 20; *P* = 0.001)				
38.4	0.148	1.357[b]	1.419[a]	+4.57
FCR, kg/kg (n = 20; *P* = 0.001)				
38.4	0.148	4.175[a]	3.963[b]	-5.08
Mortality, % (n = 19; *P* = 0.004)[1]				
38.5	0.147	17.80[a]	9.07[b]	-49.04

[1]By arcsine transformation, *P* = 0.001.

Table 2. Body weight gain, feed conversion ratio, and mortality of rabbits fed pCON or MOS diets.

Days on Test	Wtd Avg MOS, %	Diet		MOS Relative Change Versus pCON, %
		pCON	MOS	
Body weight, kg (n = 9; *P* = 0.723)				
37.4	~0.146	1.496	1.486	-0.67
FCR, kg/kg (n = 9; *P* = 0.297)				
37.4	~0.146	3.041	2.984	-1.87
Mortality, % (n = 9; *P* = 0.092)[1]				
37.4	~0.146	8.81	6.28	-28.72

[1]By arcsine transformation, *P* = 0.104.

Discussion

Based on 20 comparisons, MOS diets improved (*P* = 0.001) body weight gain and feed conversion ratio by 4.57 and 5.08%, respectively, compared to nCON diets (Table 1). In 19 comparisons, MOS diets decreased (*P* = 0.004) mortality by 49.04% relative to nCON diets. Using 9 comparisons, no significant difference was found between pCON and MOS diets for weight gain (*P* = 0.723) or feed conversion ratio (P = 0.237), indicating statistical equivalence (Table 2). The 28.72% decrease in mortality with MOS vs pCON diets approached significance (*P* = 0.092).

Conclusion

Supplementing post-weaning rabbit diets about 5 1/2 weeks with approximately 0.146-0.148% (range 0.1 to 0.2% in the diet) MOS significantly improved body weight gain (4.57%), feed conversion ratio (5.08%), and mortality (49.04%) relative to negative control (nCON) diets. The MOS diets were statistic-ally equivalent to antibiotic positive control (pCON) diets with regard to rabbit body weight gain and feed conversion ratio, but MOS diets tended to improve mortality to a greater extent (28.72%) than pCON diets. These patterns of responses in rabbits are similar to those previously reported in analyses of worldwide broiler chicken (Hooge, 2003a) and turkey (Hooge, 2003b) pen trials, and broiler commercial field trials (Sefton and Hooge, 2004), when using MOS supplemented diets compared to nCON or pCON diets.

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RATIONAL NUTRITIONAL EXPLORATION OF TRACE ELEMENTS

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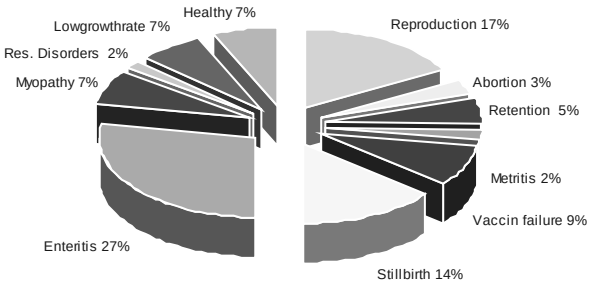
Introduction

The trace elements supply is one of the most important conditions for health maintenance(2). Animal's needs are known (INRA, NRC …) and covered by nutriments and/or mineral complementation. The evaluation of animal's status of trace elements can be obtained with different protocols : nutrients balance or dosages of trace elements in different biological supports : organs, milk, urine or blood. Our laboratory is specialized in biochemistry in blood, urine and milk. Statistical tests were realised to show relations between animal's status and health disorders.

Material and methods

We are consulted to realize nutritional explorations in blood of cattle which present an identified disorder. Figure 1 shows the disorders studied for nutritional exploration in French beef cattle.

Fig 1 : motivations of nutritional explorations, 1700 beef cattle.



Nutritional exploration can be performed through different measurement :

- Trace elements in blood (copper, zinc, selenium, iodine), but also in tissues like liver (copper) or kidney (selenium) to estimate reserves or to detect an excess.
- enzyme activity or hormones depending on trace elements : glutathion peroxydase (selenium), thyroxin (iodine).

Animal's nutritional statuses can be classified in 5 categories (1) :

- adequate : values mesured guarantee a normal homeostasy.
- marginal : shows a tendency to the exhaustion of reserves. This status can have non specific consequences like lower immune defences.
- subclinical : organism cannot correctly guarantee vital functions depending on the trace element.
- clinical : consequences can be used for diagnostic.
- excessive : revealing either excess or biological abnormalities independant on the trace element (an inflammatory status increases plasma copper)

Tableau 1 : status of 126 beef cattles situated in the west of France

Results

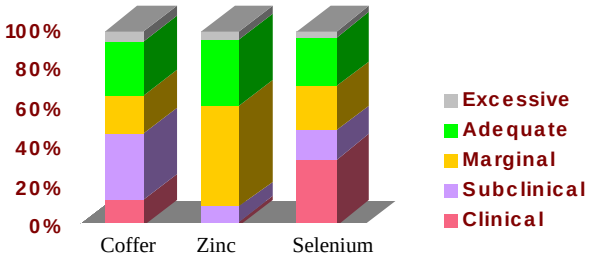


Tableau 2 : relations between deficiency and health disorders

	Coffer	Zinc	Sélénium
Reproduction	**Clinique**	Marginal	Marginal
Abortion	**Clinique**	Subclin.	Marginal
Retained Fœtal Mbne	**NS**	Subclin.	Marginal
Métritis	**??**	??	Subclin.
Vaccinal failure	**Subclin.**	Subclin.	Marginal
Still Birth Syndrome	**Subclin.**	Subclin.	Marginal
Entéritis	**Subclin.**	Subclin.	Marginal
Myopathy	**NS**	Marginal	Marginal
Respiratory Troubles	**NS**	NS	Marginal
Growth Failure	**Subclin.**	Marginal	Marginal

Discussion

Most of cattle which participated in this study present insufficient status in selenium, zinc and copper. Informations concerning iodine were not sufficient to prosperly perform a statistic analysis.

Deficiencies, also marginal, in selenium have repercussions on the disorders discribed.

This winter, we were confronted with important disorders of stillbirth and morbidity. The exploration of calve's immune status showed a failure of immunity transfert in relation to mother's deficiencies of trace elements (results presented in table 2). These results are coherent with Rollin's publication (4).

Conclusion

The exploration of nutritional status is a usefull tool in the global investigation of cattle (3). The use of trace elements is not reduced to a blindly flushing, but it participates to the managment of health and production, so in the profitability of the farms. Specific supplementations, adapted to every cattle according to the results of the analysis, can improve rapidly immune status, reproduction performance and calves vitality.

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EFFECT OF DIETARY LINSEED ON n-3 FATTY ACIDS CONTENT IN LIVER AND INTRAMUSCULAR FAT OF OVERFED DUCKS

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Introduction

An experiment was conducted on thirty mule ducks to study the impact of extruded linseed supplementation during the force-feeding period on fatty acids (FA) composition of liver and intramuscular fat.

Material and methods

Ducks were manually force-fed with boiled corn over all the duration of the experiment (13 days). During this overfeeding period, three treatment groups (10 animals per group) were established according to the feeding program : corn grains alone (Control diet -C-), corn grains with 2% of extruded linseed (L2%) and corn grains with 4% of extruded linseed (L4%). Live weight, carcass, breast, thigh and liver weights were recorded on all birds of each diet. The fatty acid profile were determined on liver (small lobe) and intramuscular fat (breast and thigh) of five individuals per diet by using gas chromatography.
Moreover, a sensory test was realized on reconstituted blocks of "foie gras" using a neighbour-designs with border plots (Azaïs and al., 1993). The panel evaluated color, flavor, juiciness, texture, granular and visual appearance of the "foie gras".
Analysis of variance (one-way ANOVA) was used to compare the three diets using the GLM procedure of MINITAB.

esults and discussion

There were no significant difference in weight parameters among diets. The sensory test showed that the L2% diet had a positive effect on the visual appearance of the "foie gras". The L4% diet gave a higher juiciness and a more granular appearance. These latter criteria are generally not appreciated by the consumer. The visual appearance was strongly correlated with color (r=0.77) and flavor was negatively correlated with granular appearance (r=-0,42). The total FA content of the different tissues was not influenced by the diet (data not shown). The linseed supplement improved the fatty acid profile by an increase in n-3 PUFA in liver and intramuscular fat and a decrease in n-6/n-3 ratio, which becomes more in line with human health recommendations. Despite a higher linolenic acid level in the linseed diets, the proportion of this fatty acid remained relatively low in liver of overfeeding ducks. We also observed that the dietary fat largely influence the n-3 PUFA (linolenic and ecosapentaenoic acids) proportion of intramuscular fats of birds receiving control or linseed diets (Table 1). The other FA proportions showed no or minor changes between the different dietary groups.This is due to the fact that *de novo* hepatic lipogenesis prevailed over dietary lipid intake to modulate lipid composition of tissues in overfed waterfowl (Chartrin and al., 2003). However, some differences in FA profile appeared between fat location (Table 1).

Table 1 : Fatty acid profile of samples of ducks assigned to three different diets

FA (g/100g)	C diet	L2% diet	L4% diet	SEM
Foie gras				
C18:3 n-3	0.090^{a}	0.228^{b}	0.354^{c}	0.025
C20:5 n-3	0.026^{a}	0.074^{b}	0.090^{b}	0.005
C22:5 n-3	0.022^{a}	0.070^{b}	0.078^{b}	0.012
C22:6 n-3	0.022	0.024	0.036	0.013
n-3 PUFA	0.158^{a}	0.396^{b}	0.558^{c}	0.036
n-6/n-3	23.4^{a}	6.7^{b}	4.5^{b}	3.01
SFA	37.8	37.6	39.3	0.59
MUFA	58.4	58.6	56.5	0.59
PUFA	3.05	3.10	3.08	0.34
Intramuscular thigh fat				
C18:3 n-3	0.654^{a}	1.788^{b}	2.548^{b}	0.073
C20:5 n-3	0.046^{a}	0.086^{ab}	0.126^{b}	0.017
C22:5 n-3	0.228	0.272	0.302	0.066
C22:6 n-3	0.282	0.256	0.258	0.050
n-3 PUFA	1.214^{a}	2.400^{b}	3.234^{c}	0.118
n-6/n-3	18.5^{a}	7.2^{b}	4.9^{b}	0.65
SFA	31.7	30.5	31.0	0.36
MUFA	48.1	49.0	47.7	1.40
PUFA	17.3	18.3	19.0	0.85
Intramuscular breast fat				
C18:3 n-3	0.526^{a}	1.448^{b}	1.912^{c}	0.061
C20:5 n-3	0.124^{a}	0.280^{b}	0.298^{b}	0.025
C22:5 n-3	0.278	0.426	0.434	0.053
C22:6 n-3	0.324	0.432	0.444	0.035
n-3 PUFA	1.252^{a}	2.586^{b}	3.086^{c}	0.126
n-6/n-3	23.6^{a}	9.4^{b}	7.0^{b}	1.03
SFA	34.7	33.4	33.0	0.45
MUFA	42.2	40.3	39.6	0.95
PUFA	19.4^{a}	22.4^{b}	23.5^{b}	0.70

^{a,b,c}Values in the same row with no common superscript are significantly different. SFA = saturated FA ; MUFA = monounsaturated FA ; PUFA = polyunsaturated FA SEM = standard error of the mean.

The "lipidic wasting" (called "fonte lipidique" in french) is a important indicator of the technological quality of the "foie gras". We supposed that the higher n-3 PUFA content in liver with linseed diets would give more elasticity to the cellular membrane and, by this fact, would reduce the lipidic wasting who is prejudicial to the "foie gras" quality. More studies should be performed to confirm this assumption.

Acknowledgements

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EFFECTS OF DIETARY ORGANIC ACIDS ON PERFORMANCE, CARCASS CHARACTERISTICS AND GUT FLORA OF BROILER CHICKS

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Introduction

The hygienic conditions provided in the poultry farms decreases the size of population of intestinal gut flora. So animals are more sensitive against pathogen bacteria from outside in comparison to wild life or free range birds. Consequently, the growth performance of broilers decreases additionally with stress factors (Mulder, 1996; Bilal et al., 1999). This situation leads animal nutritionist to investigate solutions alternative to therapeutic and/or prophylactic (Campenhout et al., 2001). Organic acids (OA) are added to food in order (1) to improve intestinal gut flora and (2) to vanish digestive disorders from stress conditions in animals (Chapman, 1988; Guerrero and Hoyos, 1991). This study was carried out to determine whether dietary organic acid have impact on growth performance, carcass and intestinal parameters on total and gram-negative bacteria numbers.

Material and Methods

The study was conducted in the research farm of 19 Mayıs University. One day old mixed sexes 360 broiler chicks (Ross 308) were used in this study. The chicks were fed with the starter diet (12.87 mj ME kg^{-1}, 240 g CP kg^{-1} as fresh matter basis) for the first three weeks of the experiment. Consequently, they were fed on grower diet (13.40 mj ME kg^{-1}, 200 g CP kg^{-1} as fresh matter basis) during the period of 4-6 weeks of the experiment. Control group was fed with basal diet without containing organic acid while the 2nd, 3rd and 4th groups were fed with the food supplemented at the rate of 0.1% (0.1 OA), 0.2 (0.2 OA) and 0.3 (0.3 OA). Total 48 representative birds (4 birds from each sub-group 2 males and 2 females) slaughtered to determine carcass parameters. For determination of intestinal parameters, 24 male birds (2 males from each sub-group) were slaughtered. For intestinal parameters, 2 male broilers representing each group (total 6), and 24 chicken broilers for total groups were also slaughtered on the 43rd day. For microbiological analysis on the 21st day of the study total 12 chicks (one male from each group), and on the 42nd day total 24 animals (two males from each subgroup) were selected slaughtered after being weighted. The data were analysed by using General Linear Model (GLM) procedures of the SPSS, release 10.0. Because experimental animals were in both sexes, sex factor was regarded as co-variant. The treatment means were compared using Duncan's Multiple Range Test (SPSS, release 10.0).

Results and Discussion

Table 1*. Body weights of broiler chickens fed diets containing organic acids, g per chicken*

Old, days	Control	0.1OA	0.2OA	0.3OA	SEM
1	41	40	40	41	0.4
7	89	90.	88	87	1.7
14	325 **a**	326 **a**	323 **ab**	310 **b**	4.7
21	721	721	715	706	9.5
28	1207	1217	1213	1198	15.8
35	1773 **a**	1789 **a**	1758 **a**	1735 **b**	24.1
42	2342	2347	2274	2321	30.9

SEM: standard error of the mean.

a, b. Values within a row with unlike superscripts differ significantly (P < 0.05).

Table 2. *Feed intake and feed conversion ratio (g feed / g gain) of broiler chickens fed diets containing organic acids*

Old, days	Control	0.1OA	0.2OA	0.3OA	SEM
Feed intake, g/bird					
7	129	129	131	127	2.7
14	483 **b**	493 **ab**	488 **ab**	504 **a**	5.1
21	1045	1060	1062	1081	12.3
28	1870	1902	1909	1935	17.2
35	2924	2963	2983	3010	26.9
42	4196 **b**	4201 **b**	4249 **ab**	4353 **a**	33.6
Feed conversion ratio, feed/gain					
7	1.45	1.43	1.49	1.47	0.03
14	1.49 **b**	1.52 **b**	1.51 **b**	1.62 **a**	0.02
21	1.45	1.47	1.49	1.53	0.04
28	1.55	1.57	1.58	1.62	0.05
35	1.65	1.66	1.70	1.74	0.04
42	1.79 **b**	1.79 **b**	1.87 **a**	1.88 **a**	0.03

a, b. Values within a row with unlike superscripts differ significantly (P < 0.05).

Table 3*. Slaughter body weight (SBW), carcass weight (CW), dressing out percentage (DP), heart weight, liver weight, gizzard weight, abdominal fat pad (AFP), AFP per 100 g body weight (BW) of broiler chickens fed diets containing organic acid.*

Variable	Control	0.1OA	0.2OA	0.3OA	SEM
SBW, g	2387	2513	2351	2403	65.3
CY, g	1745	1847	1744	1778	46.0
DP, %	73.1	73.5	74.2	74.3	0.52
Heart, g	11.2	11.8	11.0	11.8	0.53
Liver, g	44.6	44.9	42.8	42.6	1.79
Gizzard, g	31.8	31.8	31.1	33.0	1.30
AFP, g	35.5 **b**	40.6 **a**	38.9 **a**	45.8 **a**	2.54
AFP, g/100g BW	1.49 **b**	1.64 **ab**	1.67 **ab**	1.95 **a**	0.030

a, b. Values within a row with unlike superscripts differ significantly (P < 0.05).

Table 4. *Small intestine length (SIL), small intestine weight (SIW),*
SIW per 100 g body weight (BW) and pH values of duodenum, ileum
and caecum of broiler chickens fed diets containing organic acids

Variable	Control	0.1OA	0.2OA	0.3OA	SEM
SIL, cm	195.7	194.3	189.2	194.2	5.76
SIW, g	46.1	47.8	42.2	47.2	1.72
SIW, g/100 g BW	1.82	1.87	1.68	1.80	0.054
pH of duodenum	6.6	6.5	6.5	6.6	0.06
pH of ileum	6.6	6.4	6.4	6.6	0.11
pH of caecum	7.1	6.7	7.1	6.7	0.06

Table 5. *Number of total and gram (-) bacteria of ileum and caecum in broiler chickens, fed diets containing organic acid, log coloni forming unit (cfu)/g*

Old, days	Control	0.1OA	0.2OA	0.3OA	SEM
21st day bacteria counts					
Total in Ileum	9.54	8.82	9.78	9.80	0.266
Total in Caecum	10.01	9.72	9.89	9.96	0.127
Gram (-) in Ileum	8.98	8.11	7.81	8.28	0.566
Gram (-) in Caecum	9.96	9.52	9.67	9.85	0.165
42nd day bacteria counts					
Total in. Caecum	9.17	9.22	9.18	9.35	0.075
Gram (-).in Caecum	8.59	8.63	8.45	8.77	0.174

Conclusion

These results showed that organic acids supplementation did not affect the performance of broiler chicks, especially 0.20 % and 0.30% dietary organic acid supplementation decreased feed efficiency without affecting intestinal gram-negative bacteria populations. In conclusion, the dietary supplementation of organic acid had no beneficial effect on either the performance or intestinal flora.

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CONJUGATED LINOLEIC ACID (CLA) AND THE RATIO OF ω6:ω3 FATTY ACIDS ON THE LIPID CONTENT OF CHICKEN GIBLETS

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Introduction

Studies on animals have shown that CLA lowers body fat (Park et al., 1997) and increases lean body mass (Dugan et al., 1997). Brown et al. (2001) reported that culture of pre-adipocytes supplemented with CLA and sunflower oil resulted in higher content of triglycerides when compared to the cultured treated with only CLA, showing that the anti-adipogenic effect of CLA on the pre-adipocytes can be reversed. Therefore, it is reasonable to think that in studying the CLA effect on the lipid metabolism is important to take in consideration the fatty acid composition of the diet as well as the ratio of omega 6 to omega 3 fatty acids. The objetive of these studies were to evaluate the use of CLA and the ratio of ω6:ω3 in the diet on the lipid content in the giblets of broilers.

Material and Methods

Two studies were conducted simultaneously using 100 male or female Ross broiler chickens with 21 days of age at the start of the experiment. The experimental design was a completely randomized, in a factorial arrangement 2 x 5 (two oil sources, i.e., soybean or canola oil and five levels of CLA supplementation, i.e., 0.0, 0.25, 0.50, 0.75 and 1.00%). The oils used were supplied by Bünge alimentos and CLA (Lucta-CLA 60) by BASF. The control diet had 4% soybean or canola oil. CLA levels were obtained by isometrically replacing soybean or canola oil in the control diets. The lipids contained in the chicken giblets were extracted using the technique of Folch et al. (1957). The F test at 5% of significance was used to compare results between sources of oils when interactions were not detected. When there was an interaction ($P<0.05$), it was used the SNK test to compare results between sources of oils. Regression analysis was used to report the effects of CLA levels.

Results

An interaction, oil source vs CLA levels was observed on the total lipid content of heart, liver and gizzard. The use of soybean oil and growing CLA levels resulted in a linear increase ($P < 0.05$) of total lipids on the liver. Birds fed soybean oil had a higher ($P<0.05$) fat liver content (1.72%) than that of birds receiving canola oil (1.38%). These results confirm the observation of heavier ($P<0.05$) livers (1.92%) for birds receiving soybean oil in comparison with that of canola oil fed birds (1.68%). For birds fed the canola oil diets the liver fat content was better explained by a cubic response. The lowest fat liver content (0.81%) was observed for birds fed canola oil supplemented with 0.75% of CLA differing ($P<0.05$) from that of livers of chickens fed soybean oil (2.18%). This go along well with the total serum cholesterol content of males (129.6 x 156.8 mg/100 ml) and females (99.6 x 157.8 mg/100ml) for birds fed canola or soybean oil, respectively. The gizzard fat content of birds fed soybean oil with growing levels of CLA showed a linear increase ($P<0.05$). Without CLA supplementation gizzard fat content was higher ($P<0.05$) for birds fed canola oil (6.23%) in comparison to that of birds fed soybean oil (4.48%). However after 0.50% of CLA supplementation this difference did not show up anymore between oil sources. Heart fat content 7.57% was lower ($P<0.05$) for birds fed soybean oil with 0.25% CLA in comparison with 13.25% of heart fat content of birds fed canola oil with 0.25% CLA. Contrary to what was observed with fat liver content, birds receiving canola oil had higher ($P<0.05$) heart fat content (11.03%) than those receiving soybean oil (9.21%). However, as occur in the liver, there was a lower ($P<0.05$) heart fat content (8.78%) for birds receiving canola oil with 0.75% CLA in comparison with those receiving soybean oil with 0.75% CLA (10.61%).

Discussion

The use of CLA in association with oils rich in ɤ 3 fatty acids or in diets that have a balanced ratio of ɤ 6:ɤ 3 has optimized the CLA effect (Aydin et al., 2001) showing that the CLA effect depend upon the amount of fatty acids ɤ 6 and ɤ 3 in the diet. Therefore, since CLA has the potential of alter the genetic expression of the lipogenic enzymes (Bauman, 2001), it was showed in this study a synergic effect between CLA and canola oil on the lipid metabolism demonstrated by reduction of liver and heart lipid content at 0.75% of CLA when compared to that of soybean oil. However, it was observed adipogenic effect with increased levels of CLA in association with soybean oil

Conclusion

The lipid content of liver and heart is influenced by the oil source used. The CLA response on lipid content in the giblets depend upon the source of fat added to the diet.

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Biosecurity of effluents, hygiene, cleaning

Oral Communications

EXPERIMENTAL STUDY ON THE EVOLUTION OF PIG SLURRY CONTAMINATION BY *SALMONELLA* ENTERICA AND ENVIRONMENTAL IMPACT

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Introduction

Excretion of *Salmonella enterica* by late-fattening pigs may cause the dissemination of this zoonotic agent in the environment during spreading of contaminated slurry (1). Slurry spreading on pastures represents a risk of contamination of animals (ruminants), but also pollution through contaminated runoff water and even pollution of leisure areas (swimming areas) (2). In 2001, an epidemiological study (3) on pig contamination by salmonella led in French breeding-fattening farms showed that 36% of studied farms excreted *Salmonella* in the late fattening period. Even though studies have shown the purifying role of slurry (4), persistence of *Salmonella* in the soil after slurry spreading has been demonstrated (1). Controlling the risk related to the spreading of this effluent entails studying the evolution of *Salmonella enterica* slurry population according to storage time.

A preliminary study was carried out to follow the survival of various serotypes of this zoonotic agent in pig slurry after artificial contamination. Given the results of this study, experimental designs were developed in order to study the effect of different parameters (solid fraction, slurry storage temperature and time, initial *Salmonella* concentration) on the survival of *Salmonella enterica* in pig slurry.

The objective of these studies is to describe the quantitative evolution of different salmonella serotypes, compare two counting methods, describe the influence and interaction of parameters on the survival of slurry *Salmonella*, thereby gathering data that can be applied in the field with a view to propose preventive measures.

Materials and methods

Two series of pig slurry controlled free of *Salmonella* were placed into four flasks amended with four rifampicin-resistant *Salmonella* strains (*Salmonella* Typhimurium, *Salmonella* Brandenburg, *Salmonella* Derby, *Salmonella* Infantis). Using rifampicin-resistant strains makes it possible to compare the mini-MSRV MPN technique (5) to direct isolations on brilliant green supplemented with rifampicin.

Three experimental designs were developed using Doelhert uniform shell design. Three parameters per experimental design were studied at different levels. The number of experiences to be performed was determined according to the following formula: $N=k^2+k+1+n$ (k = number of parameters studied; n = number of replications at the center of the model); i.e., 16 experiments per strain and per experimental design. Two rifampicin-resistant *Salmonella* strains (*Salmonella* Typhimurium and *Salmonella* Brandenburg) were used.

Parameters studied in the first experimental design were initial bacterial concentration (3 levels), percentage of solid fraction (5 levels) and storage time (7 levels from 0 to 30 days). Flasks were amended with *Salmonella* Typhimurium and stored at 20°C. The other two experimental designs studied initial bacterial concentration (3 levels), storage temperature (5 levels) and storage time (7 levels from 0 to 18 days) for two *Salmonella* strains. Flasks were filled with 200 mL slurry controlled free of *Salmonella*, amended with *Salmonella* and stored under the conditions set by the experimental designs. In order to compare the two methods, *Salmonella* counting was done by direct isolation on brilliant green with rifampicin and by mini-MSRV MPN technique.

Results

A 2-log decrease in *Salmonella* population was observed in 28 days for the first series and 35 days for the second. One strain, *Salmonella* Brandenburg, showed a more rapid decrease in both slurries. Comparing count means in paired series ($p<0.05$) brought evidence that mini-MSRV MPN technique is adapted to count salmonella in pig slurry. Moreover, mini-MSRV MPN technique counting is possible with concentrations lower than the limit of numeration by direct isolation. Effluent volume and slurry storage mode (static or under agitation) do not influence *Salmonella* decrease under our experimental conditions. The only difference observed between the two slurries accounting for the difference in behavior was the percentage of solid fraction. This parameter will be included into experimental designs. Slurry *Salmonella* survival also seems to be strain-dependent.

After counting by mini-MSRV MPN technique and processing results using STATGRAPHICS® software, we get the significance (P value) that is set at the limit of 5% and the effects assessed for each parameter, as well as the effect of their interactions on the decrease in the amount of slurry *Salmonella*. In the first experimental design (Table 1), time clearly shows a significant influence on the decrease in *Salmonella* Typhimurium with a negative effect. Solid fraction proportion and initial bacterial concentration do not significantly influence the decrease in *Salmonella* concentration. Only the interaction between time and bacterial concentration is retained with a negative effect.

In the other experimental designs, two strains are compared, *Salmonella* Brandenburg and *Salmonella* Typhimurium (Table 2). Time, which is significant for both strains, has a negative effect. Temperature also has a significant negative effect, but this is only noted for *Salmonella* Typhimurium. For both serotypes, initial concentration does not influence *Salmonella* decrease. Temperature/concentration and time/concentration interactions are significant for *Salmonella* Typhimurium only, with a negative effect.

Table 1: results of experimental design 1

Design 1 *S.* T.	Parameters	P value
Independent parameters	A: Solid fraction	0.8636
	B: Time	**0.001**
	C: Concentration	0.1728
Interactions between parameters	AB	0.9672
	AC	0.9943
	BC	**0.0060**

Table 2: results of experimental design 2

Design 2	Parameters	P value *S.* T.	P value *S.* B.
Independent parameters	A: Temperature	**0.0033**	0.0956
	B: Time	**0.0009**	**0.0026**
	C: Concentration	0.0704	0.3203
Interactions between parameters	AB	0.3293	0.9935
	AC	**0.0155**	0.6469
	BC	**0.0203**	0.4493

*S.*T. *: Salmonella* Typhimurium
*S.*B.: *Salmonella* Brandenburg
Significant effect if P<0.05

Discussion

In all experimental designs and for both strains, time is a significant factor of *Salmonella* concentration decrease.

Temperature is significant with a negative effect for *Salmonella* Typhimurium only. This influence is confirmed by Placha's experiment (4), which describes a more rapid decrease in the summer than in the winter for this bacterium. However, this parameter does not seem to be significant for *Salmonella* Brandenburg; its influence could be strain-dependent.

Initial slurry concentration does not seem to be linked to the phenomenon of bacterial decrease. However, this parameter is still to be measured in order to determine the concentration decrease to be achieved. This observation can be partly explained by the wide limit defined for the time factor. Indeed, the response studied is *Salmonella* concentration in slurry at the time of sampling. The software analyses this response taking into account the three initial parameters, including inoculum. Yet, most results show a very low *Salmonella* count and even a complete absence of *Salmonella* in the analyzed sample, whatever the *Salmonella* amount in the inoculum.

Solid fraction proportion in slurry is a parameter that does not significantly influence the decrease in *Salmonella* concentration. Finally, the effect of time/concentration and temperature/concentration interactions is only significant for *Salmonella* Typhimurium. It may be assumed that there is a strain effect on time and temperature parameters.

Therefore, it would be appropriate to confront some of these parameters, and to include others, in experimental designs carried out over a shorter period so as to validate the effects observed and, subsequently, with different strains in order to observe a possible strain effect linked to the parameters.

Conclusion

Mini-MSRV MPN technique is a pig slurry *Salmonella* counting tool that appears to be reliable, easy to operate, inexpensive and adapted to the field. This technique enables the initial *Salmonella* concentration to be determined in slurry.

Only time stood out as a significant parameter for both strains. The effect of temperature is only noted for *Salmonella* Typhimurium. Beside a possible serotype or strain effect to be confirmed, this difference could also depend on other parameters, such as slurry oxygen content. Thus, new experimental designs will make it possible to confirm these data and quantify the effect of other parameters on the survival of salmonella using field strains as well.

In fact, a final objective is an attempt to develop a mathematical model based on these experimental designs with a view to propose to pig breeders a tool for controlling the risk of dissemination. This mathematical model could contribute to a better control of *Salmonella* dissemination in the environment.

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BEHAVIOUR OF ENTERIC MICRO-ORGANISMS DURING COMPOSTING OF RURAL SEWAGE SLUDGE

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Introduction

The spreading of sludge will only be possible on the condition of being able to guarantee to farmers and consumers the harmlessness of this practice. To minimize the potential risk for the health of humans and animals, it is necessary to coordinate sludge applications in time with planting, grazing or harvesting operations. For these reasons, sludge could not be applied continuously and must be stored in tanks during about 6 months before application. In this context, we studied the effect of sludge co-composting on survival of bacteria present in sludge stored in a tank of a rural water treatment plant.

Enumeration of bacteria covered one whole compost cycle lasting from fresh sludge to mature compost for a period of 7 months. Temperature and bacterial densities were measured in three relevant locations on the pile including the side of the incoming air flow (incoming air area), the bottom (in the middle of the pile) and the top of the pile (outcoming air area).

Material and Methods

A total of 8.1 tons of pressed sludge (15% of dry matter) were mixed with 1.4 tons of straw. The composting period carried out over 4 months with turning every month was considered as the fermentation phase, and the following 3 month period without turning, as the maturation phase.

The pile temperature profile was recorded by means of thermocouple probes inserted into the 3 areas of the pile and connected to a digital logger. The mixture was sampled upon settling, during each turning and at the end of the maturation period. Each sample was carried out in four replicates.

Enumerations of *Salmonella* and *Clostridium perfringens* were performed according to AFNOR methods[1, 2]. Enterococci, *E. coli* and *Listeria monocytogenes* were enumerated by the procedure previously described by Garrec *et al.* [5].

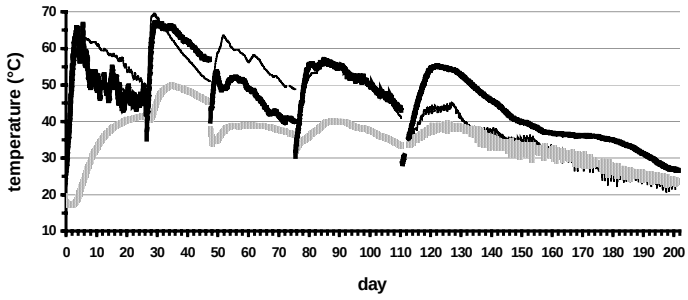
Results

During the fermentation phase, the peaks of temperature of the incoming and the outcoming air areas were rapidly reached after each turning and ranged between 53 and 69°C whereas the temperature of the bottom did not exceed 42°C except in the 2nd month (figure 1).

The maximum temperature decreased progressively during the composting process but still reached 55°C in the outcoming air area during the maturation phase. The maximum temperature decreased progressively during the composting process but still reached 55°C in the outcoming air area during the maturation phase.

Figure 1. *Temperatures inside the heap (incoming air area : — ; outcoming air area : Ƶ ; bottom : Ƶ). Days 0, 27, 48, 76, 111 and 202 correspond respectively to the settling, turnings and unloading of the heap.*

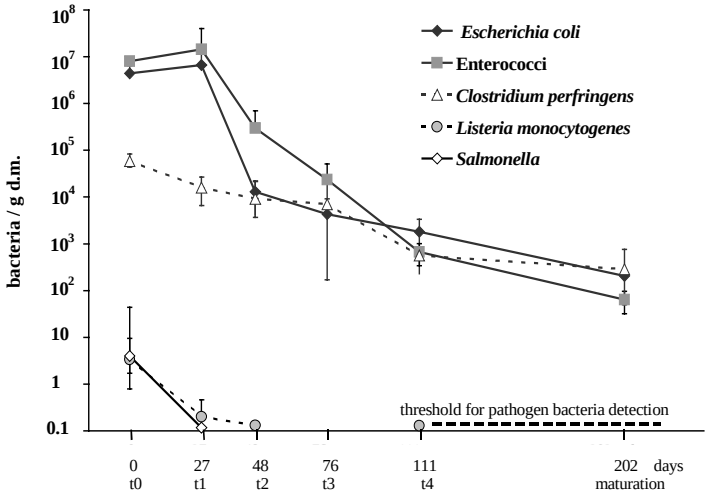
Faecal indicators, of which the concentrations slightly increased after the first turning (figure 2) were not totally inactivated although the temperature reached 66°C in the



pile. It was necessary to await the 2nd turning to observe a significant decrease.

The effect of composting on *C. perfringens* is reflected by the regular disappearance of approximately a factor 2 until the 4th turning.

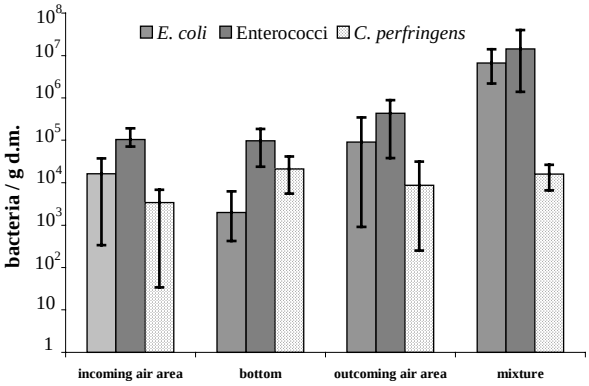
Figure 2. *Average concentrations of bacteria during the composting. Bars indicate minimal and maximal values.*



Salmonella was isolated only in one sample of the 1st turning and never afterwards whereas *L. monocytogenes* was still present in the 4 samples of compost carried out after one month of composting (data not shown).

Regardless of the period of composting, the densities of bacteria in the 3 areas of the pile before the turning did not significantly differ from one area to another as is shown in figure 3 for the 1th month of composting.

Figure 3. *Average concentrations of bacteria in the 3 areas and in the mixture before and after the 1th turning. Bars indicate minimal and maximal values*



The frequency of detection of the bacteria and their concentrations in the mixture carried out after each turning were systematically higher than those of the 3 areas. This phenomenon can be explained by the existence of zones of the compost in which concentrations of micro-organisms were probably higher. As a consequence, the integral mixture of the pile at the time of each turning could involve a slight increase in bacterial concentrations.

Discussion

In agreement with the results of Sesay *et al.* [6], we observed that both faecal indicators presented a similar length of survival during the composting of sludge, whereas longer survival of enterococci in relation to the coliforms during composting was reported by Tiquia and Tam[9]. It appears that the inactivation of micro-organisms depends not only on the type of bacteria but also on the composting process and on the origin of the biowaste.

Bacterial regrowth that we observed for faecal indicators had already been reported by Sesay *et al.* [6] who observed a regrowth of faecal indicators after each turning of compost which they put down to a contamination of the mixture by the external zone of the compost non affected by the rise in temperature. This hypothesis could explain why, in our study, the bacterial densities, after the turning of the compost, were always higher than those obtained in each zone of the pile.

Salmonella disappeared more quickly than faecal indicators as was previously observed[8] but the absence of detection of *Salmonella* is probably due to their weak level in the initial product which involves the threshold of detection being rapidly reached. In agreement with Watkins and Sleath[10], we observed that the survival of *L. monocytogenes* was greater than that of *Salmonella*. Given the high resistance of *L. monocytogenes* to environmental factors [3, 4], it is possible that the presence of *L. monocytogenes* up to the 4th turning is due to a better environmental adaptation of this species during the fermentation phase of composting.

Conclusion

The hygienic effect of composting of the sludge mixed with straw results in a significant reduction of enteric micro-organisms without however leading to the complete disappearance of faecal indicators. As reported by Sidhu *et al.* [7], the technique of composting does not guarantee the complete hygienisation of the end product obtained, insofar as it is necessary to take into account a potential regrowth of bacteria.

Furthermore, the use of *E. coli* or enterococci as indicators of hygienisation could be discussed as the survival of pathogen bacteria differs from one pathogen to another, as we have observed with *Salmonella* and *L. monocytogenes* in our study.

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COMPARATIVE STUDY ON ANTIBIOTIC RESISTANCE IN SELECTED BACTERIAL SPECIES ISOLATED FROM WASTEWATER ORIGINATING FROM SLAUGHTERHOUSES AND OF MUNICIPAL SOURCES

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Introduction

Antibiotic resistance in man may be hospital acquired or community acquired (1,2,3). The use of antibiotics in agricultural animal production is often blamed to contribute predominantly to the community acquired antibiotic resistance in the human population. One of the ways multiresistant bacteria may be introduced into the biocoenosis and into humans via environment may be by faeces of treated animals (4). Since human sources are also contributing to introduce antibiotic resistant bacteria into the biocoenosis via municipal wastewater a comparative study has been performed by characterizing selected bacterial species from municipal wastewater and wastewater from a slaughterhouse according to their resistance patterns.

Material and Methods

Wastewater was taken by an automatic sampler over the period of one year at two different sewage treatment plants. One plant was receiving only wastewater from municipal source, the other from municipal sources and from a slaughterhouse. At the latter also samples of gut content had been collected during slaughtering. The samples were characterized by determining the bacterial count of bacteria growing at 37 °C, total coliforms, faecal Streptococci and Staphylococci. In *Escherichia coli*-, *Enterococcus faecalis*- and *Staphylococcus aureus*-strains, identified on species level, the sensitivity against certain antibiotics was determined with the help the agar diffusion test (DIN 58 940 part 3/1989) and the micro dilution method (DIN 58 940 part 8/1990). A total number of 400 *E. coli* strains, 245 *E. faecalis* strains and 188 *S. aureus* strains was investigated.

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Results

While 21% of the tested *E.coli*-strains that could be isolated from the slaughterhouse waste water were as resistant, the *E.coli*-strains isolated from the municipal waste water were to 18% resistances. A seriously increasing resistance situation has been found with respect to the *E.coli*-strains, both from samples of slaughterhouse- as of municipal-waste water against Tetracyclin. 46% resistant strains could be found in the slaughterhouse waste water and 42% resistant strains were found in the municipal waste water. A more favorable situation otherwise arose for the resistances against the further tested antibiotics for both *E.coli*-strains.

In *E.faecalis*-strains a problematic resistance situation against the examined antibiotics was found. This concered resistance against Doxycyclin, Erythromycin, Bacitracin, Ofloxacin, Chloramphenicol and Penicillin. Only Amoxycillin and Vancomycin show a good effectiveness (93%-100% sensitive). In the slaughterhouse waste water, 61% of the tested *E.faecalis* were determined as resistant. In the comparison to it, the municipal waste water contains less resistant *E.faecalis* (47%).

S.aureus-strains showed with 24% also a high resistance-frequence against the penicillinase resistant antibiotic „ Oxacillin“ in the municipal waste water. It could be determined unequivocally that with *E.coli*- and *E.faecalis*-strains from the slaughterhouse waste water more multi-resistances than with those from the municipal waste water existing is introduced in the environment. With S.aureus-strains, an exactly reverse situation was found.

Discussion

The results of the agar diffusion test deliver fast statements over the antibiotics sensitivity of pathogens, against which the results worked out with the dilution test (micro dilution method) appear more exactly and therefore micro dilution method is in principle more suitable for the monitoring of resistance. Although the examinations were done under same conditions, the results with both methods are slightly different. For example *E.coli*-strains isolated from the slaughterhouse waste water were over all in the agar diffusion test to 75% sensitive, to 13% intermediate and to 12% resistant, while in the micro dilution method the tested *E.coli*-strains are found to be for 54% sensitive, for 25% intermediate and for 21% resistant.

It is still open how the ways of transmission of multi resistant bacteria via surface water or living carriers may happen and how big the practical importance of this scenario is, but it is a fact, that agriculture is contributing to the introduction of multi resistant *E.coli* and *E. faecalis* into the environment, while the introduction of multiresistant *S. aureus* seems to be mainly due to human sources.

Conclusion

The results of the sensitivity testing of the bacteria from municipal- and slaughterhouse waste water isolated *E.coli, E.faecalis* and *S.aureus* show that important quantities of antibiotic resistant bacteria can be introduced with the cleaned waste water into the environment. Adequate measures have to be taken.

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HYGIENIC ASPECTS OF BIOSOLIDS RE-USE

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Land application of biosolids provides agricultural benefits and presents a cost-effective method of sludge disposal following wastewater treatment. However, re-use of this product causes health concerns that must be addressed and satisfied before land application is an accepted practice. Health concerns include pathogen transmission to food or agricultural workers, contamination of ground water or surface water with faecal material from field run-off, and build-up of heavy metals or organic contaminants.

However, manures can contain pathogenic microorganisms which creates the potential for the spread of zoonoses from the farm environment into the food chain or aqueous environment. There is little quantitative information on the fate of manure pathogens during storage and following land application. This makes it difficult to assess whether current manure management guidelines, which are largely focused on reducing nutrient pollution, are appropriate for controlling the risks to food safety and water quality.

Concern has greatly increased about the potential for contamination of water, food, and air by pathogens present in manure, byproducts, and bioaerosols.

Effective sanitation of the environment, particularly of some of its special parts, which can be a source of spreading of diseases, plays an important role in prevention of infectious diseases. In this respect special attention should be paid to the disinfection of infected farm animal excrements. Sanitation of excrements should, on the one hand, ensure effective devitalization of infectious agents and, on the other hand, comply with the requirement of preserving the composition of the manure so it can be used in agricultural production.

Pathogenic microorganisms and helminth eggs cannot be detected simply by our senses, however, their presence in the environment presents serious risk to the health of man and animals (Juriš et al., 1989, 1991; Holoda, 1998). From this point of view, excrements of sick animals can be considered important contaminants of the environment.

Survival and transport of pathogens from manure to the environment depend on a number of complex phenomena.

Chemical and microbiological changes in the composted substrate are accompanied by an increase in temperature which plays an essential role in ensuring the devitalization of pathogenic germs. Different data were presented about the causative agents of some diseases, such as mycobacteria, with regard to their survival during composting. High resistance of mycobacteria in the outer environment has been observed by Švrļek et al. (1998) who reported that they survive in infected manure for 240 days.

Biowaste is known to contain pathogenic bacteria such as Salmonella and other microorganisms that may be a health risk for both people and animals. The biosecurity risk associated with using digested residues as fertiliser is hard to assess but this risk can not be neglected.

In recent years the fate of human and veterinary therapeutic agents as a potential pollutant of the environment has been paid increased attention. Substantial quantities of these compounds and their metabolites are excreted, flushed down the drain, discarded as waste, or left over in animal feedlots. When they enter the sewer, several of these compounds are not adequately eliminated by the methods that are currently used in sewage treatment. Substantial quantities of biosolids and livestock manure end up on agricultural land.

Four major types of human pathogens can be found in biosolids: bacteria, viruses, protozoa, and helminths. Böhm and co-workers (1999) examined a wide variety of manures, food-processing residues and household wastes for the presence of pathogenic bacteria, fungi and viruses.

The quality of treated sludge should be defined on the basis of risk to human, animal and plant life. The levels of pathogens in the treated sludge should not exceed their ambient levels in the environment. In practice this means that for the purposes of quality control realistic limits of defined pathogens must be set and well-established standard procedures have to be followed.

From the variety of bacterial pathogens Salmonella spp. are the most relevant since they can infect or contaminate nearly all living vectors from insects to mammals. Multiresistant bacteria are coming more and more into focus since their transmission via environment as well as the introduction of resistance genes into other bacteria may cause tremendous problems in human and veterinary medicine. From the viewpoint of environmental risks viral pathogens such as enteroviruses and rotavirus are the most relevant ones (Metzler et al., 1996). Special attention must be paid to the parasitic pathogens, not only to eggs of round- and tapeworms but to Giardia lamblia and especially Cryptosporidia parvum too, since the latter sometimes occurs in slurry from calves and cattle used in co-digestion. Nearly all gut related pathogens can be found in slaughterhouse effluents. If sludges are of plant origin or if they had been processed by using plant material, they may contain plant-pathogenic viruses, fungi, bacteria, parasites and undesired weeds. This will cause an additional phytohygienic risk if the final product is to be used in agriculture as fertilizer (Böhm 1999 a, b).

Recycling to agricultural land is an important outlet for sewage sludge and other organic wastes but it must be controlled in order to obtain agricultural benefit from the operation whilst protecting human and animal health and the environment at large. Current practices in Europe are based on the requirements of the 1986 Directive on the use of sewage sludge in agriculture (86/278/EEC). However, since that time new technologies have become available for sludge treatment, more pathogens associated

with the food chain have been identified and the concerns of the public relating to acceptable risk have changed.
A wide range of phyla, genera and species are likely to be present in sludges, particularly those that contain large amounts of faecal material. The identity and numbers of pathogens in municipal wastes will be dependent upon the health of the contributing population.
According to Davis et al., 2002 E. coli counts in treated sewage sludge or sludge from meat processing waste released for land use should not exceed 1000 per gram (dry weight) whereas those of C. perfingens spores 3000 per gram (dry weight). A 4 log10 reduction in numbers of added Salmonella should be achieved, and Ascaris ova should be rendered non-viable.
Of course there are some constraints on the reuse of sludges in agriculture. For example those that may contain the BSE agent should not be applied to land where animals have direct access whereas sludges from paper, vegetable and tannery waste should present no risk after mesophilic digestion or a similar treatment standard.
According to Rapp (1995) total bacterial counts as well as the numbers of Enterobacteriaceae, E. coli and faecal streptococci remained nearly unaffected under practical conditions (farm storage tanks), and even increased during storage for up to 185 days. The numbers (cfu) of Salmonellae in slurry exposed to the slurry in semi-permeable membranes were reduced by more than 105. Yersinia enterocolitica was completely inactivated within only a few days whereas the eggs of Ascaris suum as well as the oocysts of Cryptosporidium parvum also lost their viability, although very slowly.
The study by Findlay (1972) showed that Salmonella dublin in cattle slurry survived storage for between 19 and 33 weeks. Similarly Larsen and Munch (1986) found that Salmonella typhimurium survived in pig and cattle slurry stored at 8°C for more than 10 weeks with only a small decrease in numbers (from 10^6 to 10^4/ml). Yersinia enterocolitica was eradicated by week 6 while Staphylococcus aureus was reduced from 10^6 to 10^2/ml in 9 weeks. In the solid fraction of pig slurry, Salmonella typhimurium survived for 26 days in summer and 85 days in winter (Placha et al., 2001), and coliforms were reduced by 90% in 35 and 233 days during the summer and winter time, respectively. Cryptosporidium parvum oocysts required more than 90 days to become non-viable in cattle slurry stored at 4°C while at 15 and 20°C they were nearly all killed in 30 and 20 days, respectively (Svoboda et al., 1997). In the farmyard, manure stored at temperatures of 4 and 15°C, 30% and 8% of oocysts, respectively, survived longer than 90 days. When composting was encouraged and temperatures in excess of 30°C were achieved, there was no survival of oocysts after 35 days.
The two following figures (Fig. 1, Fig. 2) express the effects of temperature and time on some species of micro-organisms. In Figure 1. (Feachem et al., 1982) the Safety Zone is the area of the graph where pathogens would not survive, or the sludge would be virtually pathogen free due to a combination of time and temperature. The lowest safe temperature for the tested pathogens elimination was about 45°C. The length of time required to maintain the sludge at this temperature was over 40 days. By increasing the temperature to 50°C, the time required shortened to just over three days, at 55°C to 15 hours, at 60°C to two hours and at 70°C to seven minutes. This describes ideal conditions which are rarely achieved in full-scale treatment plants. Practical studies indicate that sludge should be held for four hours at 55°C (Fig. 2) or 30 minutes at 70°C to kill at least 99.99% of pathogens (Carrington et al., 1998). The heat distribution through concentrated sludge and solid materials is more difficult to estimate; therefore the time safety margin should be observed.

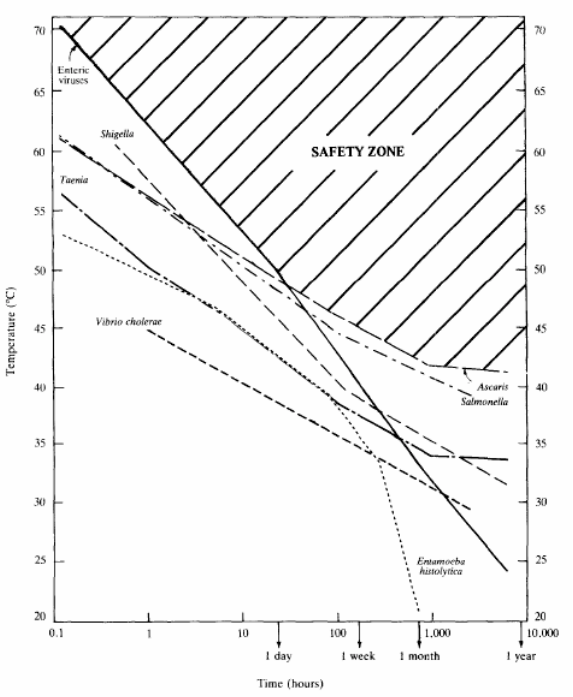

Figure 1.

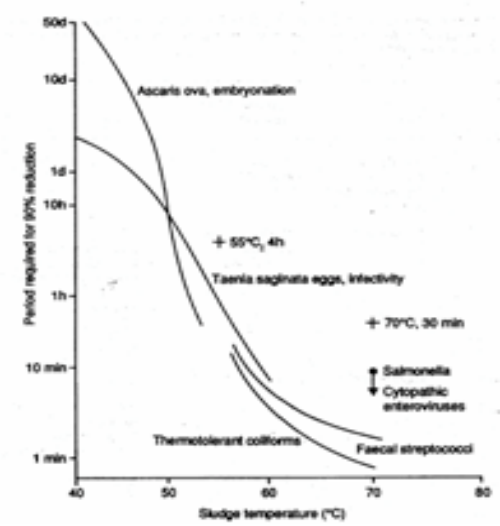

Figure 2.

Concluding it can be said that with view to the threats not only of diseases virtually or potentially transmissible to animals and man but also with view to the threats of global terrorism the hygienic aspects of the production and re-use of biosolids are of utmost importance. They present a challenge that requires further sound research and surely also global cooperation and even coordination.

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INVESTIGATIONS ON MICROBIAL INDICATORS AND/OR TEST-ORGANISMS IN SUPERVISION OF HYGIENIC SAFETY IN CO-DIGESTION OF ANIMAL SLURRY, BIOWASTES AND/OR ANIMAL BY-PRODUCTS

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Introduction

Recycling of organic material containing tissues and wastes of animal origin is connected with the risk of spreading pathogens of man and animals, especially if animal by-products are involved. Zoonotic agents of bacterial, viral, fungal and parasitic nature can often be found in raw materials as well as under certain epidemiological conditions agents of special veterinary importance as e. g. Foot and Mouth Disease Virus too. Since the aerobic or anaerobic thermophilic process is in principle effective in inactivating most pathogens (sporeformers and TSE – agents excluded) criteria have to be set up to ensure the safety of the applied treatment processes or technologies. In this context a three step procedure is demanded which has proven to be effective and administrable in certain member countries. First only technologies shall be applied which had been validated in a legally fixed procedure. The second step is the steady process supervision by continuous recording of relevant process data and the third step is the regularly supervision of the final product. Recently EU – regulations had been fixed which are dealing mainly with TSE-risks due to animal by products including those of faecal origin. Those regulations have to be regarded very carefully if they are fulfilling the intended purpose concerning risks due to other causative agents of notifiable and other infectious animal diseases as well as other zoonotic agents. The hygienic parameters given there are originating from experiences with products of rendering plants fixed 20 years ago and are only defining simple process requirements and microbiological threshold values for the final products. The given microbiological parameters and the recommended supervision strategies are up to a certain extend conflicting with actual scientific experiences and facts. This was the background for carrying out this investigations mainly for reviewing microbial indicators recommended for the supervision of the final products as well as for giving data concerning tenacity of different test organisms suitable for process-validation before an epidemiological background.

Material and Methods

To compare the inactivation kinetics of bacteria and animal viruses causing infectious diseases with those from other infectious or saprophytic agents, which may act as possible indicator organisms for investigating the hygienisation efficiency of composting and anaerobic digestion plants under practical (field) conditions, two identical composting containers as well as two anaerobic fermenters in a half technical scale were built and driven in parallel, filled with the same materials. In one of them, the inactivation of Classical and African Swine Fever Virus (ASFV), Swine Vesicular Disease Virus (SVDV), Foot and Mouth Disease Virus and Aujeszky Virus was investigated by use of different germ carrier techniques, which are described in more details by Moss and Haas (4) In the second the inactivation of Salmonellae, Fecal Streptococci, Equine Rhinovirus (ERV), Enteriic Cytopathogenic Bovine Orphan (ECBO) Virus and Bovine Parvovirus was studied in parallel. The composting containers were filled with biowastes, the anaerobic fermenters with pig or cattle slurry in a mixture with biowastes (co-fermentation), more detailed descriptions may be found at Hoferer (3). Since only the anaerobic process regarded here the following type of reactor had been used in the experiments for survival studies under mesophilic and thermophilic conditions. His set up of experiments had

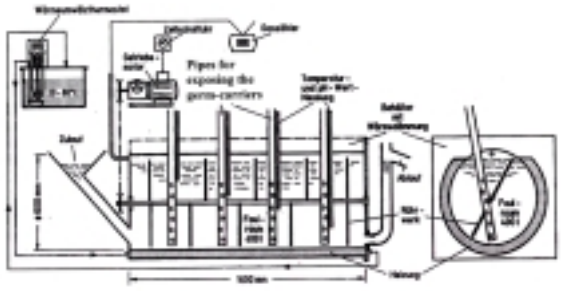

Fig. 1: Scematic representation of the semi-technical biogas reactor

been completed by bacteriological input-output analysis in co-fermentation units using different substrates, described in more details by Beyer et al. (1) and comparative experiments with plant pathogenic fungi and viruses as well as weed-seeds in pasteurization units and biogas reactors for which further details may be taken from Böhm et al. (2).

Results and Discussion

The results concerning survival of different pathogenic and indicator organisms in the anaerobic process are as follows:

- The bacteria and viruses were inactivated under both mesophilic and thermophilic temperature conditions. In the mesophilic environment, the D-value (the time necessary to reduce the number of micro-organisms by one $\log_{10}$ unit at a specific temperature) ranged from 11 hours (ECBO-Virus, 35°C, cattle slurry, volume germ-carrier) to 13 days (Bovine Parvovirus, 30°C, stored pig slurry without addition of leftovers, volume germ-carrier). This shows that the anaerobic codigestion under mesophilic temperature conditions cannot ensure a sufficient inactivation of potential pathogenic epidemic germs in the biogas substrate. Under thermophilic conditions all examined organisms and viruses were inactivated considerably faster. At a temperature of 50°C the D-values were between 7,2 minutes (ECBO-Virus, volume and sandwich germ-carrier, pig slurry) and around 30 hours (Bovine Parvovirus, volume germ-carrier, cattle and pig slurry). At 55°C D-values of 1,2 minutes (*E. coli* O157, pig slurry) and up to 8 hours

(Bovine Parvovirus, cattle slurry, volume germ-carrier) were found.
- With the exception of *E. faecium* and Bovine Parvovirus under thermophilic conditions, all of the analysed bacteria and viruses were reduced by more than four $\log_{10}$ units during a period of six hours.
- After comparing the D-values of the bacteria and viruses analysed at the University of Hohenheim with those of the viruses which where examined at the Federal Research Centre for Virus Diseases of Animals in Tübingen (0) in parallel, the Equine Rhinovirus represents a suitable test organism especially with respect to Foot and Mouth Disease Virus. However, since the differences in the D-values of both viruses were small and other viruses which are relevant for animal epidemics (SVDV, ASFV) had, in some cases, higher D-values than the Equine Rhinovirus under specific temperature conditions, ERV as sole indicator organism would not guarantee sufficient security for the validation of biogas plants. Comparing the D-values and z-values (temperature difference, which corresponds to a reduction of the D-value to 1/10) of all examined germs the faecal streptococci as well as the Bovine Parvovirus can be favoured as test organisms in validation procedures. Due to their high thermo-resistance the Bovine Parvoviruses seem more appropriate for validation of reactors and pasteurization devices at temperatures above 50°C.

Input and output analysis of different substrates used in cofermentation gave the following results:
- No representative indicator could be found which could be used for all types of substrates. Organism used in analysis of drinking water like *E.coli* as indicators for faecal pollution are not generally present in the input material, and if their quantity is high variable no decision on the hygienic status of the final product could be based on.
- Such general parameters like *Enterococci* or *Enterobacteriaceae* are not applicable for input-output analysis if biotechnological processes are involved, because organisms belonging to such groups are part of the process microflora and their propagation in the processed substrates does not necessarily correlate with those of pathogens of epidemiological relevance.
- If *Enterococci* shall be used in this context for input/output analysis in processing certain substrates of faecal origin defined species like *Enteroccus faecalis* shall be used as parameter. Suitable PCR-methods on species level are available (0).

The results concerning relationship between selected indicator and test-organisms of veterinary and public health importance to phytopathogenic organisms and weed seeds in thermophilic biogas processes and in pasteurization devices are as follows:
- The data indicate that an inactivation of *Plasmodiophora. brassicae* takes place during an exposure time of 23 hours at 55 °C in a thermophilic biogas-reactor. Thermal inactivation of tomato seed was also observed under this conditions. This correlates in principle with he behaviour of *Salmonella sp*., *E.coli* and Enteroviruses exposed under the same conditions
- Pasteurization of 1 hour at 70 C inactivates both, *Plasmodiophora brassicae* and tomato seeds as well as *Salmonella sp*., *E.coli* and Enteroviruses
- Inactivation of tobacco mosaic virus was inefficient both after incubation for 24 hours at 55 °C in a biogas-rector or 1 hour at 70 °C in a pasteurization device. Same applies for Bovine Parvovirus.
- Clostridial spores will be totally unaffected, both by pasteurization and thermophilic anaerobic treatment.

Conclusion

The system of process-validation, steady supervision of relevant process data and supervision of the final product for selected bacteriological parameter like Salmonella in 50g cannot be replaced by a simple product supervision, because microbiological properties and the occurrence of pathogens with epidemiological importance in the raw-material are highly variable and material related. If materials with high epidemiological risks concerning the presence of animal and plant pathogen are processed no representative indicator organism in the final product could be identified nor could be found, that the used process recommendations and test-organisms are representative for both groups. With respect to animal by-products this means that processes used for treatment of category 3 materials shall be validated by highly resistant viruses like bovine parvovirus as test organisms.

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EFFICIENCY AND COST COMPARISON OF DIFFERENT CLEANING AND DISINFECTING PROCESSES FOR PIG FARMS.

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Introduction

An effective scheme of cleaning and disinfection makes possible to reduce incidence and consequences of numerous diseases. Programs of cleaning and disinfection have been proposed for a long time to pig farmers but the recommendations have been more based on practical experience rather on scientifically established data. In addition, these operations considered as difficult and little gratifying are not always correctly implemented by pig farmers (Corrégé, 2002) and they represent a considerable cost, particularly in terms of working time. The aim of this study is to propose an optimised scheme of cleaning and disinfection for barns.

Materiel and methods

This study was led in the two ITP experimental farms. Various methods, at each principal stage of cleaning and disinfection of the buildings were compared in farrowing, post-weaning and fattening units (table 1). For each test carried out, the method to be tested was compared with a beforehand-defined standard program of cleaning and disinfection. This comparison was made either in the two halves of the same room, or in two identical rooms having contained the same batch. Efficiency of cleaning was approached by measurement of TPA (URL) and quality of disinfection by aerobic colony counts 48 hours after the disinfection(Corrégé et al., 2003), on 15 sites shared out within the room. (De Azevedo Araujo, 2002). Labour times and water and products consumption were recorded for each test in order to calculate the cost of these operations. Statistical analysis, both for TPA and bacterial counts, was carried out with software SAS, by variance analysis (GLM procedure) applied to logarithmic values of bacterial counts and URL.

Results and Discussion

Automatic soaking system (i.e. ramp of steeping with timer) in comparison with manual steeping (flat jet) doesn't lead to any improvement of cleaning and disinfection (table 2). Nevertheless, it reduces considerably costs thanks to a saving of labour time of 30 hours per year for 100 sows. These results don't join those reported by Foucher (1997), which allotted to automatic soaking a better effectiveness considering bacteriological results. Actually, it seems that the better general efficiency of automatic soaking thanks to the sequential water distribution is cancelled by a better local efficiency of manual soaking (the operator is spending more time when surfaces are difficult to wash or very soiled).

The use of a detergent, before or after the phase of pressure wash, allows a significant improvement of the quality of cleaning. On the other hand, disinfection is significantly improved only when the detergent is applied after pressure wash. Using detergent before pressure wash reduces time necessary to this operation, of 1,5 hours in farrowing units, 6,5 hours in post-weaning and 15 hours in fattening (for 100 sows in production). In farrowing units, the economy of time is not sufficient to compensate for the cost of the product. Yet, in post-weaning and fattening, there is a profit of 60 € per year for 100 sows.

The draining and the washing of the slurry pits improve decontamination of the rooms, and more particularly, of the grounds and the high parts of the walls. In the rooms where the slurry pits are emptied then washed, the quantity of alveolar dust (particles of diameter < 1µm) is reduced of more than 50%. Since particles measuring less than 4µm are able to carry bacteria (GUINGAND, 1994), the reduction of their number should limits the recontaminations.

Disinfecting with pulverization in comparison with foam leads to similar results in term of disinfecting efficiency. Thus, the better bacteriological efficiency of foam (in comparison with pulverization) brought back by Mahe (2002) has not been checked. The explanation could that the recommended quantities of disinfecting solution by m² of surface (0.3 l/m²) were scrupulously followed, which is unusual under farms conditions, because of the very large quantities of disinfecting solution required (120 litres / 100 m²). So time needed for pulverization is definitely higher than for foam (annual total over cost of 18.5 € per productive sow).

A second disinfection, either by foam or by thermonebulisation, results in a reduction of the bacterial contamination. Moreover, the thermonebulisation allows to reach inaccessible parts of the barn: the level of contamination of the slurry pits decreases significantly and is similar with the one obtained after the first disinfection of the pits using foam disinfection. In addition, the cost of a second disinfection by thermonebulisation is lower than the double disinfection using foam.

The heating of the room by "thermobile" at the end of disinfection process allows a faster drying of the buildings: heated rooms start to dry from the first day after disinfection and are completely dry at the end of 48 hours (temperature and hygrometry were recorded). On the contrary, in not heated rooms, drying starts later and humidity persists until day sixth after disinfection. In the second day after disinfection, the majority of the heated rooms presents a contamination lower than the not heated ones. Nevertheless, the only really significant reduction (34 observations, $p \leq 0,05$) was observed during a repetition carried out whereas the outside temperature was 2°C. Finally, heating seemed a good way to reduce contamination, at least in winter time. Its use 6 months per year represents an annual cost close to 600€.

Incidence of the heating on the level of contamination can be explained by two manners:

- Reduction of the dust level following a faster drying: the dried particles, lighter, can be eliminated more easily by ventilation and the risk of recontamination (by the deposit of this dust) is thus reduced.

- Conditions less favourable to microbial survival: the heating of the rooms allows a faster elimination of water and thus makes the bacterial multiplication more difficult. In the contrary, temperature increase (supporting the bacterial development) is in itself a factor of risk; this is why the complete elimination of water must occur quickly.

The use of a clean downtime of 6 days doesn't seem to be a good alternative to heating: indeed, average bacterial counts on day 6 is significantly higher than on day 2 (both in heated and not heated rooms). A clean downtime under our operating conditions has not allowed to reduce the bacterial contamination. On the contrary, this phase supported the recontamination of the buildings.

Several assumptions can be advanced to explain this recontamination:

- The development of the residual germs (still present after disinfection): the absence of heating, by slowing down drying, could maintain wet conditions supporting the bacterial proliferation,
- The phenomenon of sedimentation of the suspended particles, which can contaminate surfaces concerned,
- Flow of ventilation during the clean down time; being maintained at minima (20% of its maximum capacity), recontaminations could occur, coming from the roofs or of the not disinfected slurry pits. A total stop of ventilation would however not have allowed the drying of the rooms.

These results let suppose that a fast drying of the buildings (immediately after disinfection), during 48 hours at least, is more efficient than a long clean downtime.

Conclusion

The tests implemented to analyse the successive stages of cleaning and disinfection process permit to define the most adequate program regarding efficiency of decontamination; that is to say: the use of a detergent after pressure wash, the draining and the pressure wash of the pits, a double disinfection by thermonebulisation and finally the heating of the room after disinfection. In addition, the installation of an automatic soaking system, the use of a detergent before pressure wash and a foam disinfection reduce clearly the cost of the operations of cleaning and disinfection (mainly by dumping out the requested labour time).

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Table 1 : Results of the various trials and statistical tests

Trial	Method	Number of data	Means and Anova (1)	
			TPA (URL)	Bacterial counts
Soaking	Automatic	150	960 ns	51 ns
	Manual		885	30
Detergent before washing	With	120	393 *	28 ns
	Without		715	43
Detergent after washing	With	120	162 **	15 *
	Without		395	17
Disinfection	Foam	102	520 ns	101 ns
	Pulverisation		876	112
2éme Disinfection by foam	Before	158	998 ns	61 **
	After		519	28
2éme Disinfection thermonebulisation	Before	136	303 ns	27 *
	After		367	10
Heating	Yes J+2	204	2688 ns	16 a
	No J+2		2596	21 a
	No J+6		1546	35 b
Clean downtime	J+1	279	3223 ns	23 a
	J+3		2010	34 b
	J+6		2837	44 c

(1) : ns :not significant, * : p≤ 0.05, ** : p ≤ 0.001

(a, b,c) indicate groups with a significant difference with p≤ 0.001

Table 2: Annual cost price in € for 100 productive sows

Methods		Labour		Other costs (1)	Total cost
		Hours	Cost		
Soaking	Automatic	0	0	143	143
	Manual	23,6	288	14	302
Detergent		9,3	114	213	327
Disinfection	Foam	9,7	118	877	995
	Pulverisation	88,0	1080	873	1953
2éme Disinfection	Thermonebulisation	9,5	116	553	669
	Foam	9,7	118	11	1000
Heating	Thermobile	0	0	615	615

(1)water, electricity, fuel, products, investment depreciatio

BIOFILM AND THE USE OF PROTEOLYTIC ENZYMES AT SANITATION OF MILKING MACHINES

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Introduction

After the deposit of organic and inorganic molecules on the surface (*Hood and Zottola, 1995*; *Wilderer et al., 1989*), micro-organisms attach themselves to the surface containing food. The inseparable attachment to the surface is followed by the formation of micro-colonies, protected from the influences of the environment through the growing of the exopolysaccharide protective layer, forming biofilm (*Characklis and Marshall, 1990*). Due to its persistence and resistance, biofilm is unaffected by the normal cleaning agents. The researches also mention the use of enzymatic agents in the sanitation processes. Enzymes are interesting due to their efficient action at low temperatures and low concentrations, because they are degradable and disintegrate to environment-friendly decomposition substances (*Flint et al., 1999; Pintarič, 2002*).

Material and methods

The test studied the effect of using proteolytic enzymes in the cleaning of milking machines. To this end, we used a milking machine to which test segments – that we were able to take off optionally – were attached in the tubular part of the long milking tube. Test segments were exposed to milk flow during milking. After milking, the test segments were removed from the milking machine and alternatingly attached for individual manners of cleaning.

The process of cleaning the milking machine included the rinsing of milk remains with water, the main cleaning with a cleaning agent and the final rinsing with water. The whole cleaning process took 40 minutes.

Test solutions

In order to determine the effects of sanitation with proteolytic enzymes, for the purpose of comparison, during cleaning test plates were exposed to:

- running water without cleaning agents,
- test solution of proteolytic enzymes (> 5 % of serine peptidase, 15-30 % non-ionised tensides) and surfactants (5-15 % phosphates, 15-30 % carbonates, 1-5 % potassium hydroxide) (**PS**), and
- alkali (25-50 % sodium hypochlorite, 10-25 % potassium hydroxide, 2.5-10 % natron sodium silicate) and acid test solution (25-50 % phosphoric (5) acid, 2.5-10 % sulphurous (6) acid > 2.5 % cations) (**AA**).

In all cases, the sanitation process took place under equal conditions.After the cleaning process was finished, the test segments were removed and smears were taken from them for microbiological analysis and determination of ATP presence. Eluates were taken from the segments by means of sterile physiological solution to be checked for the presence of micro-organisms and the value of ATP in them. After sampling, the test segments were again attached to the milking system, where they were again poured over with milk directly during milking.

The test took 21 days. For each sample, the presence of micro-organisms and ATP value were determined in smears and eluates. Measurements were made in two replicates. A total of 84 samples were taken.

Taking and preparation of smears for microbiological investigation

Smears were taken from the entire surface of test segments by means of sterile swabs, soaked in sterile physiological solution. In the laboratory, the smears taken were first well stirred in the shaker. Then, a sterile pipette was used to take the suspension, which was applied directly to petri dishes or it was first diluted in the physiological solution in the ratios from 10^{-1} do 10^{-4}. The suspensions prepared were applied to petri dishes and poured over with milk agar (Oxoid, England). This was followed by the incubation of the samples for 72 hours at 30°C±1. The data were presented as colony units (CFU).

In the preparation of eluates, the interior of the test segments was eluted after the cleaning process with 10 ml of sterile physiological solution. The eluate was then examined for the presence of micro-organisms using the same procedure as for the smears taken.

Measurement of ATP-bioluminiscence

In order to measure the ATP-bioluminiscence on the test surfaces, we used the smears taken after cleaning according to the instructions of the producer (Charm Sciences Inc., USA). We also measured the amount of ATP in test solutions after cleaning. The apparatus Luminator T® (Charm Sciences Inc., USA) was used for measuring. In addition to the preparation of eluates for microbiological researches, eluates were also examined for ATP value for the same producer and by means of the same measurement device.

Results

Micro-organism counts in smears taken from the segments of the test milking machine

After the sanitation process, samples were taken from the segments of the test milking machine by means of smears to be used for microbiological analysis.

The results of the microbiological analyses showed occasional presence of micro-organisms on test segments, which was why the statistical analysis of data was not made.

The values of micro-organism counts in the eluates from the segments of the test milking machine

After the sanitation process, samples were taken from the segments of the test milking machine by means of eluates to be used for microbiological analysis. The average values of micro-organism counts are shown in the decimal logarithm per cm^2 ($\log_{10}(X)/cm^2$) in table no. 1.

Table 1: *Average values of micro-organism counts (log_{10}) in the eluates from the segments of the test milking machine and their mutual comparisons (n=21)*

		Water (1.42 CFU/cm^2)	**PS** (1.11 CFU/cm^2)
PS (1.11 CFU/cm^2)	diff. diff. % sig.	-0.31 -21.83% NS	*
AA (1.47 CFU/cm^2)	diff. diff. % sig.	0.05 3.52% NS	**0.36** **32.43%** **(P<0.05)**

Note: NS – no statistically significant difference

Measurement of ATP smears taken from the segments of the test milking machine

After the cleaning process, samples were taken from the segments of the test milking machine by means of smears to be used for the determination of the ATP value. The results are presented in table no. 2.

Table 2: *Average ATP values on the segments of the test milking machine and their mutual comparisons (n=21)*

		Water (1,511.00 RLU)	**PS** (1,966.32 RLU)
PS (1,966.32 RLU)	diff. diff. % sig.	455,32 30.13% NS	*
AA (1,988.64 RLU)	diff. diff. % sig.	477,64 31.61% NS	22,32 1.14% NS

Note: NS – no statistically significant difference

Measurement of ATP in the eluates of the segments of the test milking machine

After the cleaning process, ATP was measured in the eluates of the segments. The results of measurements are presented in table no. 3.

Table 3: *Average ATP values in the eluates of the segments of the test milking machine and their mutual comparisons (n=21)*

		Water (19.45 RLU)	**PS** (173.91 RLU)
PS (173.91 RLU)	diff. diff. % sig.	154,46 794.14% NS	*
AA (89.23 RLU)	diff. diff. % sig.	69,78 358.77% NS	-84,68 -48.69% NS

Note: NS – no statistically significant difference

Discussion

The test compared the possibility of using the proteolytic cleaning method with surfactants for the cleaning of a milking machine in cleaning by means of warm water. The following of the presence of micro-organisms by means of taking smears showed occasional increases in the number of micro-organisms on the surfaces of the test segments. Although it was not possible to statistically determine the difference in the count of micro-organisms between individual methods of cleaning, chart 1 shows a count of micro-organisms for PS that is, on the average, 89.74% lower than for the classical AC cleaning method. The best result has also been found in the determination of micro-organisms in the eluates of the test segments. Also in eluates, the values of micro-organism counts determined were the lowest for PS cleaning.

Along with the taking of smears for the determination of the presence of micro-organisms on test segments, smears were also taken in order to determine the ATP value by means of bioluminescence. The results indicate a similar effect of both manners of cleaning (PS=1,966.32 RLU; AC=1,988.64 RLU; P>0.05).

Surprisingly, the results of control segments, only cleaned with water, show lower values than the segments cleaned by means of the PS and AC methods. A possible explanation for the results achieved is in the formation of biofilm on the surface. With biofilm, the resistance of protective exopolysaccharide layer prevents smears to be taken from the surface, which could be the reason for the results obtained. A similar situation was found in the determination of ATP values in eluates, where the lowest average ATP value was also established in control eluates. In order to confirm the hypothesis, in further research it will be necessary to use other methods (for instance, fluorescent coloration and the use of confocal laser microscopy), able to prove the presence of biofilm on the surface. It will also be necessary to study the possibility of removing biofilm on the surface by means of agents like the peroxiacetic acid and its efficiency in removing the exopolysaccharide protective layer.

Conclusions

The comparison of the results of the microbiological representation as well as the presence of ATP in smears and eluates indicate the same or better results in cleaning by means of PS compared to the AC cleaning method. The advantages of the PS method of cleaning are also shown in the lower temperature of the cleaning agent needed, which means that less energy is necessary for the warming up of the cleaning solution. Particularly in the winter period, the PS cleaning is a more reliable way of cleaning a milking machine. An important advantage of cleaning with PS is also the 10-times lower concentration of the cleaning agent (0.04%) and the ready degradability of the active substance. Lower concentrations of cleaning agents and ready degradability reduces the burdening of the environment by the decomposition products of the cleaning agents.

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CHARACTERIZATION OF THE INFLAMMATORY POTENTIAL OF BIOAEROSOLS BASED ON HUMAN WHOLE BLOOD CYTOKINE RESPONSE

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Introduction

Exposure to bioaerosols can induce pulmonary diseases and subjective symptoms in humans and animals. Some of these diseases are of inflammatory origin, such as organic dust toxic syndrome or chronic bronchitis. The inflammatory reaction in the respiratory system is caused by various agents of the bioaerosols like endotoxins, glucans or tannic acids. These agents activate different cells in the lung tissue, chiefly macrophages to excrete inflammatory mediators like IL-1ß and TNF-a which initiate and modulate the inflammation process [Holt, 1990; Rylander, 1994].

Currently risk assessment to bioaerosol exposure is based mainly on the determination of airborne endotoxin concentration by using the Limulus amebocyte lysate (LAL) assay. Endotoxins serve as a marker for disease risk [Rylander, 1997]. However, the value of the Limulus reaction to characterize the inflammation process in mammals is under discussion for various reasons: 1) the Limulus assay is based on the coagulation reaction of amebocytes of a crab and may not reflect the inflammatory reaction in mammals, 2) the Limulus assay is limited to the detection of endotoxins.

In this study we compared the activity of typical components of bioaerosols and bioaerosol samples from animal houses to coagulate Limulus Amebocytes (LAL assay) and to induce IL-1ß in human blood macrophages (human whole blood assay) to gain information about the problems described above.

Material and Methods

- Test procedures

The LAL assay QLC-1000 (Bio Whittaker, Walkersville, USA) was used to characterize the ability of different bacteria and bioaerosol samples from animal houses to agglutinate amebocytes of the horseshoe crab *Limulus polyphemus*. The ability of these substances to activate human blood macrophages was determined by using a human whole blood assay as described by Hartung and Wendel (1995) and Weigandt (2000). Bioaerosol samples (impinger fluid, washing fluid of exposed filters, exposed filters without any preparation) are incubated with diluted human whole blood from healthy donors. After contact with relevant contaminations monocytes (blood macrophages) release pro-inflammatory signal molecules such as interleukin-1ß (IL-1ß). IL-1ß release is quantified by ELISA measurement.

- Testing of bacterial strains

Strains of bacterial species, which were frequently isolated from the airborne state of animal houses [Dutkiewicz, 1978, Chai et al., 1997, Zucker et al., 2000] were investigated in both test systems. After cultivation the bacteria were harvested by using pyrogen-free water. These bacterial suspensions were adjusted to an optical density of 1.0 at 520 nm. From these suspensions serial dilutions were prepared (dilution factor 10). After that a heat inactivation (30 min at 80°C in a water-bath) was done. In both test systems that dilution was determined that caused a just positive reaction.

- Testing of bioaerosol samples from animal houses

Bioaerosol samples from different animal houses (poultry houses, pig stables, sheep barns) were collected by filtration (PGP-dust-sampling system, Ströhlein GmbH, Germany) and impingement (AGI-30 Impinger) [Brachmann et al., 1964].

AGI-30 impingers were operated for 20 min at an air flow rate of 12.5 l min^{-1}. Air samples were collected in 50 ml of pyrogen-free water. The crude impinger fluid was centrifuged at 2000g for 10 min and directly investigated in both test systems. The exposed filters of the dust sampling system were rocked in 50 ml of pyrogen-free water for 2h and than centrifuged at 2000g for 10 min. The activity of the washing fluid was determined in both test systems.

In both test systems the activity of the bioaerosol samples was related to the activity of the same Control Standard Endotoxin (*E. coli* 0113:H10; BioWhittaker, USA). Therefore it was possible to compare the results of both test systems. The activity in the LAL assay was expressed in Endotoxin Units (EU), in the whole blood assay in Endotoxin Equivalent Units (EEU).

Results and Discussion

- Reactivity of bacteria

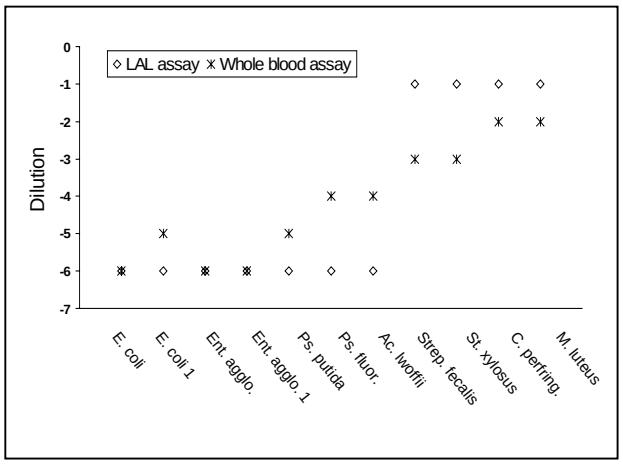
The reactivity of the different bacterial strains in both test systems is shown in figure 1. There are considerable differences between the ability of different gram-negative bacteria to induce limulus amebocyte coagulation and monocyte activation. For instance, the LAL assay overrates the inflammatory activity of *Pseudomonadaceae* and *Neisseriaceae* compared to *Enterobacteriaceae*. Similar results were found by Fennrich et al. (1998) for purified endotoxins from these bacteria. Further "Non-LAL-reactive material", such as components of gram-positive bacteria, are also able to induce monocyte activation suggesting that such components contribute to inflammation in the respiratory tract following bioaerosol exposure. However, these substances exhibited only a very weak activity in the LAL assay.

- Reactivity of bioaerosol samples

The activity detected in the whole blood assay correlated well with the endotoxic activity found in the LAL assay (figure 2). However, in some samples the inflammation-inducing potential was overestimated by the LAL assay (up to factor 8) suggesting a considerable amount of endotoxins originating from "non-*Enterobacteriaceae*" in the bioaerosol. In other samples the inflammation-inducing potential was underestimated by the LAL assay

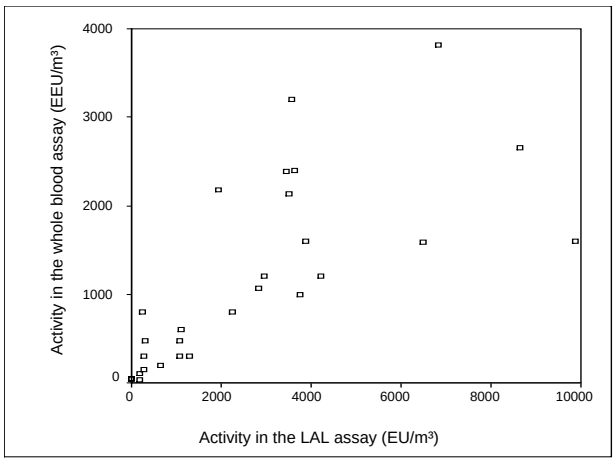
(up to the factor 4) indicating a considerable amount of "non-LAL-reactive material" in the bioaerosol.

Figure 1: Reactivity of different bacteria in the LAL- and whole blood assay



After cultivation the bacteria were harvested by using pyrogen-free water. These bacterial suspensions were adjusted to an optical density of 1.0 at 520 nm. From these suspensions serial dilutions were prepared (dilution factor 10). After that a heat inactivation (30 min at 80° C) was done. In both test systems that dilution was determined that caused a just positive reaction. (Ent. agglom = Enterobacter agglomerans, Ps. = Pseudomonas, St. = Staphylococcus, Sr. = Streptococcus, C. perfring. = Clostridium perfringens, M. = Micrococcus).

Figure 3: Reactivity of bioaerosol samples in the LAL- and whole blood assay



In both test systems the activity of the bioaerosol samples was related to the activity of the same Control Standard Endotoxin (E. coli 0113:H10; BioWhittaker, USA). The activity in the LAL assay was expressed in Endotoxin Units (EU), in the whole blood assay in Endotoxin Equivalent Units (EEU).

Conclusion

The determination of the potential of bioaerosols to activate macrophages by using whole blood models could offer new perspectives in exposure and risk assessment. This currently has two major potential advantages over the LAL assay:

1. Simultaneously detection of a broad spectrum of inflammation-inducing substances
2. Detection of these substances by using a system that is relevant to the exposed species. The whole blood assay mimics the reaction that air contaminations would cause in the exposed species.

Further experiments will be necessary to examine in more detail how well this method reflects the risk of bioaerosol exposure and to optimize the methodology of the whole blood assay.

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Biosecurity of effluents, hygiene, cleaning

Posters

FUNGAL POLLUTION IN POULTRY HOUSES ENVIRONMENT OF BATNA REGION

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Introduction

The frequency of pathogenic fungal flora capable to provoke diseases by direct or indirect action in animals (Land, 1991 ; Kluszek, 2000) and humans (Katja, 2002) must incite veterinarians to institute a sanitary prophylaxis especially based on the improvement on raising and hygiene conditions to avoid microbes proliferation. For it an optimization of environment parameters (temperature, humidity and ventilation) is indispensable (Kolbuszewski and al 1980) but also a rigorous decontamination of buildings after each end of raising (Drouin 2000). The objective of this survey is the quantitative and qualitative assessment of fungal flora in the poultry houses environment in Batna region (Algeria).

Material and Methods

The survey has been realized on 20 poultry houses with different systems of ventilation (static or dynamic) in summer. The flock population was in buildings between 5000 and 10.000 birds (broiler and started hens), either a density of 10 to 15 birds by m^2. Birds were in all cases raised on soil on organized litter of straw of 20 to 30 cm of thickness. The rearing period is between 56 to 70 days for broilers and 17-18 weeks for the started hens.

The practical work consisted in making the microbiological samples of the ambient air of buildings, the litters and feeds after the sixth week of raising. 240 samples are achieved in 20 chosen buildings at random according to the technique described by Hamet and al (1986). After incubation to 37°C during 3 days, the identification of fungal flora is made according to the conventional method (Larone, 1987) and (Teeuw and al. 1993).

Results

Tab.1 Composition of the fungal flora in the 20 poultry houses environment (%) [A]

Fungi species	Air (n=120)	Feed (n=60)	Litter (n=60)
Aspergillus spp	27.00	32.50	35.00
Candida spp	14.50	23.70	14.00
Penicillium spp	10.50	4.00	12.00
Fusarium spp	13.50	12.80	08.00
Cryptococcus	06.50	16.50	10.50
Trichosporon spp	05.60	10.50	-
Fonseceae spp	05.40	-	12.00
Curvularia spp	04.30	-	05.00
Rhodotorula	03.60	-	-
Muccor spp	03.20	-	04.00
Rhizopus spp	02.90	-	-
Alternaria spp	02.00	-	-
Cfu × 10^4	0.21/ m^3	1.7/g	1.9/g

[A] *Microclimate : 28-38°C ; 40-65 % RH*

Among the most frequent species, *Aspergillus flavus* and *Candida guilliermondii* species are present in all poultry houses with static and dynamic ventilation. *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidalans* and *Candida famata*, have been observed only in buildings with dynamic ventilation. *Fusarium spp*, *Cryptococcus neoformans* and *Penicillium spp* are met with a variable frequency in the two different systems of ventilation.

Dicussion

The major part of fungi species develops themselves when conditions of temperature and humidity are not optimized, especially in the hot and humid regions (Le Menec 1989, Le Bars 1989). The buildings without an adequate ventilation system are the most contaminated, following an increase of the internal temperature and the relative humidity. The pollution of litters is more important than the one of feeds and the ambient air (Tab.1). This doesn't correspond to results found by Lacroix (1990) and Kluszek (1997). It is obvious to note that the microclimate in which are these poultry houses is different than the one described by these two authors. Indeed microclimatic parameters have a big influence on the composition of the fungal flora in poultry houses environment (Land 1991, Rokicki 1996). The proliferation of fungi in feed and litter depend partly on the relative humidity and the temperature in the buildings. It has been demonstrated that some fungi as for example *Aspergillus* and *Fusarium* develop themselves in poultry feed when conditions of storage are bad (absence of ventilation, temperature and elevated humidity). Some isolated fungi in the poultry houses environment can cause some respiratory diseases in poultry (*A. fumigatus*) and man (Katja, 2002); and others risk to drag mycotoxicosis (*A. flavus*) in birds (Rajeswari 1991).

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SALMONELLA ENTERICA CONTAMINATION OF PIG SLURRY : A FIELD STUDY

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Introduction

In industrialised countries, *Salmonella enterica* is a frequent cause of food borne disease. About 15-25 % of all human cases of salmonellosis can be attributed to the consumption of contaminated pork and pork products (2, 3). Contamination of pork products is related to asymptomatic intestinal carriage of *Salmonella* by living pigs arriving at the slaughterhouse (4). In order to reduce the occurrence of *Salmonella* in pork, a decrease of *Salmonella* carriage at the farm level is needed. On the other hand, the spread of *S.* contaminated slurry on fields and crops may constitute a threat on an environmental point of view. Therefore, efforts undertaken at the farm level to reduce *Salmonella* shedding contribute to increase both human food safety and environmental safety. Few data are available regarding the contamination level of finishing pigs and their slurry in France. The aim of our study was to identify *Salmonella* contaminated finishing pig batches and to assess the level of contamination of their slurry.

Material and Methods

Our study was carried out from April 2003 to January 2004. 39 batches of finishing pigs from 27 farrow-to-finish French farms suspected to be positive to *Salmonella enterica* were involved in the survey. *Salmonella* shedding was assessed on the one hand with an environmental sampling procedure: sterile pairs of gauze socks (Sodibox, La Forêt Fouesnant, France) were used to wipe faecal material on the slatted floor of each pen housing the targeted batch of pigs. On the other hand, in each pen a pool of newly excreted faeces was collected on the floor and placed into sterile bags. In addition 4 litres of slurry stored in the pit below the followed pigs were collected in sterile bottles. Environmental swabs, pools of faecal matter and slurry were analysed for the presence of *Salmonella enterica* in a four stages protocol. Following a pre-enrichment step (20 hours at 37 °C in buffered peptonned water), two selective media were used : Müller - Kauffman Tetrathionate Broth (MKTB) and Modified Semi-Solid Rappaport Vassiliadis agar (MSRV), incubated respectively 24 hours at 42°C and 48 hours at 41,5°C. The migrated colonies of MSRV were isolated on Rambach agar plates and each MKTB on Xylose-Lysine-Tergitol4 (XLT4) agar plates. Both media were incubated 24 hours at 37 °C. The presumptive colonies were biochemically confirmed on Kligler-Hajna medium (AES Laboratoires, Combourg, France). All isolates were serotyped by agglutination following the Kauffman-White scheme (1). On positive pools of faecal material and positive samples of slurry, a quantification of *Salmonella* was assessed according to the most probable number method (MPN calculator VB6, 5).

Results

At least one swab sample tested positive in the environment of 13 batches (33.3 %). In 8 batches, *Salmonella* shedding was also detected in pooled faeces. *Salmonella* quantification was possible in 4 of the latter samples in which levels of 2.4 to 350 *Salmonella*/gram were detected. In 6 batches, *Salmonella* was identified in slurry samples. Quantification was achieved in only one sample of slurry and we found 1.6 *Salmonella*/ml. Quantification in pooled faeces or in slurry was only possible when at least 50 % of environmental swabs tested *Salmonella* positive. *Salmonella Typhimurium* and *Salmonella Derby* were the most common serotypes isolated. The results concerning the positive samples are presented table 1.

Table 1 : Description of *Salmonella* serotypes isolated in swabs, pooled faeces and slurry samples and *Salmonella* quantification in pooled faeces and slurry (15 positive batches, April 2003 - January 2004)

Batch	% Positive swabs and serotype	*Salmonella* serotype detected in pool faecal matter	mpn in pool fecal matter (S./gram and CI at 95 %)	*Salmonella* serotype detected in slurry samples	mpn in slurry samples (S./mL and CI at 95 %)
1	**100** ***S.T***	*S.T*	2,4 (0,66-8,5)	*S.T*	-
2	12.5 *S.T*	-	-	*S.T*	-
3	**75.0** ***S.T***	*S.T*	8,3 (2,7-25)	*S.T*	-
4	**50.0** ***S.T***	*S.T*	350 (94-1300)	*S.T*	-
5	40.0 *S.T*	*S.T*	-	-	-
6	12.5 *S.T*	-	-	-	-
7	25 *S.*Bredeney	*S. Bredeney*	-	-	-
8	**55.6** ***S. Derby***	*S. Derby*	350 (94-1300)	*S. Derby*	1,6 (0,38-6,9)
9	8.3 *S. Derby*	*S. Derby*	-	-	-
10	0.0	*S. Derby*	-	-	-
11	16.7 *S. Derby*	-	-	-	-
12	14.3 *S. Derby*	-	-	-	-
13	8.3 *S.T*	-	-	-	-
14	0.0	-	-	*S. Derby*	-
15	8.3 *S. Derby*	-	-	-	-

S.T : S.Typhimurium

Discussion

Our study indicates that pig slurry may be contaminated by *Salmonella enterica*. However, the results suggest that *Salmonella* can only be detected in slurry of highly shedding batches of pigs.

Acknowledgements

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5 - http ://members.yne.net/mcuriale/mpn

USE OF THERMAL FOGGING FOR DISINFECTION IN GREENHOUSES. WHAT ABOUT ANIMAL HOUSES ?

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Introduction

Disinfection treatment by thermal fogging has been considered since a long time as a very efficient method for hygiene maintenance. This method garantees good results in many domains as agriculture, food industry... Thermal fogging is used for the disinfection of greenhouses, shelters or grain silos, particularly against dust mites, aphids or other caterpillars since the 1980's. This method was also developped against bacteria and fungi, leading to new homologations for these uses. Until now, no official protocol for testing the virucidal activity of disinfectant applied by thermal fogging exists. This leads to the impossibility to homologate a disinfectant as virucide applied through thermal fogging.

BBV laboratory at Saint-Pol de Léon (29) has developed protocols to test disinfectants applied by thermal fogging against vegetable viruses. Since there is no chemical treatment to control viruses once plants are infected, hygiene (on tools, hands and structures) is a key aspect for an effective control. Prophylaxis and disinfectant treatments are of primary importance. In accordance with the current requirements of approval for sale for this category of usage in virucidy, as defined by the French Ministry of Agriculture, BBV develops protocols to test disinfectants. The objective is to officially approve efficient products, allowing their use by producers. During these studies, we valid protocols for testing efficiency of products to disinfect structures by fogging.

The most important virus for tomato producers is Pepino mosaic virus (PepMV). The virus was recently introduced in Europe (1999). In 2003, in Brittany, 55 ha were contaminated. In spite of bad conditions for virus development, this mechanically transmitted virus induced significant damage in tomato production. Losses of 5 % yield and 25 % quality were observed for early contamination. Because of its virulence, PepMV is classified as a quarantine pathogen on seeds in Europe (EC decision 2004 / 200). Thus, we work on another virus, as similar as possible to the PepMV: the Potato Virus X (PVX), this choice being made according to the French Ministry of Agriculture.

Materiel & Methods

Plants of *Chenopodium album* subsp. *amaranticolor* were used as an indicator plant. Plants were sowed in peat pot and transferred 3 weeks later into plastic jars (5 x 5 x 6 cm). The compost used was a mixture of fair peat, black, sandcoloured peat of fine structure. Plants were grown up to the 5-6 node stage.

- tomato leaves infected with PVX were diluted 1:5 in 30 ° F water hardness (4) sterile water and ground up using a mortar and pestle.
- The macerate was filtered on sterile stamen and presented a dilution end point superior or equal to 10^{-4}.
- 1 ml or 2.5 ml of plant sap was placed on glass Petri dish (85 x 65 mm). Dishes are then placed in a greenhouse in which a thermal fogging is applied with the Nebul'Ops (a commercial product) by means of a Thermal Fog Application System Igeba TF35. Nebul's Ops is an acid and oxydative disinfectant made of peracetic acid and of hydrogen peroxyd.
- The thermal fogging of Nebul'Ops was done on the plant sap at the concentration of 1.5 ml/m^3 with water at 1 ml/m^3 as vehicule and 2 ml of Vector to make visible the fog, and at the concentration of 2 ml/m_3 with Forneb at 5 % as vehicule. Durations of contact were 2 and 4 hours.

For each condition, the upper faces of 20 leaves (2 hours of contact) or 8 leaves (4 hours of contact) of *Chenopodium album* subsp. *amaranticolor* were mechanically inoculated by gently rubbing with a sterile compress soaked with 30 ° F sterile water dipped into the plant sap / tested product. The plants were then placed into a greenhouse compartment (12 hours light periods) and monitored for symptom development.

As a phytotoxicity control, the thermal fogging was performed at the final test concentration on 1 ml or 2.5 ml healthy plant sap on glass Petri dish. In negative control plants, sterile 30 ° F water was used instead of tested product on healthy plants sap, in the same conditions. Positive control plants consist in 1 or 2.5 ml of infected plants sap on glass Petri dish put in contact with 30 ° F sterile water during 2 and 4 hours of contact.

The estimations of the symptoms were visual and performed 15 days after inoculation. The notation of the symptoms was done according to a six points scale (0, 1, 2, 3, 4, 5) based on the number of necrotic spots by leaf. The score 0 corresponded to leaves without visible symptoms, score 1 for leaves exhibiting less than 5 necrotic spots and the score 5 to completely infected leaves. Leaves inoculated by objects were independently scored. Then, an average score by object was calculated, which determines the efficiency of the products.

Results

Phytotoxicity control

Leaves inoculated by healthy plant sap / 1.5 or 2 ml/m^3 Nebul'Ops mixture do not show any phytotoxicity. These concentrations do not thus require the use of a neutralising agent.

Control plants

The negative control plants are always free of disease, showing the absence of accidental contaminations.

In positive control plants, a strong infection, near level 5 on our notation scale, is always detected, showing that experimental conditions are relevant to evaluate the antiviral effect of products.

Test of Product

Leaves inoculated with 1 ml of infected plant sap / 1.5 ml/m^3 Nebul'Ops mixture show between 0 and 4 necrotic spots for 2 hours of contact and none for 4 hours of contact. Leaves inoculated with 2.5 ml of infected plant sap / 1.5 ml/m^3 Nebul'Ops mixture show between 0 and 12 necrotic spots for 2 hours of contact and none for 4

hours of contact. Leaves inoculated with 1 or 2.5 ml of infected plant sap / 2 ml/m^3 Nebul'Ops mixture do not show any necrotic spots for 2 or 4 hours of contact.
The desinfectant proved its efficiency to eliminate the PVX virus.
However it was found essential to properly remove the organic matter from all the surfaces before disinfection.

Discussion

From greenhouses to animal houses ?

To our best knowledge no similar protocol yet exists for testing the efficiency of disinfectants applied by thermal fogging in animal houses. In this respect, the present work could serve as first step in order to build up such protocols.
When disinfecting greenhouses and animal houses similar issues need to be addressed. Hygiene is nothing new, but it is the corner stone to good health (1) (2) (3).
The major goal of desinfection is to eliminate the specific pathogens. In both cases the latter are mostly viruses, bacteria (and fungi for greenhouses). The resistance of those pathogens to the disinfectants varies a lot depending on their physical and biological characteristics. In animals, both enveloped and non enveloped viruses can be found. Non enveloped viruses like Porcine Parvovirus and Porcine circoviruses are considered as among the most resistant within this group of pathogens. Additionnally, as well DNA as RNA viruses can be found in both animals and plants. Regarding bacteria, a broad spectrum of pathogens is involved. In animal production like in plant production, bacteria that can sporulate can be found despite those of interest do not belong to the same species.
Although there can be a huge number of equipments, the type of material used is for part similar in greenhouses and animal buildings. Concrete, metallic and plastic material can be found in both cases. The point is of course of paramount importance in respect to decontamination easiness and to corrosion.
In any case a thorough cleaning of the buildings is a prerequisite. Then, in agreement with the vet. / agronomist, the choice of a disinfectant that is independently tested and which has been officially approved is necessary.
A broad-spectrum product is often wise ie showing a biocidal activity against viral, bacterial, spores and fungal organisms. Another common issue is to avoid environmental pollution and the products as well as the application process should not show significant health and safety concerns.
Another aspect needs careful consideration. From a medical standpoint, there must not be confusion between a disinfectant and an antiseptic. The former has to be applied on inert surfaces, on non-living material, whereas the second is a treatment that can be applied onto the skin or the mucosa of humans or animals. Disinfection of livestock buildings is started after the buildings have been totally depopulated, the slurry removed and after the place has been thoroughly cleaned. One of the problems encountered with thermal fogging in animal houses and in greenhouses might be the difficulty to have air tight rooms avoiding any escape to the neighbouring rooms. Another issue which needs to be properly addressed is the method to be used to assess decontamination efficiency and eventually make official decisions on approval. Specific equipment should be adopted. In addition the difficulty of a proper residual contamination measurement still remains. In plants, bio-assays can be used when testing. In animals, for welfare reasons, such assays to reveal a residual contamination after disinfection can hardly be accepted.
An official method aiming at testing virucidal, bactericidal and fungicidal efficiency of disinfectants applied through thermal fogging is being finalized by a scientific working group (4)
Further efforts should be directed to studies about standardized protocols to evaluate disinfectant efficiency applied through thermal fogging in animal houses, in view of official approval .

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INDUSTRIAL POLLUTION IMPACT ON SURFACE WATERS QUALITY IN THE CENTRAL AREA OF ROUMANIA

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Introduction

The researches aimed the finding out of the polluant elements in the surface waters in the central area of the country, having in view to compel the attention for reducing the environmental pollution and natural habitats protection.

Material and Methods

In the central area of the country it was followed the surface waters quality by 20 samples in five sections: Pascov river, upstream of Ialomita crossing, Cricovul Dulce upstream of AGCL Moreni – Ghiordoveni point, Slanic river at Sacuieni, Ilfov river at Colanu and Cobia river upstream of Potopu point.
From sampling there were carried out: the chlorides, ammonium, CCO-Mn (O_2) and oil products quantities.

Results

The results are presented in Chart no. 1 to 4:

Chart no. 1: Chlorides in surface waters

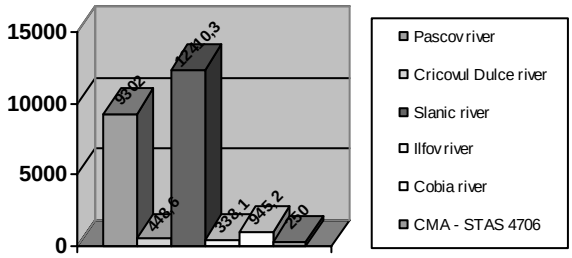


Chart no. 2: Amonium in surface waters

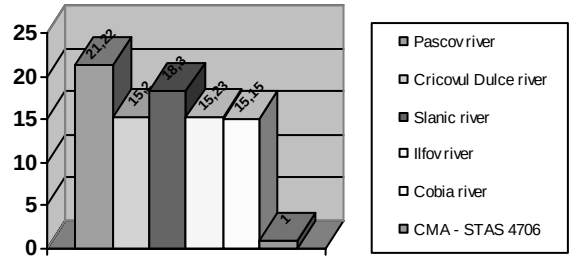


Chart no. 3: Oxygen biochemical consumption in surface waters

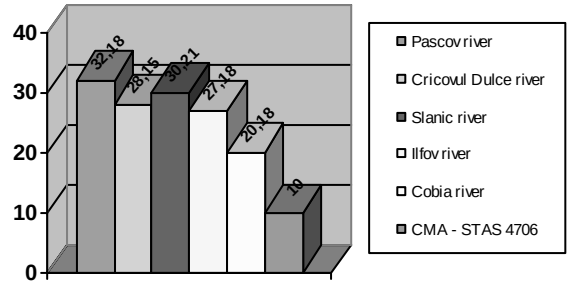
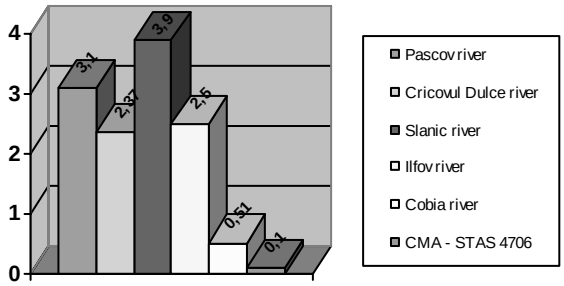


Chart no. 4: Oil products in surface waters



Discussion

Following the determinations from the water samples, there were recorded exceedings beside the standard for chlorides, ammonium, oxygen biochemical consumption and oil products (data are recorded in charts 1-4).
The exceeding of all maximum admitted values for all elements is due to the different industrial activities in area (oil extraction, textile industry and concrete production industry).

Conclusion

There were recorded exceedings beside the standard stipulated by STAS 4706/1988 in all analyzed parameters.
The chlorides exceeded the maximum admitted limits by 1,3 to 50 times in all sampling points.
The ammonium recorded exceedings beside the maximum admitted limits by 21 times in the samples from Pascov river.
CCO – Mn (O_2) recorded the highest exceedings in the samples from Pascov river and Slanic river.
The oil products exceeded the maximum admitted limit in all sampling points.

Acknowledgements

Environmental Protection Institute Dambovita

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THE SUITABILITY OF PERACETIC ACID FOR DISINFECTION OF LARGE ANIMAL HOUSES USING COLD-MIST AND FOAM SPRAYING TECHNIQUES

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Various deficiencies in disinfection may induce that chains of infection are not interrupted from one stock to the next. The goal of the present work arose from the efforts to displace usual methods in terms of the dangerous substance regulation and the TRGS 540. Cold-mist and foam spraying techniques with peracetic acid are seen as alternatives and were tested and evaluated on their suitability for disinfection in large animal houses.

In large chicken houses with ground and cage keeping samples were taken from representative subsurfaces and places in the animal house from moistened surfaces using moist swabs before and after disinfection in order to determine the number of germs; the mean germ reduction factors were determined for each experimental condition. An cold-mist generator with large throwing range (Turbostar) and a foam generator (SG 3/10), which had been specially developed by the Kesla Hygiene AG for the Wofasteril® – Kombiverfahren, were applied. An amount of 10 ml/m³ room capacity of 5% buffered peracetic acid (Wofasteril® – Kombiverfahren) were used in the cold-mist technique. In the foaming technique 350 – 470 ml use dilution /m² application concentrations of 0.8 – 1.7% Wofasteril® E 400 were used.

The cold-mist technique allows the spatial dispersion of peracetic acid, and large rooms get filled fast by the fine mist. In the practical experiments employed considerable results could be achieved even with difficult elements of equipment (Tab.1).

By the possibility of visual inspection, securing of the amount of disinfection dilution reaching the location that shall be effected and longer effecting times disinfection by foam shows crucial advantages for the success of disinfection at a high surface efficiency per time unit. The success of disinfection is proved by high germ reduction factors (Tab.1) and the incidence of 85.7 – 89.3% negative microbial samples in the swab technique.

Taking into account the constructional, device-related, economic and infection hygienic situation for the disinfection of large animal houses peracetic acid is suitable and under this assumption an alternative for active disinfection ingredients underlying the TRGS 540.

Table 1: Disinfection with buffered peracetic acid (average GKZ /cm²)

	Cold-mist technique		
		before	**after**
	n	**x**	**x**
Feeding-through	20	4450	82
Cage/ Nest	20	322	*50
Drinking-throughs	10	957700	37540
Ventilator	5	152520	*50
Ground	10	4961210	13580

	Foaming 0.8% Wofasteril® E 400		
		before	**after**
	n	**x**	**x**
Feeding-through	n.u.		
Cage/ Nest	10	1000840	*50
Drinking-throughs	5	1712000	1040
Ventilator	5	306000	*50
Ground	15	2660327	1223

	Foaming1.7% Wofasteril® E 400		
		before	**after**
	n	**x**	**x**
Feeding-through	n.u.		
Cage/ Nest	10	56100	*5
Drinking-throughs	5	6511000	504
Ventilator	5	90440	6
Ground	15	106746000	6

* below the detection limit

EXAMINATIONS OF THE PENETRATION ABILITY OF GERMS THROUGH THE EGGSHELL OF SPF-INCUBATED EGGS DEPENDING ON PRE-TREATMENT OF THE INCUBATED EGGS

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Washing of eggs is generally regarded to as very critically because it is supposed to injure the cuticle as a barrier against germs.

In order to investigate the influence of the quality of the cuticle on the penetration behaviour of germs, washed and unwashed SPF-incubated eggs were artificially contaminated with *Enterococcus faecium.*

At first the eggs were warmed to 40 °C (oviposition temperature) to simulate the so-called "suction effect" during the cooling phase. Following contamination the eggs were cooled for 2-3 h at room temperature (20°C). Afterwards they were stored for two days at 15°C.

The next step was to stain all eggs in the *MST- CUTICLE BLUE- Test.* Staining with *MST- CUTICLE BLUE* aims on indicating the quality of the cuticle.

Unwashed eggs appeared to show worse staining results than washed eggs. Nevertheless in both groups 2/3 of the eggs were colonised by *Enterococcus faecium* on the interior of the lime shell. Also within each group the colonisation by test germs did not depend on whether the cuticle was well or badly stained.

In a further experiment untreated eggs were stored uncooled (23-25°C) for three days after artificial contamination with *Enterococcus faecium.* In even 100% of these eggs *Enterococcus faecium* was isolated from the inner leaf of the shell membrane.

Assuming that the *MST- CUTICLE BLUE- Test* really allows an estimation of the quality of the cuticle, the condition of the cuticle appears irrelevant if the germ pressure is high. The germs nearly always find a way into the eggs. This occurs particularly fast if the eggs are not stored cooled.

Because the "suction effect" was not simulated in the second experiment one can assume that the germs also actively penetrate through the lime shell at high strain pressure and without cooling.

In a third experiment it was examined if the germ penetration can be prevented by disinfection of the incubated eggs. For that purpose incubated eggs (without simulation of the "suction effect") were contaminated with *Enterococcus faecium.* An experimental group passed through a two-stepped disinfection procedure where as the second group remained untreated.

The disinfection occurred in the first step as aerosol disinfection with a 1% *Wofasteril spezial – Lösung* (active ingredient: peracetic acid). After 2 h standing time an additional immersion disinfection occurred in a 0.5% *Interdes F– Lösung* (active ingredient: quaternary ammonium compounds) with addition of approx. 1% hydrogen peroxide.

In 30% of the non-disinfected eggs *Enterococcus faecium* was isolated from the inner leaf of the shell membrane. In the disinfected eggs no enterococci were isolated from the inner shell membrane.

These investigations very clearly emphasize the benefit of an as possible fast and effective disinfection of incubated eggs after laying as well as the necessity of cooling until the begin of incubation.

THE ESTIMATION OF WATER CONTAMINATION BY NON-CHOLERIC VIBRIO SPECIES

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Introduction

While on international plan have been developed numerous scientific knoledge as regards infection by Vibrio genus, in our country little data exist following the toxic infection's incidence produced by non-choleric vibrions (Vibrio vulnificus, Vibrio fluvialis, Vibrio mimicus, Vibrio alginolyticus, Vibrio metschinikovii, Vibrio parahaemolyticus, Vibrio furnissii, etc) and especially the studies concerning the presence of these bacteria into waters, on skin surface or in natural cavities of different aquatic organisms, as well as into different types of food.

Material and Methods

For a correct evaluation of contamination grade from sweet and salt waters, the water samples has been harvested right from aquatic medium into sterile receipts. After harvesting, they has been introduced into bottles with different pre-enriching mediums (saline alkaline peptonated water and saline bullion with B polymyxine).

The study was effectuated during 7 years long (1997-2003); the geographic area has been represented by hydrographic network from south and south-east of Romania: Danube Delta Biosphere Reservation, Razelm-Sinoe Complex, the seaside of Black Sea, St. Gheorghe, Sulina, downstream of Danube, different waters from south country.

Results

The complex bacteriologic exams have been allow the isolation of a total number of 127 non-choleric vibrion stems, the effectuated identification tests classifying the isolated stems in 3 species: most of stems belong to Vibrio alginolyticus and Vibrio parahaemolyticus species, while a reduced number belong to Vibrio vulnificus specie. The obtained result are presented in table no.2.

Table no. 2

The isolation frequency of non-choleric vibrions by zones and study periods

Year of study	The isolation frequency of non-choleric vibrions								Yearly prevalence / Area	
	South and southeast areas of downstream		Lake's and stagnant waters area		Danube Delta area		Seaside of Black Sea area			
	No. of isolated stems	%	No. of isolated stems	%	No. of isolated stems	%	No. of isolated stems	%	No. of isolated stems	%
1997	3	3.70	2	1.96	2	3.51	6	3.61	13	3.20
1998	5	5.95	5	4.59	7	13.21	12	7.06	29	6.97
1999	2	2.56	2	2.04	2	2.78	8	4.39	14	3.26
2000	0	0	2	1.79	3	4.41	7	3.76	12	2.69
2001	1	1.22	3	2.80	4	7.02	7	4.17	15	3.62
2002	4	.94	6	5.13	7	12.07	14	8.19	31	7.26
2003	1	1.22	2	1.75	2	3.34	8	4.73	13	3.06
Entire period	16	2.82	22	2.90	27	6.35	62	5.12	127	4.29

Discussion

From these areas the vibrions are took over by different animal species that are multiply into sweet waters (Danube Delta and flowing of Danube to Delta), where the excess of nutritive resources (brought by silts of rivers that flow to Danube) permits the abundant evolution of plankton and implicit of vibrions. The low incidence of vibrions into running waters (Siret, IalomiŞa, Prut and downstream area of Danube) could be explained by the lack of necessary conditions for evolution of these bacteria (absence of salt), stems that are isolating, being probably brought by the fishes and shell-fishes in migration. This hypothesis could be demonstrated only by epidemiological analyzing of isolated stems to make a "map" of non-choleric vibrion's movement into different types of waters.

Conclusion

1. By complete bacteriologic analysis of 2963 water samples, harvested from South and Southeast Romanian areas, it has been isolated a total number of 127 bacterial stems, identified belonging to Vibrio genus like non-choleric species
2. The identification exams practiced to the isolated stems permitted to classify them into 3 species: Vibrio alginolyticus, Vibrio parahaemolyticus and Vibrio vulnificus.
3. The statistical analysis of isolation frequency on studied areas concluded that the highest incidence is present in Danube Delta (6.35 %) and seaside of Black Sea (5.12 %), the others areas exposing an average incidence of 2.90 %.
4. The statistical analysis of yearly isolation prevalence on non-choleric vibrions has been permitted to develop the hypothesis of cyclic evolution of non-choleric vibrions, with a period of 4 years long (in the fourth year it's reach an incidence of 6.97 % - 7.26 %), in the rest of years the incidence is kept to an average of 2.69 % - 3.62 %.

Acknowledgements

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"Cantacuzino" Institute – Bucharest
Infectious Diseases "Matei Bals" Colentina - Bucharest

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FACTORS AFFECTING POST-WEANING MORTALITY ON FARROW-TO-FINISH INDUSTRIAL PIG FARMS IN GREECE: I. BIOSECURITY MEASURES

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Introduction

Preventing introduction and spread of porcine pathogens into swine herds is integral part of on-farm disease control programmes. Biosecurity measures appear to influence occurrence and severity of several diseases on intensively housed pigs and as well as the mortality rates (1, 2). The objective of the present study, as part of a broader survey dealing with factors affecting mortality in weaning pigs, was to evaluate the potential effect of some biosecurity measures under greek conditions.

Materials and Method

The study was carried out on 27 farrow to finish industrial farm over 150 sows (with a total of 17,740 sows under production which represents 23.29% of the population in industrial farms over 150 sows or 14.41% of the overall sow population in Greece). The selection of farms was based on criteria such as full or part-time veterinary consultation, existence of production records and history of collaboration with our institutions involved in the study. Data concerning application of biosecurity measures in each farm were collected by questionnaires addressed to farm veterinarians. The influence of certain biosecurity-related risk factors on post-weaning mortality in over 349,785 weaned piglets (actual capacity of sampled farms) had been investigated. The biosecurity parameters that were studied were: a) production system (all in- all out vs continuous), b) routine cleaning and disinfection programmes, c) routine parasite control, d) regular control for mycotoxins in feed, e) enforcement of quarantine for newly entered animals, f) vehicle dip, g) vaccination of stockpersons against influenza and h) restricted policy for visitors. The chi-square analysis was performed in order to determine the associations between mortality rate and risk factors.

Results and Discussion

As presented in figure 1, there was a significant ($P \leq 0.05$) increase of post-weaning mortality when: a) a continuous flow system was applied instead of all in-all out, b) regular cleaning and disinfection programme were invalid, c) routine programme for parasite control was absent and d) the controls for mycotoxins in feed were not applied in the farm (Figure 1).

Parameters such as: a) quarantine for the incoming breeding stock, b) vehicle dip, c) vaccination of stockperson for influenza, and d) restricted policy for visitors did not significantly ($P>0.05$) affect post-weaning mortality in the tested farms (Figure 2).

Figure 1: Post-weaning mortality rates (%) under different biosecurity factors

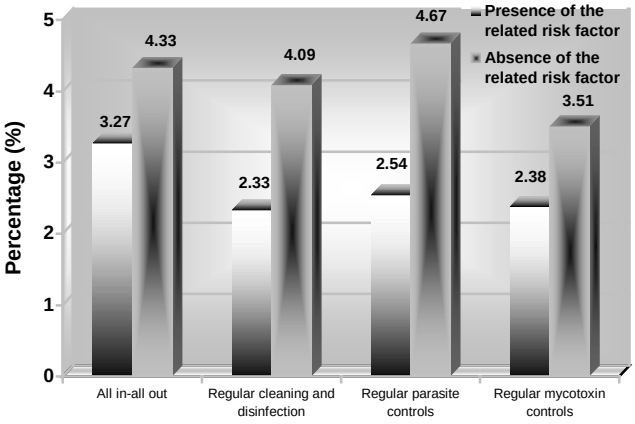
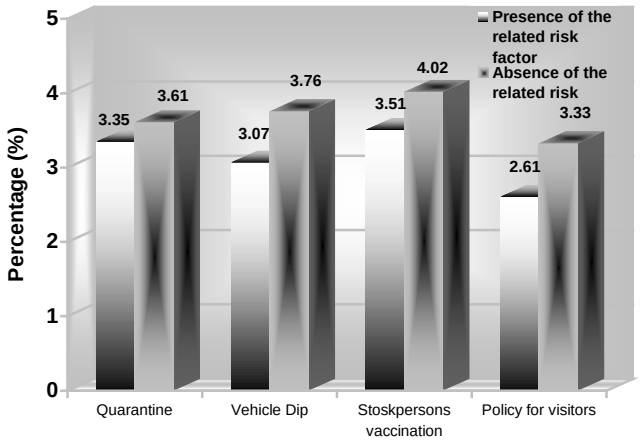


Figure 2: Post-weaning mortality rates (%) under different biosecurity factors



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THE EFFECTS OF MISTRAL™ APPLICATION ON PIGLET PERFORMANCE IN THAILAND

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Introduction

The purpose of this experiment is to measure the effects of **Mistral** application in piglet performance, that is, body temperature after farrowing, duration of drying and dropping of umbilical cord, incidence of diarrhoea and and total bacterial count in farrowing crate.

Material and Methods

Place and animals:

The experiment was conducted in a commercial breeder farm of 8,000 sows in Ratchaburi province in 2003. One farrowing house in a unit of 2-breed (Largewhite x Landrace) sows was selected to set the experiment.

A total of 456 piglets (crossbred Landrace x Largewhite x Duroc) from 43 sows was randomized into two groups. Group Mistral (Treatment), the piglets were dipped from neck to bottom with Mistral™ powder immediately after birth; while Group C (Control), no application of Mistral™ on the piglets.

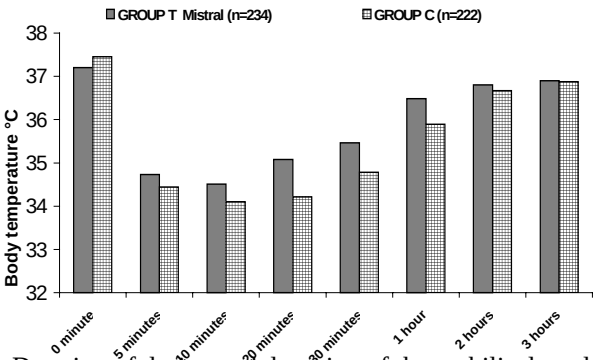
Measurements:

1. Skin body temperature using laser temperature instrument at 0, 5, 10, 20, 30 minute, 1, 2, and 3 hour after birth.
2. Duration of drying and dropping of the umbilical cords.
3. Incidence and time of recovering of diarrhoea.
4. Total bacterial count in the farrowing crates

Results

Skin body temperature

Fig. 1 Graph showing skin body temperature of piglets at 0, 5, 10, 20, 30 minutes, 1, 2 and 3 hour after birth



Duration of drying and dropping of the umbilical cords

Fig. 2 Graph showing percentage of umbilical cord drying in the piglets

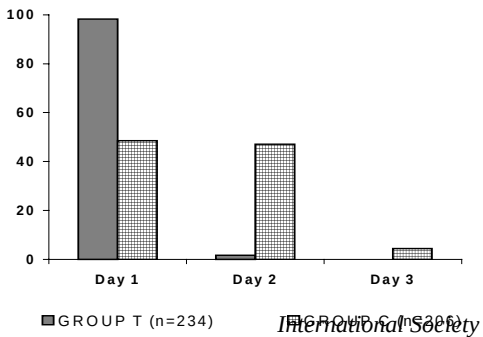
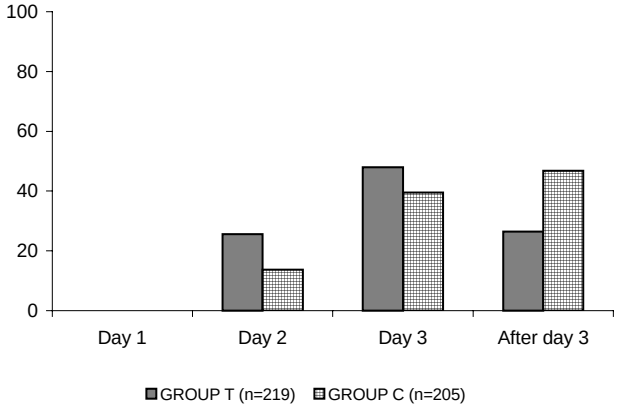
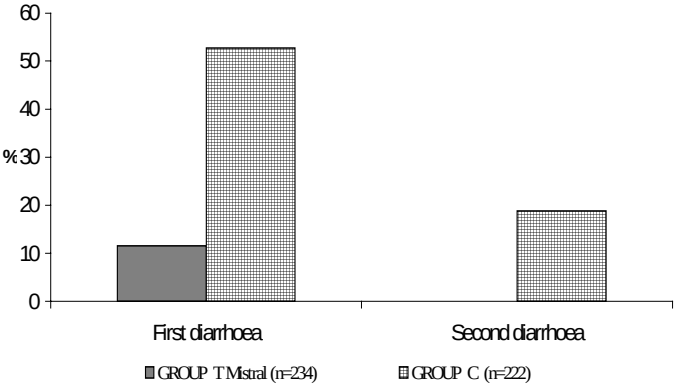


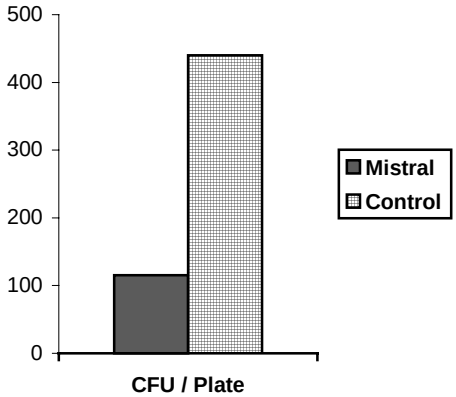
Fig.3 Graph showing percentage of umbilical cord dropping in the piglets



Graph.4 Effect of Mistral™ application on piglet diarrhoea in the first weeks of life.



Graph.5 Effect of Mistral™ application on total bacterial count in the farrowing crates



Conclusion

Using MISTRAL™ from birth in the pig farrowing pens affects significantly piglets temperature after birth, the duration of drying and dropping of the umbilical cords, the incidence and duration of diarrhoea in the first week of life, and the total bacterial counts in the farrowing crates.

Influenza : a farm animal disease and a zoonosis

Oral Communications

THE EUROPEAN SURVEILLANCE NETWORK FOR INFLUENZA IN PIGS

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The «European Surveillance Network for Influenza in Pigs» (ESNIP) was a concerted action (QLK2-CT-2000-01636) in the 5th Framework Research Programme of the European Commission.

Swine influenza viruses (SIVs) are enzootic in swine dense regions of Europe and they are a major cause of respiratory disease in fattening pigs. Until recently, however, there was no organized surveillance for influenza viruses of swine, as is the case for human and equine influenza viruses. In addition, there was no standardisation of diagnostic techniques or of the techniques used for antigenic and genetic characterisation of swine influenza virus (SIV) strains. Because of this lack of organisation and harmonisation of SIV surveillance, it was difficult to make recommendations for the control of SIV in Europe, and for the selection of vaccine strains in particular. These needs have led to the submission of a proposal for an EC concerted action by researchers from several European countries.

The ESNIP concerted action started on January 1 2001. Fourteen partners from 10 different European countries (Belgium, Denmark, the Czech Republic, France, Germany, Italy, Ireland, the Netherlands, Poland, the UK) were involved, including 2 human influenza reference laboratories and 3 industrial partners. The network was coordinated by researchers from the Institute for Animal Science and Health, the Netherlands, Veterinary Laboratories Agency, UK and Ghent University, Belgium.

The major realisations were:

1) The standardisation of protocols for SIV isolation and serology and for antigenic and genetic typing of SIV isolates.

2) The selection and production of reference virus strains and sera. These were made available to all ESNIP partners for preliminary subtyping of SIV isolates.

3) The establishment of a central SIV bank with a collection of recent SIV isolates from various geographical areas in Europe.

4) The establishment of a database with relevant information on the SIV isolates that were obtained in different countries during the network.

5) The antigenic and genetic characterisation of a number of recent SIV isolates from different European countries.

6) The organisation of a serological survey to obtain a preliminary picture of the prevalence of different SIV subtypes in various European countries.

Most important, however, is that SIV researchers and diagnosticians from throughout Europe have started to exchange information and thoughts on SIV and to speak the same language.

The concerted action has ended on December 31 2003, but it has set the stage for a further cooperation between ESNIP partners and for a more organized surveillance of SIV.

The influenza session during this symposium will in part serve to communicate the ESNIP results to the scientific community. ESNIP partners from Belgium, the Czech Republic, France, Italy, the Netherlands and the UK will present oral or poster communications during this session.

FROM ZOONOTIC INFLUENZA TO FULL SCALE INFLUENZA PANDEMIC: A WINDOW OF OPPORTUNITY NOT TO BE MISSED

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Abstract
Avians are the cornerstone of the world of influenza viruses. However other species such as pigs and humans can also be infected by influenza viruses (IV), which established themselves in their mammalian hosts. At irregular intervals, we observed the introduction in pigs or men of new IV, resulting either from an *in toto* transmission of an avian virus or from a reassortment one involving at least some genomic segments of an avian IV. In humans, such events have been associated with pandemics. The phase of the introduction of a new influenza A virus subtype in humans from the avian reservoir, with or without an intermediate species such as pigs, represents a time for urgent intervention as soon as it is detected. Measures to avoid human cases of avian influenza can be implemented such as culling sick bird domestic flocks or as implementing personal physical protective equipment for some categories of farm/veterinary workers for example.

Text
In 2003/2004, a panasian outbreak of avian influenza A(H5N1) devastated the whole poultry industry of a number of countries located on an bow linking South-Korea through to Thailand including Japan, mainland China, Vietnam, Laos and Cambodia. In Thailand as well as in Vietnam, these outbreaks were associated with human cases of influenza A(H5N1) acquired from direct transmission from sick birds.
The spectre of a new human influenza virus sprung in our collective memory as a remembrance of influenza pandemics, which plagued the earth throughout our history. It is difficult to assess whether epidemics of acute respiratory febrile illness, which occurred before the sixteenth century, were influenza outbreaks or not. From that point in time, influenza pandemics or great epidemics occurred in Europe in 1510, 1557, 1729-1733, 1781-1782, 1829-1833 et 1889-1890 and in 1900 (for review) (Potter, 1998).
The twentieth century started with the Great War and the biggest influenza pandemic ever described, *Spanish Influenza*, with casualties as numerous as 20 to 40 millions (Oxford, 2000). In total, half of the world human population was affected. In France, the first cases were reported in April 1918 among the armed forces located in Normandy. Influenza then spread to the rest of Europe and reached the United States and India. The first wave stopped in August 1918 and the second wave started in September 1918 to reach its peak at the end of October 1918, except in Australia where it continued to increase until January 1919.
The Spanish Influenza then stimulated the mind of microbiologists and René Dujarric de la Rivière, at the Institut Pasteur showed for the first time that influenza was due to an ultrafiltrable agent. In 1931, Richard Shope, puzzled by the coincidence of Spanish Influenza in men and outbreaks of a similar disease in pigs, isolated the first influenza virus, labelled as such, from pigs (Shope, 1931a, Shope, 1931b). The first human influenza virus was isolated two years later in North London, using an unexpected animal species to propagate the virus: the ferret (Smith *et al*., 1933). Two other kinds of influenza viruses, which were serologically different from what is now labelled type A, were later identified as type B (Francis, 1940) and type C viruses (Taylor, 1949). Only type A viruses are known to be associated with pandemics when a new of its subtypes is introduced in humans.
In 1957, a new pandemic virus appeared with the *Asiatic Influenza*. Although it shared common antigenic characteristics, the virus differed from its predecessors by its surface antigens. The initial outbreaks of the unfolding pandemic were first described in the south of China in February 1957 but it is possible that the new virus, identified as A(H2N2), appeared sometime sooner in this area. The virus later spread to the Yuan Nan Province and Hong Kong in April 1957, then to Singapore, Japan and the rest of the far East. The Middle-East was affected in July before Africa was hit. The new virus reached Europe during the summer 1957 but the epidemic started during the following autumn (1957). Although the number of deaths was high (2 millions worldwide (Oxford, 2000)), it never reached the scale of that of the *Spanish Influenza*. During about ten years, it circulated instead of its predecessor the A(H1N1) subtype.
Again, sometime before 1968, a new virus subtype of influenza A virus arose causing the *Hong Kong Influenza pandemic*. The outbreak of influenza A virus observed in July 1968 in Hong Kong was preceded by similar outbreaks in South-East China and was followed by influenza epidemics in Singapore, the Philippines, Taiwan, Vietnam, Malaysia (August 1968) and finally India and the North of Australia in September 1968. After this, the epidemic halted for a while, except in the United Sates where it reached California in October and then the rest of this country. Tropical countries such as Brazil, Kenya and Indonesia were hit between the last quarter of 1968 and the first one of 1969. Countries in the Southern hemisphere were affected by moderate epidemics between March and May 1969. The first episode stopped in April 1969 and in Europe influenza activity during the 1968/1969 season was usual and caused by A(H2N2) viruses. The second episode caused by the new A(H3N2) virus started in western Europe in the autumn 1969, peaked in December and stopped in March 1970. There, the epidemic was more severe than elsewhere before.
Sequence data suggest that the A(H3N2) viruses inherited six genome segments from its predecessor the human A(H2N2) virus and two other segments from a duck virus, including its haemagglutinin (H3). The scale of this last pandemic was even smaller than the previous one with a number of case fatalities less than one million.
Since then, influenza A(H3N2) viruses come back every year or so to cause epidemics in humans. Since 1977, with the re-emergence of the A(H1N1) subtype in humans, this virus has been circulating together with

influenza A(H1N1), influenza B and C viruses. Influenza A and B viruses bear two surface glycoproteins: the haemagglutinin (HA) and the neuraminidase (NA). Among influenza A viruses, the subtypes are determined by antigenic and genetic characteristics of the HA and the NA. The number of HA and NA, which are or were part of human or swine viruses, are limited to H1, H2 and H3 and to N1and N2 whereas there are 15 molecular species of HA (H1 to H15) and 9 of NA (N1 to N9). These are all found in avian viruses, particularly in wild aquatic bird viruses such as ducks and geese viruses.

Influenza viruses were isolated from birds in Asia, in Oceania, In Europe and in America. The avian species harbouring the viruses are numerous and belong to various zoological orders and families. Among these birds, some migrate over long distances from the Northern hemisphere, where they establish their nest and procreate to the Southern, where they shelter during winter. During the migration, birds enjoy pauses during which they live in promiscuity with birds of various species originating from diverse Northern regions. Among the avian migrating population, a large number of young birds do the trip for the first time in their life. They represent a population naïve to many viruses and are a target for influenza virus infection. According to some data, some quite old now, the proportion of infected birds coming down south in autumn is highest compared to other time of year. In the Baie de la Somme in France, the infection can be so widely spread (Hannoun & Devaux, 1980) that virus could be recovered from water.

Ducks such as mallards belong to migrating colonies or to wild non migrating population and even to domestic flocks. This explains how easily, influenza viruses can be transmitted from the wild world to animal populations in the closest contact with men such as pigs. Infection of humans by pigs has well been documented.

Influenza A(H1N1) and A(H3N2) as well as A(H1N2) viruses have been established for years in pigs, which can be infected either by human and/or avian viruses. Pigs can therefore play the role of mixing vessel and transmission link between humans and birds, one of the reasons of this being that swine epithelial cells of the upper respiratory tract harbour both type of "*receptors for the virus*". These are terminal sialic acids borne by glycoproteins, those preferentially recognised by human viruses (alpha 2-6) and those preferentially recognised by avian viruses (alpha 2-3). Pigs can also be "conservatories" of old human influenza A viruses such as A/Victoria/3/75(H3N2)-like viruses.

Until 1997, the direct infection of men by avian influenza viruses associated with respiratory symptoms was thought to be impossible. In 1997, the so-called "*Chicken flu*" in Hong Kong did not lead to a pandemic although a new subtype A(H5N1) was infecting men. Data on A(H5N1) viruses isolated then from men and compared with their avian counterparts suggested that these were not adapted to humans and failed to start a chain of transmission. Such adaptation can be acquired by the accumulation of mutations in various genes and/or by reassortment between two viruses, one being already adapted to humans. This must have been the case around 1957 where, possibly in pigs, the contemporary human influenza A(H1N1) virus reassorted with an avian influenza A(H2N2) virus making up a new A(H2N2) virus with genomic segments PB1, HA and NA from the avian parental strain and the other five segments from the human A(H1N1) parental virus. Again a similar situation must have occurred around 1968, with the contemporary human influenza A(H2N2) reassorting with an avian influenza A(H3Nx) virus making up a new A(H3N2) virus with PB1 and HA from the avian parental strain and the six segments from the other parental virus, the human A(H2N2) strain. Genomic reassortments are common in human viruses as it is illustrated by the emergence of the A(H1N2) in 2001 in humans (Xu *et al.*, 2002). There are also common in birds as recently shown (Hatchette *et al.*, 2004).

Reassortment is probably the biggest risk for the emergence of new influenza A virus subtypes in humans. Between December 2003 and April 2004, a total of 34 human cases of influenza A(H5N1) symptomatic infections were reported in Vietnam and Thailand with 23 deaths (WER, 2004). The case fatality rate was very high for such respiratory infections. A first paper describing human cases in 2003/2004 showed that, in all 10 cases analysed, the infection appears to have been acquired directly from infected poultry. They also show that eight out of ten patients died although none of them had preexisting medical conditions (Tran *et al.*, 2004).

Genetic data concerning the 2003/2004 avian and human A(H5N1) isolates are currently being made available and show that reassortment had not occurred between a human A(H1N1), A(H1N2) or A(H3N2) virus and the avian current dominant A(H5N1) virus causing the vast epizootics. However, a recent study (Li *et al.*, 2004) show a series of genetic reassortment events traceable to the precursor of the H5N1 viruses that caused the initial human outbreak in Hong Kong in 1997 and avian outbreaks in 2001 and 2002. These events gave rise to a H5N1 genotype (Z) dominant in chickens and ducks, which was responsible for the 2003/2004 widespread outbreak. Interestingly, their data suggest that domestic ducks in South China played a central role in the generation and maintenance of this virus genotype. They also suggest that wild birds may have contributed to the wide spreading of the virus in Asia. Furthermore, their results advocate that influenza A(H5N1) viruses with pandemic potential have become endemic in Asia and the situation we witnessed since the beginning of 2004 in the affected countries demonstrated that this virus was not easily eradicable and carries on being a Damocles' spade over our head. A recently published model estimated that, during the epidemic circulation of human influenza A viruses with 10% of the population infected at anyone time and with a probability of reassortment of 1 in case of co-infection with two viruses, 45 human cases of infection by an avian A(H5N1) influenza virus would have corresponded to a 5% risk of reassortment (Ferguson *et al.*, 2004). This figure reached 50% if 600 A(H5N1) cases were to happened. Using this model, the calculated hypothetical risk of reassortment reached 3.4%, which is probably overestimated but was nevertheless not negligible. That was the time to cull all sensitive animals in infected farms to prevent any human/avian virus reassortant virus to appear. In the same paper, the authors suggest that instead of

considering the risk of reassortment by observing the total cumulated number of human cases of A(H5N1) infections, the size of the clusters of A(H5N1) human cases were most relevant to monitor. With various hypotheses on the reproductive rate of the infection, thresholds were calculated above which, epidemiological data would point out an unusual event corresponding, most probably to a virological event (Ferguson *et al.*, 2004) to investigate in priority.

As in 1997, in 2004 influenza A(H5N1) viruses did not established themselves in the human population and human to human transmission chain never started or, if it did it aborted very quickly. The culling of millions of poultry was probably necessary and was this far successful to avoid more human cases and possibly viral reassortment and/or cumulated mutations (2004).

Although emphasis has recently been put upon the role of some bird species such as hens for their role in the amplification and transmission of avian influenza viruses to humans, pigs should not be ignored as a zoonotic source of influenza or as a mixing vessel, which could facilitate the transmission to human of a new pandemic influenza A reassortant virus.

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THE MOLECULAR EPIDEMIOLOGY AND EVOLUTION OF INFLUENZA VIRUSES IN PIGS

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Introduction

Swine influenza (SI) is a highly contagious acute viral disease of the respiratory tract in pigs that is distributed worldwide. The disease is economically damaging, primarily due to weight loss and reduced weight gain. In the UK the financial loss resulting from reduced weight gain in finishing pigs alone due to SI has been estimated at approximately £7 per pig, equivalent to a total loss in the UK per annum of £60 million. Increasingly SI is considered one of the most important primary pathogens of swine respiratory disease worldwide. There are significant variations in virus epidemiology in different continents and regions together with approaches to swine husbandry and disease control.

Reservoirs of influenza A viruses

Influenza A viruses infect a large variety of animal species including humans, pigs, horses, sea mammals and birds. Aquatic birds are known to be the source of all influenza viruses for other species. Pigs are an important host in influenza virus ecology since they are susceptible to infection with both avian and human influenza A viruses, often being involved in interspecies transmission, facilitated by regular close contact with humans or birds. Phylogenetic studies of influenza A viruses have revealed species-specific lineages of viral genes. The evolution of influenza genes in species-specific gene lineages is an invaluable characteristic in studying influenza virus epidemiology.

The methods of swine husbandry make it likely that the virus is maintained by continual passage to young susceptible pigs. Swine husbandry practises influence directly the evolution of influenza viruses in pigs leading generally to reduced genetic drift, particularly in the genes encoding haemagglutinin (HA) and neuraminidase (NA), compared to those of similar viruses in the human population.

Epidemiology

Influenza A viruses of subtypes H1N1, H3N2 and H1N2 have been reported widely in pigs, associated frequently with clinical disease. These include classical swine H1N1, 'avian-like' H1N1 and 'human'- or 'avian-like' H3N2 viruses and H1N2 viruses of numerous origins. These viruses have remained largely endemic in pig populations worldwide. Although usually regarded as an endemic disease, epidemics may result when influenza infection occurs in an immunologically naive population (which can be linked to significant antigenic drift) or through exacerbation by a variety of factors such as poor husbandry, secondary bacterial or viral infections and cold weather. Serosurveillance results in Great Britain indicated that more than half of adult pigs in the national population had been infected with one or more influenza A viruses during their lifetime [1].

Classical H1N1

Classical swine influenza viruses, or their antibodies, have been reported from many parts of the world including North and South America, Europe, Asia, and Africa [reviewed 2].

'Human-like' viruses

Infections of pigs with the prevailing human subtypes also occur under natural conditions. Since 1984, outbreaks of clinical influenza in pigs due to these viruses have been observed throughout Europe with infections frequently characterised by high seroprevalence. In 1998 a novel H3N2 virus emerged in the USA, following genetic reassortment of avian, human and swine viruses and spread widely [3].

'Avian-like' viruses

In Eurasia, there have been several introductions of avian H1N1 viruses to pigs that have led to the establishment of stable lineages. These viruses have spread widely in pigs in this region and are often associated with disease epizootics. Since 1979 these viruses have become the dominant strain in European pigs. In addition, some of the H3N2 viruses isolated from pigs in Asia since the 1970's have been entirely 'avian-like'.

H1N2 viruses

Influenza A H1N2 viruses, derived from classical swine H1N1 and 'human-like' swine H3N2 viruses have been isolated in Japan and France but viruses of this genotype have not persisted within European pigs. Subsequently an H1N2 influenza virus (*see* Genetic reassortment) related antigenically to human and 'human-like' swine viruses has emerged and become endemic in pigs in Europe [4,5]. In North America another unique genotype of H1N2 virus has appeared and become established in recent years [6].

Emergence and variation of influenza viruses in pigs

Emergence of new strains or modifications to existing viruses has occurred in pigs by three mechanisms (i) an influenza A virus from another species transmitting *in-toto* to pigs. (ii) an influenza virus undergoing antigenic change or drift as a result of accumulating mutations with time in the genes encoding the major viral antigens (iii) co-infection of a pig with two unrelated influenza A viruses can result in the production of a new virus derived by genetic mixing of the progenitor strains leading to the potential emergence of a new virus with different antigenic and genetic characteristics.

Interspecies transmission of virus to pigs

In Europe, avian H1N1 viruses that were transmitted to pigs established a stable lineage, spread widely causing significant economic losses. All of the gene segments of the prototype viruses were of avian origin [7] indicating that transmission of a whole avian virus into pigs had occurred. Phylogenetic analysis of the genes of these viruses has revealed that they have retained an entirely avian genetic composition throughout their maintenance

in pigs. Influenza A viruses of H3N2 subtype related closely originally to a human strain from 1973, continue to circulate widely in pigs long after their disappearance from the human population [1,2]. The appearance of a H3N2 subtype variant strain in the pig population of a country appears to coincide with the epidemic strain infecting the human population at that time. Recently, H9N2 viruses have apparently been introduced to pigs in South-East Asia, most probably from land based poultry [8]. Currently there is no clear evidence supporting their independent maintenance in pigs through pig to pig transmission. In 1999, an avian H4N6 virus was isolated from pigs in Canada with respiratory symptoms but there was no apparent spread [9]. The potential of avian viruses novel to pigs including H9N2 or H4N6, to spread and persist within pigs remains unknown, substantiating the need for good surveillance of swine populations worldwide.

Genetic reassortment

The pig has been the leading contender for the role of intermediate host for influenza A viruses. Pigs are the only mammalian species that are domesticated, reared in abundance and are susceptible to, and allow productive replication of avian and human influenza viruses. Given the worldwide interaction between humans, pigs, birds and other mammalian species there is a high potential for cross-species transmission of influenza viruses in nature. Continued co-circulation of influenza A viruses in pigs can result in the production of new reassortant viruses. This is an ongoing process with frequent genetic exchange between co-circulating variants of the same virus that may give rise to 'new' viral genotypes with the potential for spread including to other species. Evidence for the pig as a mixing vessel of influenza viruses of non swine origin was first demonstrated by Castrucci et al. [10], who detected reassortment of human and avian viruses in Italian pigs. Further evidence for the emergence of new strains that are able to spread widely in pigs following genetic reassortment was the appearance of H1N2 virus in Great Britain in 1994 before apparent spread to the rest of Europe. The H1N2 viruses derived from a multiple reassortant event over a number of years involving human H1N1, 'human-like' swine H3N2 and 'avian-like swine' H1N1 [11].

Since 1998 H3N2 viruses isolated from pigs in the USA have contained combinations of human, swine and avian genes. Strains have possessed significant antigenic and genetic heterogeneity due to the acquisition of HA genes from the prevailing human H3N2 viruses [12] rather than through independent genetic drift in swine viruses. These newly emerged triple reassortant H3N2 viruses are now well established in pigs in North America and have reassorted with classical H1N1 viruses producing another unique genotype of H1N2 virus that has subsequently spread within pigs in this region [6].

Genetic variation

Following transmission to pigs influenza virus genes evolve in the pathway of the host of origin but diverge forming a separate sublineage. Genes that code for the surface proteins HA and NA, are subjected to the highest rates of change. Current epidemic strains are clearly distinguishable from the prototype strains. The HA gene of both the classical and 'avian-like' swine H1N1 viruses is undergoing genetic drift, being more marked in the latter. The more limited antigenic variation in the HA gene of swine viruses is probably due to the lack of significant immune selection in pigs because of the continual availability of nonimmune pigs.

'Human-like' swine H3N2 viruses appear to be evolving independently in different lineages to those of human and avian strains [13,14]. The rates of genetic drift in HA and NA genes is equivalent to those of H3N2 viruses in the human population but in contrast to the latter the changes are not generally associated with antigenic sites.

Adaptation of 'new' influenza viruses to pigs

The mechanisms whereby a virus from another host species is able to establish a new lineage in pigs remains unclear, although following the introduction of an avian virus into European pigs in 1979 the virus was relatively unstable for approximately ten years. Furthermore, adaptation of this virus to pigs resulted in the virus acquiring altered receptor specificity, preferentially recognising receptors with α2,6 linkage [15], the native linkage in humans. The avian H4N6 and H9N2 viruses detected recently in pigs had some modifications in the receptor binding pocket on the HA gene which may have facilitated binding to receptors with α2,6 linkage [8,9]. Furthermore, the continual genetic exchange between viruses is likely to result in the emergence of 'genetic variants' with a higher fitness and therefore potential selective advantage. It would appear that the adaptive processes can take many years as occurred following transmission of both avian H1N1 and human H3N2 viruses to pigs.

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SEROPREVALENCE OF SWINE INFLUENZA IN EUROPE AND INTERPRETATION OF SEROLOGICAL FINDINGS

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Introduction

In recent years the epidemiology of swine influenza (SI) in Europe has changed. The relatively novel H1N2 subtype appears to have become widespread and to circulate concurrently with H1N1 and H3N2 swine influenza viruses (SIVs). Until recently, there was limited knowledge of the current prevalence of each subtype in different regions of Europe. The circulation of 3 subtypes has also made the interpretation of SIV serology more challenging. In this paper, we first report the results of a SIV serosurvey that was undertaken during the EU concerted action «European Surveillance Network for Influenza in Pigs» (ESNIP). In the second part, we present our personal viewpoints on the use of SIV serology in practice.

Seroprevalence of different SIV subtypes in various areas of Europe

The major aim of the serosurvey was to determine the current prevalence of the H1N2 virus throughout Europe and to compare it with that of the «older» H1N1 and H3N2 virus subtypes. ESNIP partners from Belgium, the Czech Republic, France, Ireland, Italy and Poland, and 2 diagnostic laboratories from Germany and Spain participated in this study. The areas sampled included 12 out of the 19 main swine production regions in Europe. A total of 4377 sera (5 to 10 sera per farm) from 695 commercial swine farms (55 to 101 farms per country) were collected between 2001 and 2003. The sera were from adult fattening swine in France, from first parity sows in Italy and Poland, and from sows of various ages in the remaining countries. None of the pigs were vaccinated against influenza.

All sera were examined in haemagglutination inhibition (HI) tests against H1N2, H1N1 and H3N2 SIVs at a starting dilution of 1:20. The H1N2 strain (sw/Scotland/94) used was identical in all countries, but H1N1 and H3N2 strains differed. Experimental infection studies in pigs have demonstrated that there is no serological cross-reaction between subtypes in the HI test and that HI antibodies to a given subtype provide evidence of infection with that subtype (3). Thus, HI antibody titres Ó20 for a given subtype were considered indicative of an infection with that subtype.

Seroprevalence in sows: Table 1 shows seroprevalence rates in sows at the individual and at the herd level. In most herds only part of the animals tested positive for antibodies to a given subtype, so that seropositivity rates were higher for the herds than for the individual sows. The percent of positive reactions to the H1N2 virus was highest in Belgium, Germany and Spain, and somewhat lower in Italy. These 4 countries also had high seropositivity rates for H1N1 and H3N2 SIVs. In the Czech Republic and in Ireland, only few sera reacted with the H1N2 virus and seroprevalences of H1N1 and H3N2 were lower than in the previous countries. The sera from Poland were negative for H1N2 and H3N2 antibodies and the prevalence of H1N1 antibodies was lower than in any other country.

Table 2. Seroprevalence of different SIV subtypes in fatteners in France (Brittany) and Belgium (Flanders).

	% of seropositives					
	France		Belgium			
Sub-			2001-2002		2003	
type	pigs	herds	pigs	herds	pigs	herds
H1N2	25.7	41.8	17.9	25.0	50.6	67.9
H1N1	28.9	43.6	52.4	82.1	55.4	92.9
H3N2	0.0	0.0	39.3	57.1	15.5	32.1

Table 3. Prevalence of antibodies to multiple SIV subtypes in sows and fattening swine in Belgium.

		% of animals with antibodies to … subtypes			
		0	1	2	3
Sows		6.0	25.8	38.3	29.8
Fattening	2001-02	23.8	44.0	31.6	0.6
pigs	2003	16.7	47.0	33.3	3.0

Seroprevalence in fattening swine: The serological data from fattening swine in France are compared with those of similar examinations in Belgium (Table 2). The farms in Belgium were first sampled between June 2001 and May 2002 and resampled in March 2003. The pigs in France had antibodies to H1N1 and H3N2 SIVs, but not to H3N2. Though antibodies to all 3 subtypes were found in fatteners in Belgium, seropositivity rates for H1N2 and H3N2 varied significantly between the 2 sampling periods.

Incidence of infections with multiple SIV subtypes in sows and fattening swine: The serological data from Belgium were further analyzed so as to get an idea of the % of animals that had been infected with multiple SIV subtypes during their lifetime (Table 3). Most sows showed antibodies to a combination of 2 subtypes or to all 3 subtypes. Most fattening swine were seropositive for one subtype or for a combination of 2 subtypes, and few had antibodies to all 3 subtypes.

Table 1. Seroprevalence of H1N2, H1N1 and H3N2 viruses in different European countries.

SIV	% positive in …													
subtype	Belgium		Czech Rep.		Germany		Italy		Ireland		Poland		Spain	
	sows	herds	sows	herds	sows	herds	sows	herds	sows	herds	sows	herds	sows	herds
H1N2	57.8	89.0	3.2	15.4	32.5	71.3	13.8	35.5	0.6	2.0	0.0	0.0	52.8	87.0
H1N1	80.8	97.0	16.4	39.7	65.8	90.8	46.5	82.9	17.8	41.8	7.8	8.9	38.5	65.0
H3N2	53.8	86.0	0.8	5.1	57.7	87.4	41.7	68.4	4.2	16.3	0.0	0.0	38.0	67.0

Conclusion: All 3 SIV subtypes appear to be widespread in the main swine production regions in Europe, and they circulate concurrently. H1N1 is the dominant subtype in countries with lower SIV prevalences. Both sows and fattening swine may become infected with more than one subtype, but infections with all 3 subtypes are most frequent in sows. This can be explained by the fact that sows have a longer lifetime than fatteners and thus a higher chance to become exposed to multiple SIV subtypes.

The use of SIV serology in the veterinary practice

Though many infections remain subclinical, SIV is a major cause of acute respiratory disease outbreaks in swine (2) and an important contributor to more chronic, multifactorial respiratory problems (1). In the veterinary practice SIV serology is used for various purposes: to confirm the involvement of SIV in respiratory disease, but also to assess the SIV immune status on a farm or to optimize vaccination schedules. However, the interpretation of serological data is often frustrating and it has become increasingly complex with the emergence of a third SIV subtype. In this section, we will review some frequently asked questions about HI test results, as this remains the most used and best serologic test for SIV.

Question 1. How sensitive is the HI test? The HI test is relatively sensitive, provided that suitable strains are used as antigens in the laboratory. The test detects antibodies that bind to the receptor-binding site on the haemagglutinin protein of the virus, and this binding is subtype- and to a lesser degree strain-specific. Therefore, at least one representative of each of the 3 circulating SIV subtypes (H1N1, H3N2, H1N2) should be included in the test. It is best to use relatively recent strains that match antigenically with the strains circulating in the field, because this will increase the number of positive reactions and the antibody titres measured.

Question 2. How specific is the HI test, or can it distinguish between H1N1, H1N2 and H3N2 SIVs? As mentioned higher, the test is highly subtype-specific. In experimental studies, 97% out of 116 pigs that had been infected with one or with 2 different SIV subtypes only showed HI antibodies to the infecting subtype(s) (3 and unpublished). In the few pigs with serologic cross-reactions, antibody titres were very low (10-20) when compared to those against the infecting subtype (Ó160).

Question 3. What is the significance of low (1:10 and 1:20) HI antibody titres? Because these antibody titres may be due to non-specific inhibition of the haemagglutination, some labs set the cut-off value for the HI test at 1:40. On the other hand, even pigs with HI antibody titres of 1:10 or 1:20 may have been truly infected with SIV. After experimental infection of SIV seronegative pigs, serum HI antibodies to the infecting strain peak at 1:160-1:320 by 2-3 weeks PI, but they may have declined to 1:10-1:20 by 18 to 24 weeks. In the field, young pigs with residual maternal antibodies usually develop lower post-infection antibody titres compared to completely seronegative pigs. In the authors' lab, a farm is considered positive for a given SIV subtype when Ó2 out of a group of 10 animals have HI antibody titres Ó20.

Question 4. How to confirm an SIV infection by serology or which titres are indicative of an acute infection with SIV? Paired sera (an acute serum collected when disease is seen and a convalescent serum taken 2 to 3 weeks later) from approximately 10 pigs are required. If an infection with SIV occurred, antibody titres to the causative SIV subtype will be absent or negligible in the acute serum and rising or Ó4-fold higher in the convalescent serum. Convalescent sera alone are unreliable for the diagnosis of SIV. One complication is that serological responses to a given SIV subtype can be different in pigs with antibodies to other subtypes compared to those in fully seronegative pigs. For example, experimental H1N2 infection of previously H1N1-infected pigs may boost already existing H1N1 antibodies and vice versa (3). More examples will be presented during the symposium.

Question 5. How to evaluate the SIV immune status on a (non-vaccinated) farm? For a detailed picture of the SIV immune status on a farm, we recommend to sample pigs from 3 different age categories: 1) 10 adult fattening swine, which will show HI antibodies to the SIV subtypes that circulated during the previous 3-4 months; 2) 15 randomly selected sows, which may show antibodies to subtypes circulating more than 4 months ago; 3) 10 nursery pigs. These pigs may have maternal antibodies until 6-8 weeks of age. The presence of antibodies in 8-10 week old pigs may point to the continuous circulation of SIV on the farm. It should be stressed that the SIV serological picture is usually very heterogeneous.

Question 6. Will the HI antibody profile differ on vaccinated versus non-vaccinated farms? All of the above questions apply to the situation in non-vaccinated farms. The current SIV vaccines contain H1N1 and H3N2 SIV strains and they stimulate HI antibodies to those subtypes. Many vaccinated sows in the field have previously been infected with SIV, and vaccination has been shown to cause a dramatic booster of post-infection antibody titres under experimental conditions. This may explain why antibody titres in vaccinated sows in the field are significantly higher (frequently Ó1:160) than in unvaccinated sows and relatively uniform. Maternal antibodies in pigs from these sows may last until 14-16 weeks. Another point is that serologic cross-reactions with H1N2 seem to occur more readily in vaccinated than in unvaccinated pigs.

Conclusion: Serologic findings in a pig herd are usually not uniform and they need to be evaluated at the group level. Knowledge of the SIV vaccination status is essential for a correct interpretation of serologic data. If results of the HI test remain unclear, one can perform additional serologic tests such as the virus-neutralization (VN) test, which is more sensitive.

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RECOMMENDATIONS ON SWINE INFLUENZA VACCINES AND VACCINATION STRATEGIES

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Introduction

Vaccination remains the primary means of preventing SIV in pigs. Commercial, inactivated SIV vaccines have been on the market in Europe since the early 1980s, and they contain both of the subtypes that were prevalent at that time, H1N1 and H3N2. However, the recent changes in the epidemiology of SIV have raised many questions with regard to the efficacy of these vaccines against the current strains. In this paper, we will first present basic information on the immune response to SIV infection and vaccination, because this is necessary to understand how vaccines work and what one can achieve with vaccination. Thereafter, we will review a few questions that are frequently asked by vaccine manufacturers and swine practitioners. The viewpoints presented are based on data from the EC concerted action «European Surveillance Network for Influenza in Pigs», as well as on pig experiments performed in the authors' laboratory.

The immune response to an infection with SIV

An infection with SIV induces a rapid and efficient immune response, which results in complete elimination of the virus within a week and a very solid protection against reinfection. The specific immune response to SIV includes the production of antibodies in the circulation and at the mucosae of the respiratory tract, as well as a cell-mediated immune response. Antibodies develop to the haemagglutinin (HA), neuraminidase, matrix and nucleoprotein proteins. However, only antibodies to the globular head region of the HA, which are detectable by virus neutralization (VN) or haemagglutination inhibition (HI) assays, can neutralize the virus and thus prevent an infection. These HA-specific neutralizing antibodies are highly efficient mediators of protection against reinfection with a similar virus strain, but they will not protect against strains belonging to another SIV subtype. In recent infection experiments, however, we have demonstrated some type of cross-protection between the current European H1N1, H3N2 and H1N2 SIV subtypes in the absence of cross-reactive HI antibodies (1). In contrast to pigs that had been previously infected with either H1N1 or H3N2, pigs with infection-immunity to both subtypes showed a solid protection against H1N2 infection. Still, these pigs only had HI antibodies to H1N1 and H3N2 at the time of H1N2 challenge. This suggests that some of the other immune mechanisms, which are generally less effective but more cross-reactive between influenza viruses, contribute to this cross-subtype protection. The cell-mediated immune response, for example, which is largely directed against the conserved internal viral proteins, is probably involved.

The current SIV vaccines and the immune response to vaccination

SIV vaccines are based on whole, inactivated influenza virus, or on highly purified disrupted virus particles («split» vaccines) and an oil adjuvant. Most of the current vaccines still contain the older human New Jersey/76 (H1N1) and Port Chalmers/73 (H3N2) strains, but no H1N2 component. The primary vaccination should consist of two intramuscular injections 3 to 4 weeks apart, and bi-annual booster vaccinations are recommended for sows.

As for other inactivated vaccines, the immune response to SIV vaccines differs from that following replication of infectious virus in the host. SIV vaccines mainly induce circulating antibodies to the HA of the vaccine strains, while mucosal or cellular immune responses are barely stimulated (2). The presence of high titres of neutralizing antibodies in the serum, which can reach the lungs by diffusion, is sufficient to block or significantly reduce SIV replication in the lungs in case of an infection, and to prevent disease. Such a reduction of lung virus titres appears to result in a reduced production of proinflammatory cytokines in the lungs, which are thought to be essential mediators of the typical SIV symptoms (3). Experimental data have clearly shown that a minimal reduction of virus replication in the lungs will strongly reduce cytokine levels and thus protect against disease.

Because protection following vaccination is almost entirely dependent on HI antibodies in the circulation, antibody titres to the infecting strain and protection are tightly correlated. In vaccination-challenge studies by the authors, all pigs with HI antibody titres >160 were completely protected against virus replication in the lungs and disease (4). Pigs with lower antibody titres showed a significant reduction of lung virus titres when compared to unvaccinated controls, and they were still completely protected from disease. However, we used a very severe challenge method in these studies ($10^{7.5}$ EID50 virus intratracheally), and antibody titres Ò160 may be effective against challenge with a lower virus dose or under field conditions. On the other hand, protection induced by vaccination is somewhat more specific than that after infection and this issue is further discussed in the questions below.

Is there a need to update H1N1 and H3N2 vaccine strains?

It is generally accepted that antigenic drift of circulating influenza virus strains in comparison with vaccine strains may render vaccines less effective, and human or equine influenza vaccine strains are therefore regularly updated. Replacement of the New Jersey/76 (H1N1) and Port Chalmer/73 (H3N2) strains in SIV vaccines has also been considered, based on reports of antigenic drift in European H1N1 and H3N2 SIVs during the late 1990s (5,6). On the other hand, antigenic analyses performed during the ESNIP concerted action have clearly shown that antigenic drift in swine influenza viruses is minimal when compared to that occurring with human influenza viruses over a 20-year period. Most important, commercial New Jersey/76 (H1N1) and Port Chalmers/73 (H3N2) based vaccines were still very efficacious against more recent strains in pig experiments. In studies by the authors, a double vaccination with such a vaccine conferred excellent protection against a severe intratracheal challenge with H1N1 or H3N2 viruses isolated in Belgium in '98 (4,7). Despite the antigenic differences between vaccine and

challenge strains, the commercial vaccine still induced high antibody titres to the field strains. Challenge virus replication in the lungs was undetectable or strongly reduced and there was no disease. Similar results were obtained in challenge studies with an H3N2 challenge virus isolated in The Netherlands in 1996 (2). There are thus no scientific arguments to update the H1N1 or H3N2 vaccine strains.

Do the current vaccines protect against H1N2?

Under experimental conditions, the commercial SIV vaccine that protected so efficiently against recent H1N1 and H3N2 strains did not protect against challenge with the H1N2 subtype (1). The vaccine induced little if any HI antibody to H1N2, and it could not prevent H1N2 virus replication or disease upon challenge. In contrast, the addition of an experimentally prepared H1N2 component to the vaccine conferred significant protection from H1N2 infection and disease. It is still unknown how the absence of an H1N2 component in the vaccine affects vaccine performance in the field, but the H1N2 subtype has clearly become widespread throughout Europe. Therefore, the inclusion of an H1N2 strain in SIV vaccines must be considered.

The failure of (H1N1+H3N2) vaccines to protect against H1N2 also points towards a role of cellular and/or local immunity in the protection to H1N2 in (H1N1+H3N2) infection-immune pigs.

How much antigenic drift is needed before vaccine strains become obsolete?

This is not exactly known. The vaccine strains must show some antigenic overlap with the infecting strains to be protective, but antigenic (cross HI tests) and genetic analyses are not the most accurate predictors of vaccine strain performance. In fact, many of the antigenic and genetic variations found within H1N1 and H3N2 SIV subtypes appear to have little impact on vaccine efficacy in the pig, as illustrated by the experiments mentioned higher (2,4,7). On the other hand, dramatic antigenic differences, such as that between the current H1N1 vaccine strain and the circulating H1N2 strains, will compromise vaccine efficacy. In genetic analyses, we found as much as 99 amino acid changes between both strains, and 39 of them were located in antigenic sites. This compares with 28 amino acid differences in five antigenic sites between the H1N1 vaccine and challenge strains used in pig experiments (1, 7). Unfortunately, genetic analyses of influenza viruses are rarely combined with in vivo vaccination-challenge studies and there is still a significant lack of knowledge concerning the impact of genetic drift on vaccine efficacy. Another important issue is that factors other than the nature of the vaccine strains, such as the antigenic dose and adjuvant, can also have a dramatic effect on vaccine efficacy. Therefore, challenge tests in pigs remain essential to evaluate vaccine efficacy.

How efficient is vaccination in the field?

There are few published data on SIV vaccine efficacy in the field. While experimental studies generally use SIV seronegative pigs and an optimal time interval between vaccination and infection, this may be different in the field. Maternal antibodies, for example, frequently interfere with effective vaccination of feeder pigs. Furthermore, the cost-benefit of SIV vaccination is often questioned. Though an acute SIV outbreak can cause serious disease and weight loss in fatteners, recovery is rapid in uncomplicated cases and pigs may catch up on their weight within 2-3 weeks.

Should we vaccinate sows or fattening pigs?

Serological data indicate that vaccination of the sows is likely to be beneficial for both the sow and her offspring. Indeed, significantly higher H1N1 and H3N2 antibody titres are seen in vaccinated (frequently 1:160-1:640 or greater) than in unvaccinated sows. This results in high and long-lasting maternal SIV antibody levels in the piglets from vaccinated sows. In a study by Thacker (2000), SIV passive antibody levels dropped below 1:40 by 6 weeks of age in nearly all pigs from unvaccinated sows, which had only low HI titres. In contrast, antibody titres in pigs from vaccinated sows were frequently detectable until 16 weeks of age. As mentioned previously (see paper on SIV serology), the high antibody titres in vaccinated sows may have been stimulated by previous infections with SIV. In experimental studies, SIV vaccination of infection-immune pigs caused a dramatic increase of HI and VN antibody titres to all subtypes with which the pigs had previously been infected. It is unlikely, therefore, to encounter problems with SIV in sows that have been routinely vaccinated or in their newborn pigs.

Vaccination of feeder pigs is less commonly performed. This strategy may be recommended in herds where influenza is a problem in growers or finishers. One difficulty is that even very low levels of residual maternal antibodies can interfere with vaccination of young pigs. Vaccination of feeder pigs is therefore difficult to combine with vaccination of sows, since prolonged passive immunity may interfere with effective vaccination of piglets.

Conclusion: Of all vaccines against respiratory viruses of pigs, SIV vaccines are among the most effective. One weakness of the current vaccines is that they do not protect against the novel H1N2 subtype under experimental conditions. Still, the available field data suggest that vaccination of sows is highly efficient in controlling disease in suckling pigs and may protect pigs throughout the nursery phase.

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NEW STRATEGIES FOR THE CONTROL OF "NOTIFIABLE" AVIAN INFLUENZA

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Avian Influenza (AI) is an infection of birds by influenza A viruses of the family Orthomyxoviridae. Many species of birds have been shown to be susceptible to this infection. Whereas wild aquatic birds form a major reservoir of low pathogenic (LP) viruses, infection of domestic poultry by AI viruses may be asymptomatic or produces clinical signs. In this case, they range from mildly pathogenic infection to systemic disease with near 100 % mortality (referred to as highly pathogenic : HP). In a very recent past, HP AI caused the loss of 50 millions poultry in the Netherlands/Belgium (in 2003) and more than 100 millions poultry died or were culled in South Asia (in early 2004 without taking into account the new losses officially notified since this summer). Although 16 haemagglutinin (H1-16) and 10 neuraminidase (N1-10) molecular species are known at present, to date, HP AI has been associated only with the H5 and H7 AI viruses (with two exceptions). In fact, although most H5/H7 AI viruses are LP they can easily mutate to HP. Beside the economical consequences mentioned above, on rare occasions H5/H7 viruses have displayed zoonotic properties and (altogether) have been involved in the death of at least 30 persons. Therefore, to take into account the future OIE AI definition (still to be discussed), we will presently refer to notifiable AI (NAI), every infection with HP or H5/H7 AIV.

Thus targeted control strategies aim at avoiding introduction in poultry farms and further spread of H5/H7 viruses irrespective of their virulence. Taken the recent increasing number of epizootics in the world, new strategies of control are considered. The present communication will overview them.

At the international and national level risk assessment is more and more implemented to identify the main sources and ways of transmission and to put in place suitable measures. Reinforcement of the requirements for international trade is being discussed and a new concept such as compartmentation, that is based on a management approach, is also considered (3).

A regular surveillance of AI is achieved through compulsory targeted serological surveys in poultry at risk and virological surveys in wild bird species at risk (1). In addition, Member States can decide on complementary surveillance. For instance, France has also implemented a virological surveillance of the most at risk poultry holdings to be able to define the characteristics of the eventual viruses that may be isolated, such data being useful in case of prophylactic vaccination (see below).

At the same time, a revision of the Community policy measures to control AI is being undertaken (2) and tools based on veterinary, economic, social-ethical issues are being set up to assess the efficacy of control strategies.

Emergency vaccination -that could prove itself and reveals itself as much less expansive then culling- is restored to favour in Europe as a supplement to biosecurity measures to avoid NAI secondary spread from known cases. However its use is dependent on the authorization of the European Commission that will be limited in time and space and will be given only on the basis of a detail, well-argued protocol provided by the concerned member State (2). The present communication will emphasize the situations that can justify such an approach and list its limitations depending on the vaccine made available and its efficacy with respect to the prevention of infection. In fact, commercial available vaccines do not completely prevent infection with wild viruses and investigations (which our staff is contributing to) are carried on to improve their efficacy. The lessons to be made from previous experiences of field vaccination will examplify the proper conditions to be recommended with the actual vaccines. The minimal requirements are a well scheduled and controlled vaccination using a vaccine allowing differentiation of infected/vaccinated birds together with a serological/virological monitoring to assess the efficacy and eliminate every new infected flock immediately (not to lead to an endemic situation with a permanent virus circulation) favouring the selection of new viruses. Thus, it is essential that the veterinary Authority of the concerned Member State has the command of the whole operation. Under such conditions, the vaccination of birds can help to control the spread of the epizooty while avoiding mass culling of poultry (particularly when high poultry density areas are concerned) and protecting endangered, rare species (such as those kept in ornithological parks for instance) and high value poultry (such as the stocks obtained after genetic selection).

Even prophylactic vaccination to prevent primary introduction in poultry, is being considered in Community policy (2), although its modes are still to be discussed. In addition, the restraints imposed up to now to vaccinated poultry and their products with respect to international commercial exchanges should be released, under specified guarantees. However, the application of prophylactic vaccination will probably require the ability to ensure the best fitness between the vaccinal strain to be used and the characteristics of the AI strain likely to emerge, to prevent any infection of poultry. Our preliminary results show that, actually, it cannot be recommended in France to prevent the infection of poultry holdings at risk.

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TRANSMISSION OF A HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS TO SWINE IN THE NETHERLANDS

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Introduction

At the end of February 2003 a highly pathogenic avian influenza H7N7 was introduced in the Dutch poultry population. In the early stages of the epidemic it became apparent that humans could also be infected with this virus. Although this mainly resulted in mild symptoms of conjunctivitis, also one fatal case was recorded[2]. In early April a serological surveillance program was started to determine whether the virus was also infecting pigs. Infections of pigs may have consequences for both animal and human health.

Material and Methods

Field surveillance:

Three categories of swine herds inside the protection zones were sampled:

Category 1: Mixed herds with swine and poultry; poultry infected.

Category 2: Mixed herds with swine and poultry; poultry pre-emptively culled (no infection established).

Category 3: Swine herds with no poultry present.

The first part of the surveillance consisted of 13 herds from category 1 where a possible infection had the highest chance of detection. The poultry was culled 3-5 weeks before the pigs were sampled, so there had been enough time for any serological responses, had the virus infected the pigs. Eight of the herds were sampled according to a representative sampling schedule, allowing for the detection of even small outbreaks (60-118 samples per herd). The five smallest herds were sampled completely, to allow detecting even very small outbreaks (62-376 samples per herd.

After the first results from these herds, five herds were sampled again. Eleven days after the first sampling, all individual animals above 4 weeks of age were sampled and tested (173-713 per herd, total 2373 samples).

After that, all category 1 herds were tested serologically after at least 3 weeks had passed since the culling of the infected poultry. They were sampled according to the same representative sampling schedule used during the first part of the surveillance in pigs (60-217 samples per herd). Including the first 13 herds, in the end a total of 46 herds in this category were identified and tested during the avian influenza outbreak.

During the outbreak the surveillance was soon expanded to swine in mixed herds where poultry was preventively culled (category 2), and to swine in swine herds with no poultry at all (category 3). All these herds were located in the protection zones, within a 3 kilometer radius of an infected premises. From category 2 a total of 21 herds were sampled and from category 3 a total of 23 herds. In each herd 60 samples were randomly taken, resulting in a total of 2640 blood samples.

Pilot experimental infection:

An experimental infection of pigs with influenza subtype H7N7 was carried out to determine the serological response of an infection and to get a first indication of transmission. Four 10-week-old SPF piglets were inoculated intranasally (I.N., 2ml per animal, 10^6 EID_{50} per ml). Two piglets were inoculated intramuscularly (I.M.) with the same doses and two piglets were added 24 hours later as non-infected contact pigs. Blood samples were taken at days 0, 7, 11, 14, 18, 22, 28 and 32 for antibody detection in an Np-ELISA and a haemagglutination inhibition (HI) test. Oropharyngeal swabs were taken daily at days 0 to 9, and furthermore at days 11, 14 and 18 for virus isolation.

Transmission experiment:

To quantify transmission between pigs, a transmission experiment was carried out with 40 pigs. Half of these pigs were inoculated intranasally (2ml per animal, 10^6 EID_{50} per ml). The other half was added 24 hours later as non-infected contact pigs. Blood samples were taken at days 0, 4, 7, 11, 14, 18, 21, 28 and 35 for antibody detection in an Np-ELISA and a haemagglutination inhibition (HI) test. Oropharyngeal swabs were taken daily at days 0 to 9, and furthermore at days 11 and 14 for virus isolation.

With these numbers of pigs, a contact infection of up to 4 pigs results in an R_0 (reproduction ratio as a measure for transmission) below 1.

Testing:

Samples were tested in a haemagglutination inhibition (HI) test, using the avian H7N7 as antigen. Sera were pre-treated with cholera toxin and chicken erythrocytes. 969 blood samples from swine herds in the Northern parts of the Netherlands, from before the introduction of H7N7 in the Netherlands, were tested to determine the test specificity. From these samples the specificity of the HI-test, based on the titre 40 threshold level, was determined at 97.4%. To determine whether a herd was infected , the seroprevalence was corrected for this specificity and an estimated sensitivity of 80%. Around this estimated true seroprevalence a 95% confidence interval (CI) was calculated[1].

While decisions during the outbreak had to be made mainly based on HI-results, on selected field samples also a virus neutralisation test (VNT) was carried out to confirm the HI-results.

For the experimental infections an Np-ELISA was used that detects antibodies against all influenza subtypes. Virus isolation was carried out on embryonated chicken eggs.

Results

Field surveillance:

The results of the first part of the surveillance (13 category 1 herds) showed that in five of these herds the seroprevalence was significantly above 0, indicating that the H7N7 virus was introduced into the swine population. Seroprevalences in these herds (counting titers of 40 and higher) were 5.1%, 5.6%, 8.3%, 15% and 27% respectively.

The results of re-sampling these 5 herds showed no significant increase in seroprevalence (now 2.5%, 7.7%, 6.9%, 14% and 29% respectively). In the first three herds the seropositives were found scattered across the farms, rarely more than one seropositive pig in one pen. In the herd with the highest seroprevalence, these seropositives were correlated with feeding of broken eggs from infected poultry in two compartments. Paired blood samples from over 200 individual animals showed 5 conversions from positive to negative and 3 conversions from negative to positive. All other results showed to be reproducible. In all cases the titres of these conversions were around the threshold value of 40 and test variation and aspecific responses were most likely to be the cause of these 'conversions'.

Further testing of all category 1 herds, up to a total of 46, revealed an additional 8 herds with an estimated true seroprevalence of more than 0. This brought the total number of likely infected herds at 13, with estimated true seroprevalences ranging from 3-42% (table 1).

From 5 of the 13 infected herds, samples were tested in a VNT to confirm the HI-results. All 5 herds were positive in the VNT as well. From all herds with very few (presumably false-)positives, positive samples in the HI were retested in the VNT and turned out to be negative.

The test results for the category 2 and 3 herds showed an overall seroprevalence of 1.1%, with no significant difference between both categories. Most of the titres found were at the threshold level of 40 and could well be explained by non-specific reactions. An additional 457 blood samples were taken on 5 herds from category 2 and 3 with one or two titres >40. Two percent of these samples were positive, again with mainly low titres and within what could be expected, based on the specificity of the test. Confirmation of all HI-positive samples in a VNT showed that they were indeed false-positives. In the end, no antibodies were detected in category 2 and 3 herds.

Table 1 : Results on 13 herds with an estimated true seroprevalence significantly above 0% (Prev=measured seroprevalence, True prev=estimated true prevalence).

Herd	Samples	Positive	Prev.	True Prev.	95%CI
1	60	5	8.3%	7.4%	3.4-11
2	60	9	15%	16%	11-21
3	61	16	27%	32%	25-38
4	118	6	5.1%	3.2%	1.0-5.5
5	72	4	5.6%	3.9%	0.9-6.9
6	60	17	28%	33%	26-39
7	60	21	35%	42%	35-49
8	60	18	30%	35%	29-42
9	60	9	15%	16%	11-21
10	116	5	4.3%	2.2%	0.1-4.3
11	60	5	8.3%	7.4%	3.4-11.3
12	60	4	6.7%	5.3%	1.7-8.9
13	60	6	10%	9.6%	5.3-14

Pilot experimental infection:

After inoculation of the pigs, either I.M. or I.N., no clinical signs related to influenza were ever noticed. After 7 days, all inoculated pigs were seropositive in the Np-ELISA. In the HI-test, pigs showed higher titres after I.N. inoculation than after I.M. inoculation (table 2). Virus could be isolated from oropharyngeal swabs at 1 to 4 different days between D1 and D5 from all four I.N. inoculated pigs.

Table 2 : Serological results of the pilot experimental infection. HI-titers are given (< means <10) and positive Np-ELISA results are shown in shades (Con=contact pig, I.M.=intramuscular, I.N.=intranasal)

DPI:	0	7	11	14	18	22	28	32
Con	<	<	<	<	<	<	<	<
Con	<	<	<	<	<	<	<	<
I.M.	<	<	40	40	40	40	40	20
I.M.	<	<	<	<	<	<	<	<
I.N.	<	<	160	320	160	160	80	80
I.N.	<	40	80	80	40	20	20	20
I.N.	<	<	160	160	80	80	80	40
I.N.	<	<	40	40	40	40	20	20

Transmission experiment:

All 20 inoculated pigs became seropositive in both the Np-ELISA and the HI-test. None of the 20 contact pigs seroconverted. No clinical signs were seen in any of the infected pigs.

Discussion

During the Dutch outbreak of avian influenza, subtype H7N7, it was found for the first time that pigs also could get infected with this particular influenza strain. The results from the field surveillance already indicated that a high exposure to the virus was necessary to infect the pigs. Only pigs in mixed herds with infected poultry were at a significant risk for introduction of the avian influenza strain. No evidence for (ongoing) transmission between pigs was found in the field. No evidence was found that the virus was able to remain for long in the swine herds after the source of infection, the infected poultry, was removed.

The experimental infections confirmed the results from the field and no transmission at all was noticed during both experimental infections. This means that without further adaptation of the virus, and even without additional measures, the infection will always come to an end and the virus will disappear from the pig population.

Conclusion

Pigs were infected during the Dutch outbreak of avian influenza in 2003. Transmission between pigs was however very low to negligible. Therefore, without further adaptation of the virus, there was no chance that it could have become endemic in the pig population

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Influenza : a farm animal disease and a zoonosis

Posters

DETECTION AND SUBTYPING OF SWINE INFLUENZA VIRUS BY RT-PCR AND STANDARD METHODS

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Introduction

Influenza virus causes outbreaks of acute respiratory disease in man and animals; three virus types (A, B, C) can be distinguished on the basis of antigenic differences of nucleoprotein (NP) and matrix (M) proteins. Influenza A virus infects a wide range of avian and mammalian species. Type A viruses are further divided into subtypes, based on the antigenic nature of their surface glycoprotein haemoagglutinin (HA) and neuraminidase (NA). Ducks and other waterfowl birds are the principal natural hosts of influenza A virus (10). From this natural reservoir viruses are transmitted to other species; among these an important role in influenza epidemiology is held by swine (8). Swine influenza virus (SIV) causes serious economic consequences because of the increased time needed for infected animals to obtain slaughter weight. SIV is a zoonosis for which pigs may act as intermediate host and mixing vessel for genetic reassortment between human and avian viruses (8). The passage of influenza A virus from animal host to man may lead the emergence of new pandemic strains; the prompt detection and identification of such events are paramount in the surveillance of influenza viruses. In Europe three major influenza A subtypes (H1N1, H1N2, H3N2) actually circulate in swine population. Diagnosis of SIV includes application of various methods. Isolation by inoculation of fertilized chicken eggs with pathological samples is considered as "gold standard" method (7). Cell culture system showed to be a reliable substrate for influenza virus replication (3, 6). Viral isolation and identification by eggs or cell culture inoculation are however time consuming methods. To have more rapid results in virus influenza detection, rapid enzyme immunoassay tests (9) and RT-PCR assays could also be used (2). Antigenic characterization of influenza A virus isolates is traditionally performed by serological tests, Haemoagglutination Inhibition (HI) and Neuroaminidase Inhibition (NI) tests. Application of diagnostic techniques based on molecular studies recently has supported serological tests by development of two RT-PCR assays to detect and subtype swine influenza virus (SIV). The aim of this study is to compare standard virological methods, eggs and cell culture inoculation, with RT-PCR based on the matrix (M) gene and to compare two multiplex RT-PCR methods for subtyping SIV isolates with HI test.

Materials and methods

Samples. 441 pathological samples (lung, nasal and tracheal swabs) were collected between June 2001 and December 2002 in 177 outbreaks of respiratory diseases in intensive breeders in Northern Italy. Samples were examined for presence of SIV by virus isolation (VI) and RT-PCR, specimens belonging to the same outbreak were pooled (max 10) for RT-PCR detection of SIV.

Virus isolation. Virus isolation (VI) was carried out by inoculation of embryonated chicken eggs (EEI) and infection of two cell culture (4): NPTr (Newborn Pig Trachea) (3) a swine origin cell line, and MDCK (Madine Darby Canine Kidney)(6) which is the most common cell culture system used for propagation of influenza virus. Two serial passages were performed. Amnio-allantoic fluids and cell supernatants, both at first and at second passage, were submitted to HA (7) and to ELISA sandwich assay (5) to evaluate the presence of influenza virus antigens.

Detection of viral RNA. Isolation of viral RNA were performed using a High Pure Viral Nucleic Acid Kit (Roche Molecular Biochemicals) according to the manufacturer instructions. cDNA was synthesized from total RNA and an aliquot was used in nested PCR reaction to amplify a conserved region of matrix gene (2). PCR products of secondary amplifications were separated using 1.5% agarose gel and visualized by staining with ethidium bromide. Sample purification, RT-PCR reactions preparations and agarose gel analysis were performed in separated laboratories. Negative controls were processed and run with each assay.

Molecular typing. Multiplex-PCR reactions to amplify haemagglutinin (HA) and neuraminidase (NA) genes were performed as previously described (1) on 56 SIVs collected in 2001-2003 years.

Serology. HI tests were performed using chicken antisera as previously described (7).

Results and discussion

Results of detection of SIV in 441 pathological samples from 177 respiratory outbreaks by diagnostic methods used in this study are shown in Table 1. RT-PCR showed the highest positivity rate (23.1%). EEI gave a percentage of positivity of 14.1, lightly lower than MDCK cell line positivity rate (15.2%), while NPTr cells showed the lowest sensitivity (11.3%). MDCK cells gave the best results in viral detection, even if this result was increased overall by using two serial passages. Considering that 46 SIVs were isolated in 177 respiratory outbreaks, it is possible to evaluate the incidence of influenza virus infection in respiratory disease complex occurred in intensive swine breeders (25.9%). RT-PCR, compared with EEI was able to detect SIV further 18 samples, even if it failed to detect the virus in two EEI positive samples. MDCK cell line allowed us to detect SIV in 7 specimens EEI negative although failed in viral isolation in 5 EEI positive cases. Furthermore inoculation of NPTr cell culture allowed SIV isolation from samples resulted EEI negative. Positivity rates of one or more methods in different combinations, among a total of 46 SIV positive specimens are showed in Figure 1. From some samples it was possible to detect SIV just by only one method when all others failed. It would be interesting to evaluate if this remark could be related to a peculiar genetic character of the isolate.

Figure 1. Detection rates of SIV in infected samples by different techniques applied alone or in combination

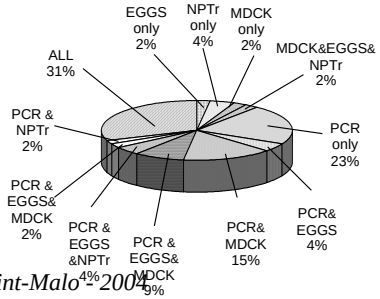

Table 1. Detection of SIV in 441 pathological samples from 177 respiratory outbreaks by various methods

	Samples examined	RT-PCR	Embryonated chicken eggs			MDCK			NPTr		
	177		I *	II	tot	I	II	tot	I	II	tot
Positive samples	46	41	20	5	25	15	12	27	13	7	20
positivity	25.9%	23.1%	14.1%			15.2%			11.3%		

*serial passage

Table 2. Results of RT-PCR subtyping of 56 SIV isolates compared to HI tests.

RT-PCR typing	N° samples	Concordant with HI	Discordant with HI	% of concordance	Sequence analysis
H1N2	25	24	1	96%	N1
H1N1	5	4	1	80%	N2
H3N2	22	22	0	100%	/
H3N2 and H1N1	4	4 (H3N2 only)	4 (H1N1 only)	/	/

Once more, adding the percentage of positivity by RT-PCR in the various combinations, it is demonstrated that this method shows the highest rate of positivity (90%). The concordance rate of the applied methods is 31%. Besides if it would be necessary to choose two combined methods for SIV detection, it is important to note that RT-PCR combined with MDCK cell line test or EEI allows to detect SIV in 94% of positive samples, while RT-PCR associated with NPTr cells inoculation reaches 96% of viral detection. The results pointed out that RT-PCR can be considered a useful tool for SIV detection in pathological samples because of its high sensitivity and short time performing. Furthermore this test could be applied to one or more pools of specimens collected in the same respiratory outbreak for a first screening before virus isolation.

At last 56 SIV isolates were RT-PCR subtyped and results were compared with HI tests (Table 2). RT-PCR typing was not in agreement with HI test in two cases: two field viruses, previously characterised as H1N2 and H1N1 by HI, were typed respectively as H1N1 and H1N2 by multiplex RT-PCR . The RT-PCR results were confirmed by sequencing. Due to the high variability of HA sequences, even if primers were designed on conserved regions obtained from multiple alignments, RT-PCR failed to amplify H1 gene in two samples. In four samples, naturally infected by two different subtype of SIV (H1N1 and H3N2), HI test detected only H3N2 subtype while RT-PCR was able to detect the presence of both subtypes. Multiplex RT-PCR showed to be useful to subtype swine influenza viruses. Results highlighted the specificity of this test to identify subtype isolates. Moreover molecular typing test resulted to be a rapid method: while for serological tests it is necessary to perform further passages to have a high HA titre viral stock, RT-PCR didn't require a high HA titre or a large amount of virus with the advantage to examine viral suspension at the first isolation. Comparison with serological tests and analysis of discordant data could be of concern for further molecular studies of particularly interesting viruses.

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HOW TO MANAGE AN OUTBREAK OF AVIAN FLU IN A REGION

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Introduction

Outbreaks of highly pathogenic Avian Influenza can be extremely difficult to control, even under favourable conditions . To be successful in controlling outbreaks of animal diseases, it is necessary to be prepared, to take immediate action and to limit the financial consequences. This means a coordinated approach and contingency planning.

Never the first time

Avian Influenza is not likely to disappear.* In waterfowl, faecal contamination of water supplies is considered to be a very efficient way of transmitting the virus.
Although no evidence to date has conclusively linked the current outbreaks to wild migratory birds; several outbreaks have been linked to contact between free-ranging flocks and wild birds, i.e. the shared use of water sources. Infected poultry present a major risk of further spreading of the virus, both to other susceptible poultry and to other animal species, including humans. So culling must be completed as safely, quickly and humanely as possible. Avian Influenza itself will not disappear, so we have to be prepared for its sudden appearance in domestic poultry. When this happens the only option is to fight Avian Influenza with all means, and to minimise the risk of avoidable spreading to other animals. If Avian Influenza is not dealt with correctly and efficiently, one of the worst consequences can be infection of human beings. Specialised knowledge, based upon recent experience, is needed to be successful in fighting Avian Influenza, and experts should be consulted whenever possible.

Bio-security

- *Contingency Planning*, based on standard international procedures and protocols for eradicating measures
- *Eradication procedures*, based upon local circumstances, different types of housing and animal related factors such as the type of animal, age etc.
- *Development of culling techniques and equipment* to carry out eradication procedures with respect to animal welfare
- *Training and management development* for personnel and staff responsible for contingency planning, eradication procedures and culling
- *World wide consultancy* on the above mentioned issues, in close cooperation with United Nations organisations, such as the WHO and FAO
- *Reducing financial risks* for farmers, farmers organisations and governments

Based upon the understanding of these recent outbreaks, the European Community should develop a contingency database with vital information about critical factors that are of major influence on the choice of the most appropriate culling technique under certain conditions. These techniques were already tested by independent researchers at Wageningen University in The Netherlands

Pilot light concept

These concepts contain ‘pre-outbreak planning’ that includes:

- Pre-consulting and auditing of farms
- Contingency planning in close cooperation with farmers, farmers organisations, local and regional governments and disease control organisations
- Building a local operation to carry out emergency culling within 24 hours of confirmation of an outbreak of an animal disease
- Creating a local service network to assist during an outbreak
- Setting up local training programmes for management and staff
- A yearly training course to test and update all practices

Avian Influenza Insurance

Poultry production contributes greatly to the economies and food supplies of affected countries. Outbreaks of animal disease can result in immense financial consequences. The direct costs of the latest outbreak in The Netherlands are estimated to be one billion Euro. The agricultural sector therefore faces the challenge of minimising losses.
We should develop a Culling Database based upon experience. This database contains all knowledge and expertise on culling, taking into account the local circumstances, the amount of poultry, the type and age of the animals, etc. To calculate the risks one carries out an audit at the premises of all participating farmers (at least 80% have to participate to be successful.) This results in the issue of a certificate of clearance, which will enable culling to take place within 24 hours in the event of an outbreak of a contagious animal disease, and an estimate of the costs involved.

Some recent outbreaks of H P avian flu

2002; Hong Kong (China), H5N1
2002; Chile, H7N3
2003; Netherlands, H7N7
2003; Belgium, H7N7
2003-2004; South East Asia Region H5N1

ANTIBODIES AGAINST HUMAN INFLUENZA VIRUSES IN PIG POPULATION IN THE CZECH REPUBLIC

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Introduction

In the mid-1990s, serological surveillance of pig herds revealed no antibodies against swine influenza virus. However, in 1996, several herds showed antibodies against human influenza virus A/Praha/625/95 (H3N2), a causal agent of the 1995 influenza epidemic (Pospisil et al., 2001). In the surveillance carried out in 2002 as part of the ESNIP project, we detected antibodies against swine influenza viruses with H1 haemagglutinin, and occasionally also against H3 virus and the new variant H1N2 in the sera of sows.

Material a Methods

Considering the findings from 1996, we tested these sera against human influenza viruses A/New Caledonia/99 (H1N1) and A/Plzen/2000 (H3N2) and compared the results with antibody levels against the swine influenza viruses
A/Sw/Gent/132/86 (H3N2),
A/Sw/England/117316/86 (H1N1),
A/Sw/Scotland/410440/94 (H1N2) and
A/Sw/Finistiere/82 (H1N1 avian-like) used in the ESNIP project. Haemagglutination inhibition tests were used (Final Report ESNIP, 2004). Sera with a titre of 1:40 and higher were regarded as positive. The specific pig antisera against viruses A/Sw/Gent/132/86, A/Sw/Scotland/410440/94 and A/Sw/Finistiere/82 obtained from the ESNIP project were used as positive controls. These sera reacted against homologous viruses at titre levels of 1:320 to 1:640. None reacted with any of the human viruses.

Results

Out of 666 sera examined, we detected antibodies against the human influenza virus A/Plzeŕŕŕ2000 (H3N2) in 47 samples at titre levels ranging from 1:40 to 1:320, and against the virus A/New Caledonia/99 (H1N1) in 7 samples at titre levels ranging from 1:40 to 1:80. In 14 samples with antibodies against A/Plzeŕŕŕŕ2000 (H3N2) we also found antibodies against A/Sw/England/117316/86 (H1N1). However, none of the sera that tested positive against virus A/New Caledonia/99 (H1N1) were also found positive against any of the swine influenza variants with haemagglutinin H1 or H3, table 1.

Table 1

Number Positive/ Total number (% positive)		
Viruses	Samples	Herds
A/Plzen/2000	47 / 666 (7,1%)	24 / 85 (28,2%)
A/Plzen/2000 and A/sw/Eng/86 or A/Sw/Fin./82	15 / 666 (2,3%)	6 / 85 (7,1%)
A/New Caledonia/99	7 / 666 (1,1%)	6 / 85 (7,1%)
A/New Caledonia/99 and Sw/Gent/84	0 / 666 (0%)	0 / 85 (0%)

Conclusion

Our results show that infection of pig herds with both human and swine influenza viruses is currently possible in the Czech Republic and that there is a potential of the development of new influenza variants.

Acknowledgements

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Special Plenary Session

Biosecurity and Hygiene in a situation of crisis

(with the support of SOGEVAL Laboratory)

A SCIENTIFIC APPROACH TO INCREASING BIOSECURITY AWARENESS IN SWINE PRODUCTION

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Introduction

Pork production in the United States has changed dramatically over the last 20 years. Today, the largest 1% of all U.S. pork producers represents approximately 60% of all annual production while the smallest 60% represent only 1% of annual production. Intensification of the U.S. swine industry has allowed most producers to raise hogs at a lower cost by placing greater emphasis on facility throughput to spread fixed costs. With the high pig density on most modern hog farms, proper biosecurity is essential to protect swine against the transmission of disease between or among herds.

Biosecurity decisions should be based on facts from practical scientific data rather than relying on speculation. Producers should always be searching for ways where they can minimize their risk of exposure to disease. Establishing a disease surveillance protocol allows for routine monitoring of herd health. An isolation procedure is essential for protecting the existing herd and should include monitoring the health status of incoming replacement stock. Further considerations should include ways to minimize risk of exposure to disease transmission by biological vectors, mechanical vectors, and area spread. Any living organism that can carry a pathogen and allow it to replicate is considered a biological vector. With a mechanical vector, the pathogen is only carried and does not replicate. Defining area spread is less clear. Often whenever a disease outbreak cannot be linked to definite cause, area spread from a neighboring herd is implicated. Area spread could include wind currents as well as unknown biological or mechanical vectors.

On-Farm Biosecurity

The use of boot baths containing disinfectant solution is common to many farms. Recent research has shown that improper use of boot baths are a waste of time and money.[1,2] Bacterial contamination is not reduced by stepping through a pan of disinfectant solution when boots are soiled with manure. Even standing in a pan of disinfectant for 2 minutes did not reduce bacterial numbers on the sole of the boot if they were covered with manure. If boot baths are to be used properly, all manure should be removed manually prior to contact with disinfectant. Manure and other organic material prevent decontamination by encasing and protecting pathogens from disinfectant exposure. Also, organic material inactivates the active ingredients contained in most disinfectants. The amount of contact time required to sanitize the boot varies with the disinfectant. Based on personal experience, a good estimate would be that 99% of all boot baths are used improperly. Based on this research, boot baths containing more manure slurry than disinfectant solution are obviously ineffective.

Nursery problems such as swollen joints and umbilical abscesses have lead some producers to consider increasing hygiene during tail docking and/or teeth clipping. A recent experiment examined the best method to reduce contamination on tail docking pliers.[3] Wiping pliers with a clean cloth was more effective than no treatment and dipping them in a disinfectant solution containing chlorhexidine diacetate for 3 seconds.

Transmission of Infections Agents

Porcine Reproductive and Respiratory Syndrome (PRRS) virus. PRRS virus has plagued the U.S. swine industry for more than 10 years. Most swine genetic companies and some commercial farms have a down time requirement for personnel prior to entering a production facility. The most common requirement is to avoid contact with pigs from other farms for a minimum of 3 nights before entering a production unit. Many trials have been published recently that suggest down time is not necessary to prevent transmission of PRRS. In one such trial,4 people who did not shower after contact with PRRS virus-infected pigs did not transmit the virus after contacting naïve pigs housed in another room. In another trial5, PRRS virus was transmitted from infected pigs to naïve pigs when caretakers did not shower or change boots and coveralls between rooms. In this study, caretakers that washed their hands and changed into clean boots and coveralls did not transmit PRRS. In both of these studies,4,5 down time was not required to prevent transmission of PRRS from infected pigs to naïve pigs.

PRRS virus has been shown to be inactivated quickly on certain fomites.[6] PRRS virus was recovered less than 30 minutes after contamination of stainless steel, plastic, boot rubber, wood shavings, alfalfa, straw, fecal slurry, swine saliva, and swine urine but not when sampled the following day. PRRS virus could not be recovered from denim material, ground corn, or pelleted starter feed contaminated 30 minutes prior to sampling. PRRS virus was recovered from well water daily up to 9 days following contamination and from city water daily for at least 11 days.

Needles, houseflies, and mosquitoes have been documented as potential mechanical vectors for transmitting PRRS virus.[7] Aerosol transmission of PRRS was deemed to be capable of traveling short distances in one review[8] but did not occur under the conditions of one trial.[7] In another study involving a coordinated sequence of events in cold weather, PRRS virus remained viable in 8/10 replicates after being placed into a snowball, compressed beneath a truck fender, subjected to a truck wash, stepped onto by a person's boot, thawed onto the floor, and transferred onto one of several containers by dragging them through the puddle of thawed snow.[9] Using the same protocol except with warm weather and a dirt ball instead of a snowball, PRRS virus remained viable in only 1/10 reps.[10] In these previous two studies,[9,10] some questions remain regarding the practicality of the model compared to actual events that would likely occur on the farm. Further, the use of a swine bioassay where pigs were injected with the PRRS virus-contaminated snow or mud is not representative of an event likely to occur on the farm. If pigs were to be exposed, it would likely be by touching the contaminated object rather than through injection.

Transmissible Gastroenteritis (TGE) virus. One study evaluated the ability of people to act as mechanical vectors to transmit TGE virus from infected pigs to naïve pigs housed in a separate room.[11] TGE virus was transmitted when the caretaker walked directly from infected to naïve pigs. However, TGE virus was not transmitted when the pig caretaker washed hands and changed into clean coveralls and boots. No down time was necessary to prevent transmission of TGE.

Mycoplasma hyopneumoniae. A field-based study concluded that showering and changing into clean boots and coveralls was sufficient to prevent transmission of M. hyopneumoniae from a naturally infected farrow-finish herd to a naïve finishing facility without down time16. Three veterinarians visited the infected farm every week for 20 consecutive weeks and contacted pigs for 3-4 hours per visit to collect nasal swabs and blood samples from nursery and finishing pigs. They wore disposable coveralls and rubber boots during contact with infected pigs but did not wear gloves, facemasks, or hairnets. After contact with the infected pigs, the three veterinarians showered, changed clothing, and drove approximately 60km to the naïve herd where they donned cloth coveralls and rubber boots but did not shower again. At the naïve site they collected nasal swabs and blood samples from four month-old pigs for approximately one hour. Pigs from the infected farm were seropositive to M. hyopneumoniae and positive by nested PCR. Pigs from the naïve were seronegative and negative by nested PCR at the beginning of the study and remained seronegative and negative by nested PCR 154 days later.

Pathogenic Escherichia coli. An evaluation of people as mechanical vectors for transmitting pathogenic E. coli from infected weaned pigs to naïve pigs showed that showering and donning clean outerwear was required to prevent transmission.12 Naïve pigs contacted by a person that did not employ any biosecurity procedures directly after exposure to the infected group developed diarrhea and were culture positive for the same pathogenic strain of E. coli. Pigs contacted after the caretaker washed hands and donned clean outerwear following exposure to the infected group showed less severe signs of diarrhea but were culture positive for the same pathogenic strain of E. coli. No down time was required to prevent transmission of pathogenic E. coli.

Foot and Mouth Disease (FMD) virus. A study[13] was recently conducted to investigate the transmission of FMD virus, one of the most contagious veterinary pathogens known. This study evaluated transmission of FMD virus (the same strain responsible for the 2001 UK outbreak) by people from infected pigs to naïve pigs and sheep. Showering and donning clean outerwear following exposure to FMD virus-infected pigs prevented transfer to naïve pigs and sheep. Further, under the conditions of this study, washing hands and donning clean outerwear following exposure to FMD virus-infected pigs was sufficient to prevent transmission to naïve pigs but not to naïve sheep. No down time was required to prevent transmission of FMD virus.

Other Modes of Disease Transmission

An exhaustive review of published literature outlines the possibility of aerosols, rodents, insects, birds, dogs, and cats to transmit swine pathogens.[8] It was concluded that *Actinobacillus pleuropneumoniae*, hog cholera virus, PRRS virus, and swine vesicular disease virus could likely travel by aerosol over relatively short distances. FMD virus, pseudorabies virus, and *Mycoplasma hyopneumoniae* can likely travel by aerosol over longer distances. In the same literature review, rodents were found to harbor *Bordetella bronchiseptica*, *E. coli*, *Leptospira*, rotavirus, *Salmonella* spp, *Toxoplasma gondii*, and *Brachyspira hyodysenteriae*. Neither pseudorabies virus nor PRRS virus were isolated from rodents on endemically infected farms. Further, it was concluded that under laboratory conditions, insects could transmit African swine fever virus, *Eperythrozoon suis*, hog cholera virus, pseudorabies virus, *Streptococcus suis*, swinepox virus, and TGE virus. Birds on infected farms were found to harbor *B. bronchiseptica* and *Mycobacterium avium*. Experimentally, birds have been shown to transmit hog cholera virus, PRRS virus, and TGE virus. Dogs in contact with infected pigs have been shown to harbor *B. hyodysenteriae* and *Brucella suis*. Cats have been well documented as the definitive host for *Toxoplasma gondii*.

Disinfectant Use

Cleaning and disinfection protocols have been well established as critical components of effective disease control in modern swine production systems. However, cleaning and disinfection efficacy is more commonly based on speculation than on scientific fact. Few research studies have been published that have evaluated disinfectants under typical farm conditions. One study attempted to assess on-farm cleanliness using tests capable of producing feedback within a few minutes[14]. Unfortunately, these tests lacked adequate sensitivity and specificity for on-farm use. For this reason, it is important to determine which disinfection principles can be backed up with scientific evidence and to identify knowledge gaps where additional research is necessary.

A study evaluating transport vehicle sanitation clearly demonstrated the importance of drying time for inactivating PRRS virus[15]. Four different treatment groups with 10 replicates each were used to evaluate cleaning efficacy in scale model trailers where PRRS-infected pigs were kept for 2 hours. Treatment one had bedding material consisting of wood chips removed manually with a scraper. No further cleaning was performed. Treatment two consisted of bedding removal, washing with hot pressurized water, and disinfection using a phenolic disinfectant 1:256 with 10 minutes contact time. Treatment three was cleaned as described in treatment two with the addition of a freeze and subsequent thaw. Treatment four consisted of bedding removal, washing, disinfection, and drying. PRRS virus was detected by PCR in all trailers prior to treatment. All trailers from treatment groups one, two, and three contained PRRS virus detected by PCR. PRRS virus was not detected by PCR from trailers in treatment four where they were thoroughly cleaned, disinfected, and dried.

The presence of residual organic matter along with differences in farm water properties are two major factors affecting disinfectant activity and will vary on every farm. Organic material such as manure, feed, and

secretions can encase and protect infectious organisms as well as inactivate many disinfectant ingredients. Additionally, farm water properties such as hardness and inorganic compounds can alter the activity of many disinfectants.

Two basic *in vitro* procedures exist for evaluating disinfectant efficacy. *In vitro* suspension testing involves the addition of disinfectant solution to a known number of organisms within a test tube. Disinfectants are considered effective if organisms are reduced by a pre-determined amount. Suspension tests simulate on-farm usage conditions very poorly. *In vitro* carrier testing involves the addition of disinfectant solution to a surface containing dried organisms. Disinfectants are considered effective if all organisms on the surface are inactivated. Carrier testing simulates on-farm usage better than suspension testing but some inadequacies remain. *In vitro* testing where organisms are in constant contact with disinfectant solution may not correlate well to on-farm usage where disinfectant dries rapidly on surfaces. Further, the use of horse serum or yeast solutions to simulate the effects of organic matter in many *in vitro* tests represents a very poor simulation of actual organic material present in swine facilities. Many disinfectant testing procedures use sterile, distilled water or synthetic hard water to mix disinfectant solutions, something completely impractical for use in production facilities. On-farm testing provides better information regarding disinfectants than other testing methods. Sampling surfaces in facilities before and after disinfection provide the best results of disinfectant efficacy. Unfortunately, this is very labor-intensive and not practical in many circumstances.

Manufacturers' disinfectant label claims do not necessarily correlate to efficacy under on-farm use conditions because *in vitro* testing cannot perfectly simulate the disinfection process in a swine facility. No disinfectant can be considered efficacious against all swine pathogens under all circumstances.

Conclusions

Modern pork production has become very sophisticated with constant evolution over the years. Large populations of animals located in a relatively small area make effective biosecurity protocols absolutely necessary to safeguard swine herds. A breach in biosecurity can have huge economic implications. Unfortunately, a concept that is so basic and simple can sometimes be overlooked: never underestimate the importance of being clean. When caretakers make a conscious effort to keep themselves clean, recent research reports make it seem quite apparent that evidence is lacking supporting the need for down time to prevent transfer of many swine diseases. Biosecurity considerations should be based on scientific fact rather than speculation. The science behind biosecurity is becoming clearer every day but many questions remained unanswered and further research is necessary.

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THE CHALLENGE OF FMD CONTROL IN THE 2001 UK FMD EPIDEMIC

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Introduction

The 2001 UK foot-and-mouth disease (FMD) epidemic resulted in 2030 cases of disease (2026 on mainland GB) being confirmed over a seven month period between March and Sepember 2001. A review of the course of the epidemic, the successes and failures and the results of post-epidemic analysis, are presented in an effort to better aid the understanding of the problems encountered, so as to guide and inform future control programmes.

The virus

Foot-and-mouth disease is a highly contagious disease of cloven-hoofed animals, in particular cattle, sheep, pigs, goats and domestic buffalo, as well as wild ruminants such as deer. It is characterized by fever and vesicles on the mouth, feet and udder of lactating animals. Pregnant animals may abort and young stock may die suddenly due to myocardial infarction. FMD is caused by strains of aphthovirus, in the family Picornaviradae, of which there are seven immunologically distinct serotypes, namely O, A, C, SAT1, SAT2, SAT3 and ASIA1. Animals that have recovered from infection with a strain of one serotype remain fully susceptible to infection with strains of the other six. Within each serotype there are a substantial number of strains showing a variable degree of antigenic diversity. The genome of the virus contains a single strand of positive-sense RNA, of approximately 8.2 Kb, and in common with other RNA viruses has a high mutation rate, which together with the apparent 'plasticity' of the major neutralising sites on its surface, explains a high antigenic variability.

The clinical severity of FMD varies with the strain of virus, as well as the infecting dose, the route of infection, the species and individual susceptibility of the host. It is clinically most apparent in high-yielding dairy cattle and intensively-reared pigs, in which the lesions can be severe and debilitating. In adult sheep and goats, FMD is frequently only a mild disease, with transitory clinical signs which can easily be missed by the stockman or veterinarian, or confused with other diseases presenting similar lesions (De la Rua *et al*, 2001; Watson, 2002; Ayers *et al*, 2001) but can cause a severe clinical picture in lambing flocks including high levels of neonatal mortality (Hancock and Prado, 1993). The virus replicates to a high titre in epithelial cells, particularly those undergoing repair, and consequently lesions may also be seen on the hocks or elbows of pigs being housed on concrete flooring where damage to legs is common.

The most common method of spread of FMD virus is by contact between an infected and a susceptible animal. An infected animal produces a large amount of virus in exhaled breath. Cattle and sheep are particularly susceptible to infection by the aerosol route, requiring as little as 10 Tissue Culture Infective Doses 50 ($TCID_{50}$) (Donaldson *et al*, 1987). Pigs are considerably less susceptible to aerosol infection, possibly requiring as much as 6000 $TCID_{50}$ (Alexandersen *et al*, 2002a). During the clinical phase all excretions and secretions contain huge quantities of virus, and infection can occur either across damaged epithelium or orally. Pigs produce up to 3000 times more aerosol virus per day during the acute stage of infection. Under appropriate weather conditions, infectious levels of aerosol virus can potentially spread a considerable distance, particularly if the source is a large infected pig herd (Donaldson *et al*., 1982). Prediction models have been developed which can predict the likely dispersion of infectious levels of aerosol virus if the number and species of animals infected and the weather conditions at the time of virus excretion are known. The animals most at risk are usually cattle since they are especially susceptible to infection by the aerosol route, and because they have a higher respiratory volume than sheep.

When an animal infected with FMD virus is slaughtered, all meat and organs contain FMD virus. The build-up of lactic acid post-mortem kills any virus in the meat by reducing the pH to below 6, however no reduction in pH occurs in the glands or bone marrow in which virus may survive for 120 days at $4^{o}C$ (Cottral, 1969). Milk from infected animals contains large quantities of live virus and semen from infected bulls and ova from infected cows may also be contaminated with live virus.

It is possible for virus to survive days or weeks in the environment if kept moist and at neutral pH. The hands, clothes or nasal passages of personnel handling infected animals may become contaminated with live FMD virus, and mechanically carry virus and infect susceptible animals by close contact. Vehicles can carry infected material between farms, although, for transmission of infection to occur, there is the necessity that the material makes direct contact with a susceptible animal. Milk tankers venting during filling operations can create an aerosol of virus contaminated milk droplets and spread disease.

Convalescent ruminants and those that have been vaccinated against FMD and subsequently exposed to live virus, may become carriers remaining infected for a variable period of time. Cattle may carry the virus for over three years, sheep for up to nine months and goats for up to four months in the epithelial cells of the pharynx (Zhang and Kitching, 2001; Kitching, 2002a), despite there being high levels of circulating neutralizing antibody. The mechanism by which the virus is protected from the host immune response is not understood (Salt, 1998). Nor is it known what risk these carrier animals represent in terms of causing new outbreaks of FMD. It has not been possible experimentally to show transmission from a bovine carrier animal to an in-contact susceptible animal. However there is circumstantial field evidence that carriers may initiate a new outbreak (Kitching, 2002a, Thomson, 1996).

The PanAsia O strain responsible for the 2001 GB epidemic was first identified in India during 1990. It spread northwards into Nepal in 1993 and westward into Saudi Arabia during 1994 and then throughout the Middle East, becoming essentially endemic and progressively replacing the other Type O strains in circulation. In 1996 it reached Bangladesh and Turkey

from where it spread into Greece and Bulgaria. The virus reached mainland China by 1999, as well as Taiwan, and in 2000 it was identified in South Korea, Mongolia, eastern Russia and Japan. In September 2000 it caused the first outbreak of FMD type O in the Republic of South Africa where the origin was attributed to the feeding to pigs of untreated shipping waste. Phylogenetic analyses showed an extremely close relationship between the UK and South African virus isolates, and between them and virus from the Far East, with the Japanese isolate being the closest.

National Control Policies

The control policy operated by a country depends very much on its individual disease status and geographical location. Countries free of the disease with well defined boundaries, such as the UK, have traditionally relied on stamping-out policies. These require a well developed State Veterinary Service for the early recognition of the disease complemented by swift slaughter and carcase disposal, efficient cleansing and disinfection procedures and effective movement controls. Such policies are backed up by strict import controls on animals and their products in an attempt to prevent the importation of virus, backed up by veterinary checks of imports and waste feed controls should virus be inadvertantly imported. Members of the OIE adopt veterinary certification and disease notification procedures so as to underpin international trade. Many countries have no national geographic barriers protecting their borders allowing free movement of nomadic herdsmen, wild animals and disease, thus countries in Africa, the Middle and Far East have no choice but to control FMD by mass vaccination. Between the two approaches of stamping out and mass annual vaccination many variations are practised, notably strategic vaccination in the face of an outbreak where barrier or ring vaccination is applied.

Overview of the 2001 FMD epidemic in GB

A total of 2,026 cases of FMD, caused by the PanAsia O strain of virus, were confirmed in Great Britain between 20 February and 30 September 2001. This marked the end of the country's longest period of freedom from FMD in recent history, the last epidemic on the mainland occurring in 1967-'68.

Although the first case to be confirmed in GB in 2001 was in pigs at an abattoir in Essex, in south east England, this was not the index case in the epidemic. The oldest disease found in any animals during the epidemic (and therefore the index case) was in pigs, on a waste-food feeding premises 400 km to the north, near Newcastle-upon-Tyne in Northumberland. There was unprocessed waste food on the premises to which the pigs had access, moreover cutlery was found in the troughs and pens with the pigs. Investigations in April 2001 discovered commercial quantities of illegally imported, air-dried, bone-in, pork legs from Asia, (DEFRA, 2002) on the premises of a wholesaler supplying local restaurants in the Newcastle area, from which the index farm collected waste.

It was estimated that clinical disease had been present on this farm since at least 12 February 2001 (Alexandersen *et al* 2002b, Alexandersen *et al* 2003, Gibbens *et al* 2001). Sufficient virus could have been released to form a viral plume from about this time and analysis of the meteorological conditions during early February showed they favoured spread of virus to farms up to 10km away, particularly in the period 12 to 13 February (Gloster *et al* 2003). Airborne dispersal of virus from the pig farm is considered to have been the most likely method of introduction of virus into sheep and cattle on a Ponteland farm 5 km distant. Exhaustive investigations into the source of infection for this farm found no evidence of disease on any farm with which there had been any contact from 1 January 2001 nor on any farms within a 3 km radius (DEFRA, 2002).

It seems likely that the sheep and cattle on the Ponteland farm were exposed to infection shortly before 19 sheep from the farm were sold for slaughter at Hexham livestock market, on 13 February. Nine of the 19 sheep went for slaughter (introducing disease to two premises) whilst the remaining 10 were bought (unfortunately) by a livestock dealer who mixed them with 174 other sheep. The 184 sheep remained in close contact for almost 48 hours at Hexham then nearby Longtown markets, before entering the national sheep marketing system (Mansley *et al* 2003). This is a sophisticated, interlinking network of livestock markets, dealers and hauliers capable of collecting, processing and rapidly transporting tens of thousands of animals daily. February is traditionally a busy sheep marketing time in Britain as ewe replacements are being bought, there is a demand for over-wintered hoggs for further fattening and there is a market for barren ewes and cast tups underpinned by the export trade. Longtown market is one of Europe's biggest sheep markets, selling animals originating predominantly from the north of England and southern Scotland, and attracting livestock dealers from throughout the British Isles who supply the UK and Europe. By definition, dealers buy and sell commodities, often on the same day, and livestock trading is little different. Groups of animals, particularly sheep, are bought, split up, resold (either privately or through markets, sometimes on the same day), transported long distances, mixed with more animals, resold, and so the cycle continues. Each dealer often has several premises between which stock and personnel regularly move. Some premises are used by more than one dealer and trading between dealers is frequent. The conditions of close contact between animals found in market pens and livestock transport vehicles are particularly favourable for virus transmission, both directly between susceptible animals and indirectly between animals and virus contaminated surfaces, as the potential for FMD virus to survive outside the host is well documented (Cottral, 1969). It is therefore a most efficient means of spreading infectious agents, especially one as contagious as foot and mouth disease virus, which in sheep may produce little clinical evidence of its presence whilst replicating and being released in large quantities into the environment.

Epidemiological investigations at the two markets concluded that the subsequent movement of the 184 sheep was responsible for the introduction of infection, before 20 February, to as many as 79 premises in GB, 20 of which were operated by large-scale dealers, in 10 of the 12 separate geographic epidemiological groups of IPs

that were identified during the epidemic (Mansley *et al*, 2003, Gibbens *et al*, 2001). Virus was further disseminated from these premises by the subsequent movement of animals, particularly sheep, and fomites, both locally and over longer distances. Once it became apparent that the disease was not confined to Essex, national animal movement controls were imposed on 23 February. It has been estimated that at this point in time animals on as many as 150 farms could have been exposed to infection. In reality Britain was faced with what amounted to multiple-seeded cases of FMD, scattered widely across the country, from which virus had already begun to spread, seven days before the first case was confirmed. The national animal movement controls were a draconian measure, the implementation of which so early in an outbreak was without precedent in the history of FMD control in Great Britain. It is without doubt that this single control measure played a pivotal role in minimising the potential for further distribution of disease and greatly reduced the scale of the epidemic.

The scale and temporal pattern of FMD cases in the first months of the 2001 epidemic was similar to that in 1967/68 (Gibbens, *et al*). Both reflected the practical problems of controlling epidemics characterised by initial multiple seeding followed by local spread. However the evidence suggests that in the 2001 epidemic, the index case was the source of infection for all other cases, whereas the 1967/68 epidemic had a multi-centric origin in which a number of pig farms were infected concurrently from the same source. The two epidemic curves differ only slightly in that the peak of the 1967/68 epidemic was greater and occurred slightly sooner after the first case than in 2001.

The national epidemic curve of confirmed FMD cases shows a steep rise over time until 27 March; this high level of 40 to 50 cases per day was maintained for about a week. Case numbers then fell, more steeply than they had risen, to reach a steady 5 to 10 cases per day for a month from 26 April. Of the approximately 1600 IPs that were confirmed in this time period almost half were in the county of Cumbria in the north-west of England. Epidemiological investigations concluded that over 100 farms, spread widely throughout the county, could have been infected before the first case had been confirmed in the area on 1 March. The national peak on 27 March was largely due to the effect of the Cumbrian cluster of cases, the peak being earlier in other areas; 22 March in Dumfries and Galloway (D&G) in Scotland and in Devon in south-west England. Using a conservative 5-day incubation period (Kitching, 2002b) it could be said that the spread of disease had been brought under control in Cumbria (and nationally) by 22 March and somewhat earlier elsewhere e.g. 17 March in D&G and Devon. The early intense part of the epidemic was virtually over by the end of April.

The 2001 epidemic however, was characterised by a prolonged 'tail' comprising almost 400 cases, confirmed over a 20 week period from May to September, appearing as a series of sporadic outbreaks in previously unaffected, widely separated, geographic areas of the country. The source of many of these defied identification although long distance fomite spread and inapparent infections of sheep were implicated. Local fomite spread was believed to have perpetuated the disease during the epidemic 'tail' as most of the outbreaks occurred in areas of farm fragmentation. At this time of year most farm animals had been turned out to grass and essential seasonal activities, such as silaging and sheep shearing, were in full swing, resulting in an increased frequency of movements by people and vehicles.

The disease was eventually controlled in the 'tail' following the introduction, in late July, of legislation to enhance biosecurity measures in cartographically delineated Restricted Infected Areas (RIAs). The special measures applied in the RIAs included:

- Proper cleansing and disinfection of all vehicles entering or leaving all farms
- Licensing of feed lorries and milk tankers, the latter to be accompanied by DEFRA staff
- Cleansing and disinfection of agricultural vehicles entering or leaving the RIA
- Continuous biosecurity patrols by the Police and DEFRA
- Slurry and forage movement only by license
- Licensing of sheep shearing and agricultural contractors' activities
- Structured sero-surveillance of all sheep flocks

The last case in the epidemic was confirmed on clinical grounds in sheep on 30 September 2001 in Cumbria; laboratory samples were negative for this and the three preceding cases.

Post-epidemic analysis revealed that in 86% of confirmed cases sheep were present on the premises and that 25% of IPs were laboratory negative. The high incidence of sheep on IPs may well reflect the underlying population, although the distribution of sheep on farms in Great Britain is not clear. It is not possible to give a clear picture of the relative risk of infection in sheep and cattle, as the speed of imposition of control measures often prevented complete examination of all stock on a premises. During the bulk of the epidemic, if disease was detected in cattle on a holding, it wasn't always possible to closely examine all the sheep in detail, or test them serologically, due to lack of resource and the overwhelming requirement for rapid slaughter. The evidence suggests that they have a similar risk of infection. During the early weeks infection was confirmed more frequently in sheep, reflecting its early dissemination, then the disease moved into the cattle herds which became the predominant species affected although disease continued to be identified in sheep. Once cattle became involved the amount of virus being released would have increased drastically; this seemed to be particularly the case once dairy herds were involved. Widespread serological testing of sheep during the 'tail' of the epidemic, and afterwards in the national sero-surveillance programme to demonstrate country freedom from disease, found little evidence to support the belief that cryptic infection in sheep was responsible for perpetuating the epidemic.

Animal movements, rather than fomite or airborne transmission, infected most of the major geographic clusters of cases before restrictions came into force. Thereafter the fomite-mediation appears to have been the predominant method of secondary transmission between

IPs during the epidemic. Infected Premises often had several potential sources of infection and there remains no doubt that proximity to an infected place is an important risk factor for becoming infected with FMD. However, spatio-temporal analyses of the epidemic in Cumbria (Taylor *et al* 2004) concluded that spread of infection beyond 1.5 km occurred in over 50% of cases, indicating that limiting disease control measures to contiguous premises (i.e. within 1.5 km) was unlikely to stop the epidemic. Similar conclusions were reached by Thrusfield *et al* (2004) in Dumfries and Galloway This emphasises the need to limit contact between farms and to ensure that adequate cleansing and disinfection procedures are implemented and maintained to achieve disease control, hence the apparent success of the RIAs.

The size and scale of the 2001 epidemic can be attributed to a variety of factors

- There was an initial delay in reporting suspicion of FMD on the index case
- 90% of the 540 pigs on the index farm were affected
- There had been windborne spread from the index farm to sheep on a nearby farm
- Inapparently infected sheep from this farm entered the livestock marketing system
- The movement coincided with a seasonal peak in sheep marketing
- The GB sheep dealing and marketing system is sophisticated and complex
- Sheep bear no individual identification and movements may be poorly recorded
- FMD is often difficult to detect clinically in sheep
- Sheep are susceptible to the PanAsia O strain
- Farm size and fragmentation have increased in recent years
- Stock numbers on each holding have increased
- There is a greater reliance on shared or contract labour and equipment
- The prevailing cold, damp climatic conditions favoured virus survival
- The State Veterinary Service had been progressively reduced in size
- The widespread dissemination of virus rapidly stretched resources beyond their limit

Control procedures adopted in GB in 2001

Stamping out:

- Rapid slaughter and disposal of all susceptible animals on IPs and on premises considered by veterinarians to be at risk of being exposed to infection ('Dangerous Contacts': DCs)
- National animal movement restrictions
- 3km Protection Zone and 10km Surveillance Zone farm restrictions
- Enhanced biosecurity
- Veterinary inspections of 'at-risk' livestock e.g. on contiguous farms
- Veterinary epidemiological investigations to identify potential sources and spread of infection.

Novel policies:

- Pre-emptive contiguous culling was practised around IPs from 26 March
- Culling of all small ruminants and pigs on premises within a 3km radius of IPs in Cumbria and D&G began on 23 March, the 3km cull
- Confirmation of disease on clinical signs, only, without recourse to laboratory confirmation, became a policy on 26 March, along with the new category, 'Slaughter on Suspicion' (SOS)
- Restricted Infected Areas were implemented from 27 July
- Post-epidemic sero-surveillance of small ruminants was carried out

An assessment of the control policies used in GB in 2001

Import Controls – failed, breached by smugglers

Waste Food Controls – failed, breached by indifference, greed, idleness

Early Notification – failed, breached by indifference, ignorance, idleness

National Animal Movement Ban – successful, instituted sooner than ever before

Stamping-out Policy – successful in controlling disease spread

– nationally 22 Feb to 22 March (30 days)
– locally, Cumbria, 1 March to 22 March (21 days), Dumfries and Galloway, 1 March to 17 March (16 days)
– failed to prevent the 'tail' occurring
– successful in helping to control the 'tail'

Novel Policies

3km cull

– untargeted, 6 weeks to complete, began after epidemic peaked (5 days after in D&G, 1 day after in Cumbria)
– sero-surveillance of 3km culled sheep, 2 of 115 flocks positive (1/32 low positive, 9/56 positive)

Contiguous cull – began after epidemic peak, (9 days after in D&G, 4 days after in Cumbria)

– not implemented in north Cumbria (700 IPs), 50% of premises survived with stock, epidemic curve identical to rest of GB

Compulsory clinical confirmation/SOS – to what benefit?, led loss of support of Farmers and vets (in extensive sheep populations FMD is usually self-limiting, virus output is low, could restrict and bleed)

Restricted Infected Areas – successful, helped control the 'tail'

Other Difficulties Faced During the 2001 Epidemic

In the first eight weeks of the epidemic there was a serious lack of resources. Most of the staff of the State Veterinary Service had had no experience of foot-and-mouth disease and had just completed long spells working away from their homes during the outbreak of classical swine fever. There was a general impatience with the apparent lack of success of the control measures in place, usually driven by Press elements more interested in sensational headlines, fuelling public disenchantment and misunderstanding. Some of the novel control procedures met serious opposition from farmers, veterinarians and the public. Vaccination was continually presented as the only solution. There was an inability to measure the progress of the Control Policies in 'real time'. As in most crises a wide range of instant "experts"

appeared (comprising veterinary scientists, biological scientists, mathematical scientists and non-scientists). The Canadian CVO observed in 1952, "I find it truly amazing, the number of foot-and-mouth disease experts (self proclaimed) who appeared almost overnight." (Childs 1952). Theoretical disease simulation models, prepared by bio-mathematicians, containing improbable assumptions (especially veterinary), were widely publicised and used for the first time in a major disease epidemic.

Real Time Data Analysis During An Epidemic

The key distributed epidemiological analyses should be calculated routinely, on a local basis, as daily counts, 3 or 5 day retrospective rolling averages and automated to allow the rapid review and assessment, as near to real time as possible, the pattern of disease spread, the effectiveness of control measures and the formulation of new strategies.

Data to be collected

- Species and number present on each IP
- Species and number clinically affected on each IP
- Species with oldest lesion on each IP
- Location of species on IP (housed, fields)
- Location of affected species on IP (housed, fields) and location of animal with oldest lesion
- Type of farm in PZ/SZ/IA (sheep, cattle, beef, dairy) provides data for calculation of attack rates
- Type of farm affected (sheep, cattle, beef, dairy) as a count and as a proportion of all farms of that type within the PZ/SZ/IA
- Laboratory results for each IP, positive or negative
- Numbers of other culls (DC) and numbers found to be infected

Routine analyses

- Epidemic curve
- EDR / Case ratio
- Average age of oldest lesion
- First lesion to slaughter
- First lesion to report
- Report to confirmation
- Report to slaughter
- DC:IP ratio
- Case finding (Report, patrol, tracing, DC cull)
- PZ area added by each new IP
- Rate of increase of area within protection zones
- Source of infection
- Cluster and sub-cluster analysis
- Nearest possible source (based on shedding and incubation windows)

Swift and accurate data gathering from IPs, its recording and collation allows real time analysis to be completed. The parameters selected are best calculated as retrospective 3 and 5 day rolling averages and should be completed for each spatio-temporal cluster of cases as this allows the distinct differences of the epidemiology and application of control measures between heterogeneous clusters to be assessed.

The Estimated Dissemination Rate (EDR), or a similar ratio of current cases compared to cases in a previous time period, are good indicators of the progress being made. Thus when this falls below 1 it can be said that the spread of infection has been controlled. Honhold and others 2004 (in press) concluded that the time from the estimated date of the first lesion to the date of slaughter (FLtoS) was a valid predictor of EDR. This is a simple calculation from data routinely collected on each IP by the veterinarians investigating the disease. FLtoS can itself be split into two periods, which provide information on the performance of the control programme. The time between first lesion and report of suspect disease to the authorities measures the speed with which disease is being detected. Time from report to end of slaughter measures the speed with which infected farms are being depopulated.

The area added by each new 3km Protection Zone is a useful indicator of the spatial spread of disease. The initial control procedures can have little effect against the first and second waves of infection, as these have already taken place and there whereabouts are unknown, and are instead intended to minimise subsequent spread from them. Once the increase in area added by new PZs indicates that the maximum spatial extent of infection has been discovered, and that newly reported cases are tending to occur within that area ('in-fill'), other control measures such as targeted veterinary surveillance visits or vaccination areas can be formulated.

Conclusion

The initial dissemination of FMD starkly illustrates the ability of the virus to be spread through the movement of infected animals showing little or no clinical signs. The virus entered the country at a time when the prevailing cold, damp climatic conditions favoured its survival away from the host, when the meteorological conditions favoured airborne dispersal from the index case and when sheep sales and movements were entering one of their seasonal peaks. However, the early imposition of movement restrictions, coupled with the rapid slaughter of infected animals and their contacts, and the implementation of strict biosecurity measures effectively contained and eventually halted the epidemic. The rapid collection, collation and analysis of field data is of paramount importance when trying to follow the course of the epidemic and judge the effects of the control measures being used.

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Veterinary Public Health

Contents

DO WE NEED MEAT? MEAT CONSUMPTION – WHERE DOES IT GO TO?

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Introduction

The aim of this paper is the attempt to answer the question "do we need meat?". This question can be discussed by nutritional scientific knowledge, statistical data analysing the meat demand and the probable meat consumption in different European countries. As the meat demand is not only a process influenced by prize and income level, but also by emotions, attitudes, motives and knowledge a new psycho-social model explaining the consumers` uncertainty will be introduced.

Nutrient components of meat and nutritional value of meat

Meat and meat products are a rich source of protein, minerals and B-group vitamins including niacin, thiamine (B1), riboflavin (B2) and vitamin B 12 (table 1).
They contain essential nutrients, which appear exclusively in meat (vitamin B12) and micronutrients for which meat is the major source because of either high concentration or better bioavailability (selenium, zinc).

Table 1: Nutrient components of meat

Nutrient components of meat				
Per 100 g food	**Chicken (breast with skin)**	**Beef** (muscles only)	**Pork** (muscles only)	**Sheep** (muscles only)
Energy in kJ	606,8	454,42	442,82	490,5
Energy in kcal	144,6	107,44	105	116,5
Protein in g	22,2	22,0	22,0	20,8
Total fat in g	6,2	1,9	1,86	3,7
Water in g	70,6	75,1	74,7	74,7
Cholesterol in mg	66	58,41	65,26	63,0
Iron in mg	1,1	2,2	1,09	1,6
Zinc in mg	*	4,29	2,00	2,9
Vitamin B 1 in mg	0,07	0,23	0,90	0,15
Vitamin B 2 in mg	0,09	0,260	0,230	0,370
Vitamin B 6 in mg	0,53	0,186	0,565	0,130
Vitamin B12 in µg	4,0	5, 0	2,04	2,7
Niacin in mg	10,5	7,5	5,0	6,2
Selenium µg	6,46	5,24	8,73	4,1

** no data*
Source: Souci SW, Fachmann W, Kraut H (1994): Food Composition and Nutrition Tables. Medfarm Scientific Publishing: Stuttgart

Meat is also a high quality protein food with a good balance of essential amino acids. In comparison, plant proteins have lower levels of at least one essential amino acid and so they are considered as lower quality protein. Plant proteins need to be combined to give a more appropriate balance of amino acids.
Modified breeding methods and well-balanced animal food caused a reduction of the fat content in meat during the last years. Beef contains 8,5% fat on average, lean pork cuts (filet, steak, loin) consist of not more than 5% fat and pork cuts with a median fat-content including 6-12% fat. The fat content of poultry without skin is only 0,7%, with skin it makes up 6,2%.

A certain amount of fat in meat is preferable as carrier of fat-soluble vitamins.
In the last years the conjugated linoleic acids (CLA) in meat are of special interest, as CLA have anti-carcinogenic, anti-oxidative and anti-sclerotic effects. CLA can be found basically in meat of ruminants, e.g. in beef 3,1-8,5 mg/g fat, also in milk and dairy products (CMA 2001).

The main type of iron in meat (haem iron) is more efficiently absorbed by the human body than the iron in plant foods (non-haem iron). As much as 15 to 35% of the iron in meat is absorbed - depending on iron stores- compared to 1 to 2% of iron in plant foods. The body will

absorb more haem iron if iron stores are low. The redder the meat is, the higher the iron content.
Iron is an essential mineral found in every cell. It has three key functions in the body: to carry oxygen, to provide chemical reactions and to ensure a healthy immune system. Iron deficiency is the most common nutrient deficiency in the world, affecting mainly older infants, young children and women of child-bearing age. The iron intake is still the weak point in the supply of women of child-bearing age. Their average iron intake is usually distinctly lower than the recommended quantity (DGE 2000).
Zinc is also a component of every living cell in the body. It is essential for the structure and function of over 50 metalloenzymes. It is important for growth and reproduction, night vision, digestion and appetite, sense of taste and smell, for maintaining the body's immunity and for the healing process. Zinc is not widely distributed in foods, so meat is an important source of this micronutrient.
B group vitamins regulate many chemical reactions necessary to maintain health.
Animal products are the only reliable source of the important vitamin B 12, which is required to make new cells and maintain nerve cells. Vegans may become deficient in this vitamin (Biesalski 2002).
It has been claimed for many decades that meat is a risk factor for cancer, especially because of its fat and cholesterol content. But epidemiological data does not confirm this claim. Evidence of the role of meat in human carcinogenesis is weak. It comes from different kind of studies, which can only hardly be compared and come to different answers. For the European context there is no significant evidence for the relation between meat intake and colorectal cancer. Some support for such a relation is available from American studies, but only at the high intake levels of more than 140 g per day. Present international studies analysing meat as risk factor for cancer have to be discussed again regarding evidence based data as shown in the following table 2.

Table 2: Red meat intake and cancer mortality

Country	Red meat intake (kg / person / annum)				Cancer mortality (deaths / 100.000 / annum)		
	Cow	Sheep/Goat	Pig	Total	Large bowel	Prostate	Breast
Austria	23,1	1,1	66,4	90,6	23,5	17,3	21,8
Belgium	21,3	2,0	53,2	76,5	19,2	18,3	25,8
Denmark	2,4	1,0	64,8	86,2	23,3	19,5	27,2
Finland	19,1	0,3	29,4	48,8	13,9	18,1	16,5
France	26,4	4,4	35,8	66,6	20,8	16,6	19,7
Germany	17,8	0,9	54,4	73,1	22,6	16,6	22,1
Greece	**20,3**	**14,4**	**21,2**	**55,9**	**9,5**	**8,8**	**15,5**
Ireland	17,5	9,8	32,7	60,0	24,8	18,4	26,5
Italy	**26,5**	**1,7**	**34,2**	**62,4**	**19,4**	**11,4**	**20,4**
Netherlands	18,6	1,3	58,1	78,0	20,1	18,7	26,8
Portugal	17,4	3,5	34,6	55,5	18,8	15,2	18,1
Spain	**13,2**	**6,3**	**53,2**	**72,7**	**16,7**	**13,5**	**17,4**
Sweden	17,3	0,6	33,4	51,3	15,2	21,1	17,4
UK	16,8	6,8	24,3	47,9	21,1	17,2	27,1

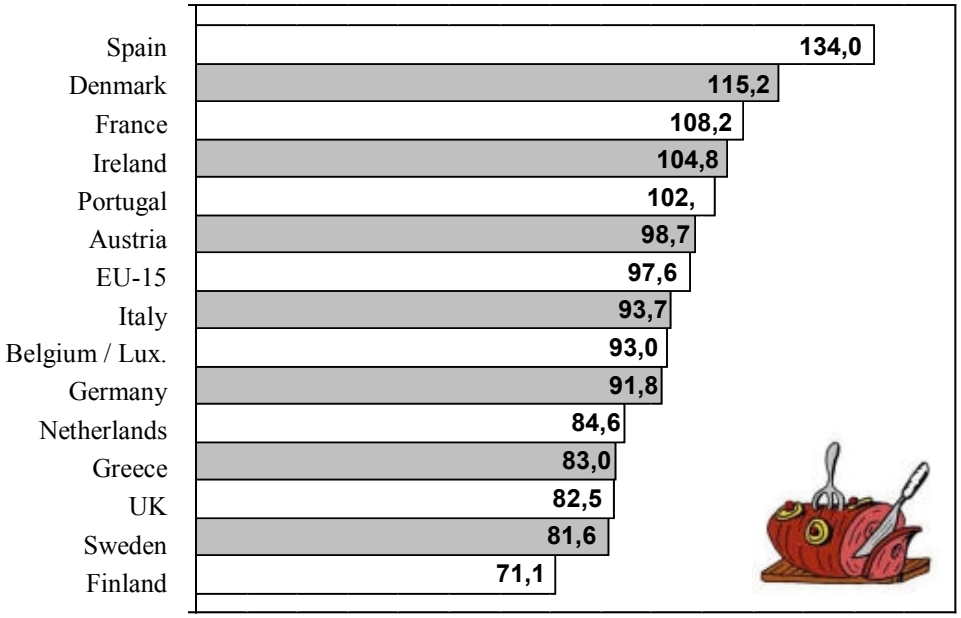
Source: Hill M (2002): Meat, cancer and dietary advice to the public. European Journal of Nutrition (2002) 56, Supll 1, S. S37

Meat consumption in the UK is less than that in any of the EU Mediterranean countries and yet the colorectal cancer risk is much higher. In Spain, Italy and Greece the total red meat intake is higher, however the cancer mortality lower (table 2).
A large Japanese prospective study even came to the conclusion, that meat is a major protection against gastric cancer. A possible explanation is that meat is only a risk factor for those, who do not eat sufficient amounts of other cancer-protective factors like fruit and vegetables (Hill 2002).
Comprising it can be said that a balanced diet rich in fruits and vegetables, including meat and meat products in moderate quantities (2-3 servings per week) and a normal body weight as well as a reasonable amount of exercise is a good advice for healthy living and this kind of diet provides the body with macro- and micronutrients it needs. As a nutrient-dense food meat is an important component of the human diet. It cannot be said that meat is per se carcinogenic, one has also to take into account its preparation, if it is fresh or preserved meat, the amount of meat eaten per day and the diet in a whole.

Meat consumption

As figure 1 shows Spain, Denmark, France, and Ireland have the highest meat consumption in the EU-15 countries with over 100 kg per capita per year.

Figure 1: Meat consumption 2003 (per capita intake per year in kg)



Source: ZMP; Agriculture data 2004, report center for market and price, Bonn, Germany, 2004
(ZMP Zentrale Markt- und Preisberichtstelle GmbH (Hrsg.) (2004): Agrarmärkte in Zahlen. Europäische Union 2004 und EU-Beitrittsländer), p. 19

As table 3 documents meat consumption shows an increasing tendency in all EU-15 countries except in the Netherlands and Portugal where the total meat consumption decreased according to the Food Balance Sheets. However in both of the countries mentioned there was no change in the consumption of sheep- and goats meat during 2001-2003. In Greece the overall meat consumption decreased which is mainly due to a reduction of pork consumption during 2001 and 2003. Nevertheless the poultry meat consumption increased in the same time. The Greeks show the highest per capita consumption in sheep- and goats meat. Comparing
France and Germany the pork consumption in Germany is higher than in France. On the other hand the beef and veal consumption in France is more than twice as much as in Germany. As the pork consumption in the United Kingdom stays far behind Germany and France whereas the consumption of poultry meat is higher. After the BSE crises the beef consumption in 2001 and 2003 was higher in the United Kingdom than in Germany. However the French consume more beef per capita and they are the second largest beef consumers in the EU-15 behind Denmark.
There is no general cluster in the meat consumption of the Mediterranean countries visible, compared to all the other EU-15 member states.
The heading “other meat“ includes exotic meat which is imported by EU-member-states e.g. bison imported from the USA, kangaroo from Australia, crocodiles respectively alligators from Australia, Florida and Israel, snakes from the USA or China and ostriches which come from South Africa, Israel, the USA, Australia or even Europe. Additionally frogs and horses are consumed however they don’t have to be imported as they can be produced in Europe (DGE 2004).

Table 3: Human consumption, meat, supply balance sheet (kg/ capita/ year)

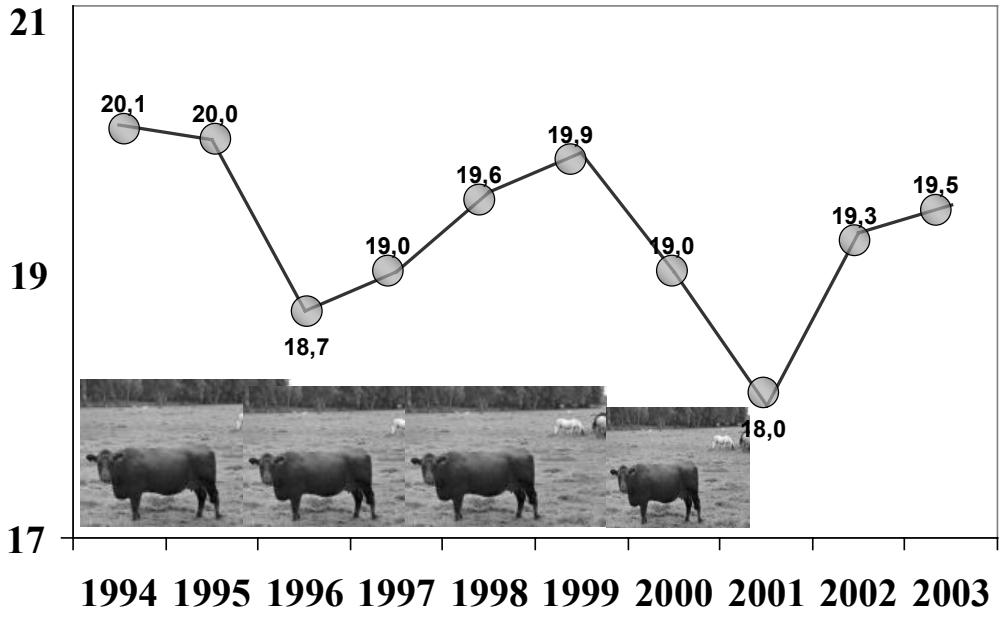
Country	Meat total (incl. Offals)		Beef and Veal		Pork		Sheep- and Goatsmeat		Poultry meat		Other meat
	2001	2003	2001	2003	2001	2003	2001	2003	2001	2003	2001
Belgium/ Lux.	94,2	(93,0)	20,0	(19,9)	45,9	(45,4)	1,8	(1,7)	17,7	(20,0)	3,6
Denmark	113,9	(115,2)	22,5	(28,5)	63,1	(62,2)	1,3	(1,1)	20,8	(21,0)	0,7
Germany	88,4	(91,8)	10,3	(12,4)	53,8	(55,9)	1,1	(1,0)	18,5	(17,9)	1,5
Greece	91,2	(83,0)	18,7	(17,3)	32,3	(28,6)	13,5	(12,8)	19,6	(20,5)	1,2
Spain	/*	(134,0)	/	(16,0)	/	(67,6)	/	(5,8)	/	(33,0)	/
France	107,2	(108,2)	25,2	(28,1)	36,7	(36,6)	4,2	(4,4)	26,1	(24,0)	5,6
Ireland	/	(104,8)	/	(20,2)	/	(38,4)	/	(5,3)	/	(31,7)	/
Italy	90,5	(93,7)	22,7	(25,2)	37,9	(38,9)	1,6	(1,5)	18,3	(18,0)	4,7
The Netherlands	86,9	(84,6)	19,4	(19,1)	42,6	(41,4)	1,4	(1,4)	22,2	(21,3)	0,2
Austria	97,6	(98,7)	18,3	(18,7)	56,4	(57,7)	1,2	(1,1)	18,3	(17,5)	0,8
Portugal	103,1	(102,3)	14,8	(16,5)	43,7	(43,2)	3,4	(3,4)	31,5	(30,0)	3,4
Finland	63,0	(71,1)	12,3	(17,9)	32	(32,3)	0,3	(0,2)	14,5	(15,5)	2,6
Sweden	73,3	(81,6)	20,6	(24,4)	34,7	(36,3)	1,0	(1,0)	13,5	(14,0)	2,6
United Kingdom	82,6	(82,5)	18,6	(17,2)	25,1	(25,0)	5,7	(6,0)	28,9	(29,5)	0,2
EU-15	/	(97,6)	/	(19,5)	/	(43,8)	/	(3,4)	/	(22,0)	/

Source: ZMP; Agriculture data 2004, report center for market and price, Bonn, Germany, 2004
(ZMP Zentrale Markt- und Preisberichtstelle GmbH (Hrsg.) (2004): Agrarmärkte in Zahlen. Europäische Union 2004 und EU-Beitrittsländer), pp. 19-42

During 1994 and 2003 the consumption of beef and veal decreased (figure 2). Due to food scares such as BSE and scrapie the lowest intakes were reached in 1996 and 2001. The meat demand recovered after the crises but never reached its level of 1994.

It is not obvious if this development is caused by hard facts like price and income or by qualitative aspects like behavioural determinants as consumer uncertainty.

Figure 2: Beef and Veal intake in the EU-15 (intake per capita in kg)



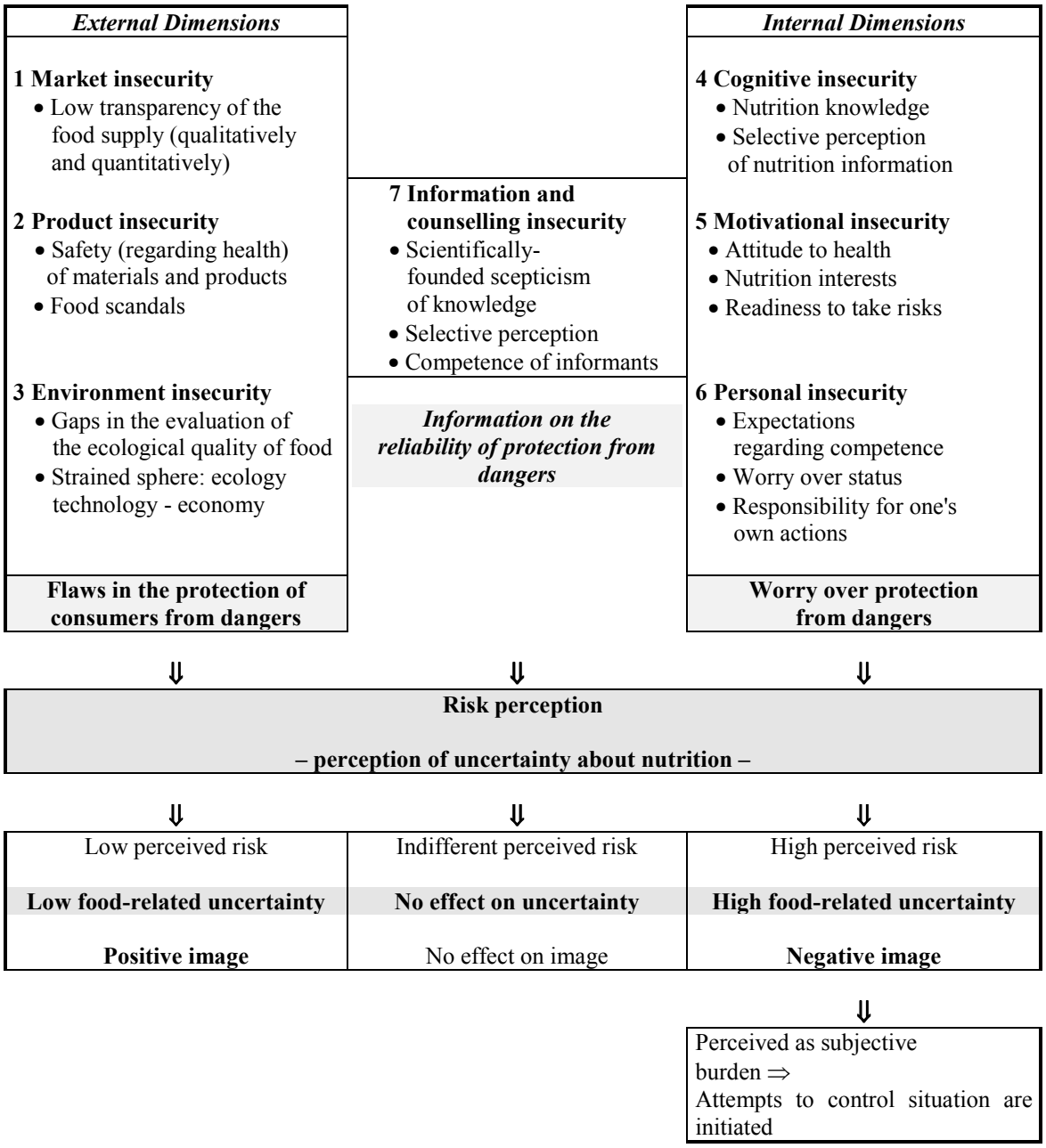
Source: ZMP; Agriculture data 2004, report center for market and price, Bonn, Germany, 2004
(ZMP Zentrale Markt- und Preisberichtstelle GmbH (Hrsg.) (2004): Agrarmärkte in Zahlen. Europäische Union 2004 und EU-Beitrittsländer), p. 23

Consumer uncertainty

Food-related consumer uncertainty is a major topic of public discussion in different European countries. This uncertainty appears more often in connection with new food products as well as with food processing, food technologies, and food scares. According to representatives of European consumer organisations and consumer protection food distributed on a global market provides a basis for consumer uncertainty too. Experts state that the underlying reasons are subjective (Chatard-Pannetier, Rousset et al. 2004) and, thus, do not meet the requirements of objective and scientifically-based criteria for influencing the state of health. As systematically conducted studies and data regarding this topic are

lacking, there is an urgent need to explore empirically the phenomenon of consumer uncertainty. On the basis of an empirical study focussed on consumer uncertainty by the example of convenience food the following model (figure 3) shall be introduced.

Figure 3: Model of food-related uncertainty



Source: Bergmann K (2002): Model of diet-related uncertainty. In: Dealing with Consumer Uncertainty. Public Relations in the Food Sector. Heidelberg: Springer Verlag, p. 40

It puts the dimensions and effects of food related uncertainty into proportions and provides a structural transparency of the phenomenon. Consumer uncertainty is caused by external and internal components. As hypotheses, these are reflected as deficiencies in the protection of consumers. It is modelled with seven sub-areas: Market insecurity, product insecurity, environment insecurity, cognitive insecurity, motivational insecurity, personal insecurity, and information and councelling insecurity (Bergmann, Dorandt, Leonhäuser 2004). The model describes the complexity of the consumers` behaviour as a research objective and it allows to analyse the meat related decision making process and its different determinants within their probable correlation.

Conclusion

Using statistical data the development and structure of meat consumption in different European countries has been illustrated. Nutritional scientists attest that meat is a good source of protein, readily available iron, calcium,

magnesium, selenium, zinc and a range of B vitamins. Since the evidence for any role in carcinogenesis is weak, the benefits of meat in the diet should not be ignored. The conclusion should be to encourage increased intake of fruit, vegetables and whole grain cereals. If the intake of those products is sufficient, "then there is no need to worry about meat intake" (Hill 2000, p. S40).

In order to be able to reduce consumer uncertainty in the future, a new comprehensive concept for communication between producers, retailers, and consumers is necessary. Food safety and information about the physiological value of meat as part of a balanced and varied diet as well as the traceability with regard to meat production can have a positive effect on the consumers` confidence.

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SAFETY AND FEEDING VALUE FOR FARM ANIMALS AND THE FOOD CHAIN OF GENETICALLY ENHANCED PLANTS.

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In a controversial world with the quick adoption of genetically modified GM crops covering nowadays 67.7 million hectares, the question for the scientists responsible for health and consumer protection is not to be in favour or against the products issued from new technology. Users and consumers must be objectively informed on the safety of the genetic constructs, of expressed proteins and finally of new feeds/foods. New recombinant genes as rDNA representing 1 10^{-4} % of the total nucleus DNA mainly code for functional proteins conferring tolerance to herbicides or insect resistance or both traits for a few. From the consumer point of view, the safety, concerned by the presence of the rDNA, the new proteins and eventually of other substances not intended and synthesised in the plant. Scientific authorities or agencies only authorised their dissemination on the basis of results on risk assessment derived from appropriate tests. Examples on the compositional analysis, substantial and nutritional equivalence, long term safety tests are summarised and discussed in the present review. Results on the quality and the safety of animal products, milk and meat issued from animals fed GM plants have been also interpreted .

1. Typical genes, expressed proteins and substantial equivalence

New proteins such as Bt CryIA(b,c), Pat and Cp4epsps are generally expressed in low amounts (0.1 to 10 µg/g fresh weight in leaves) representing 0.00005 to 0.0005 % of total proteins (agbios.com 2003). Acute toxicity tests in mice revealed a high safety factor for expressed proteins which are easily degradable in vitro at low pH in simulated gastric or intestinal fluid. Similarly, because of the presence of low levels of rDNA in genetically modified plants and its massive destruction into small nucleotides during fermentative processes, functional rDNA (> 2000bp) is low or absent from the silage and from the small intestine of ruminants. No significant modification of the chemical composition of plants in macro and micro-nutrients and toxicants have been observed between GM plants and their near isogenic parents. This is valid for the protein and amino acid content, the carbohydrate or fibrous content, the fat and fatty acid in their leaves, the whole plants, beans and kernels. It has been almost always concluded that new GM plants are substantially equivalent to their near isogenic parents. However, the concept of substantial equivalence identifying known nutrients and toxicants is neither a safety assessment *per se* but that characteristics and composition of the novel food as equivalent to the conventional food with a history of safe consumption.

The resistance of maize to the damage of the corn borer, prevents the plant in the development of mould contamination before and after harvesting. A typical unexpected and unintentional bonus in favour of the insect protected maize leads in its substantially lower level in several mycotoxins.

2. Safety and nutritional equivalence of GM plants and feeds.

Results of long term experiments corresponding to toxicological studies systematically run on high producing animals such as the dairy cow for forages and silage , the fattening steer, the growing chicken and the growing-fattening pig for maize kernels, oilseeds and oilmeals are available in the referred literature. None of the comparative performance of the dairy cows including average daily dry matter intake, fat corrected milk and milk composition are modified by the use of Bt or herbicide resistant maize silage. GM cotton seeds bearing various rDNA and fed as raw seeds at the level of 2.3 kg/ cow/day did not affect either milk production or milk composition. Moreover, nitrogen and rumen metabolism have not been modified in cows fed raw soybean resistant to glyphosate. No physiological and hormonal disturbance have been associated with feeding GM beets to dairy cows. The content of milk in total protein, but also the proportion of casein, non protein nitrogen, α-lactalbumin and β-lactoglobulin are not modified, leading to the absence of effect on the physicochemical characteristics of the curd and of the cheese made with milk issued from cows fed GM maize.

Additional digestibility trials in adult rams confirmed the nutritional equivalence of GM maize forage and sugar or fodder beets to their near isogenic parental plants. Similarly, across 8 long term studies lasting from 101 to 234 days and undertaken on fattening steers fed up to 96 % of their dietary dry matter intake with GM maize silage or grain, no deleterious effect on performance, health, frequency of liver abscess have been found in hundreds of experimental animals. As a consequence, the nutritional equivalence, carcass performance, dressing percentage, the 12^{th} rib fat thickness and ribeye area have not been modified by the ingestion of insect and glyphosate resistant maize. The chemical composition of the *Longissimus dorsi* muscle in steers as in pigs fed glyphosate tolerant maize or soybean, respectively are also not modified by feeding GM grains.

3. Safety of animal food.

Animal food produced by animals fed GM plants has been suspected to be of lower quality, on the basis of the transient presence of foreign plant DNA fragments (140bp) in organs (liver, spleen, ovary) and tissues (muscle, blood). The first observation in mice species has been confirmed in farm animals without an explanation on their physiological significance. Opposed to that, all samples of milk, muscle and eggs (yolk and albumen) of animals fed Bt maize silage, Bt cotton or soybean resistant to glyphosate were negative of the presence of transgenic DNA for either traits or fragment thereof and the protein encoded in the GM plants.

Because GM plants have only been grown recently (www.isaaa.org), few toxicological data are available on the long term effect on reproductive performance of

animals. However, recent data demonstrated the absence of effect of feeding Bt maize grain to the quail over 4 generations.

4. Conclusion and the near future.

Nowadays, maize (26% of requests sent to the EFSA panel), oilseed rape (21%), sugar beets (16%), potatoes and even wheat are concerned by genetic modification. Major modifications in the proportion and in the balance of nutrients could be particularly concerned (18 % of the new requests) as abiotic stress yield (13%) or resistance to pathogens which are still in the laboratory phase. Further experimental tests and experimental approaches concerning the safety and even more importantly the nutritional value of the new plants will be required. The safety and the nutritional value for animals must be tested and the results published. The quality and the safety of animal food must also be considered for health and consumer protection. Methods for testing and guidance documents on the information need for the risk assessment of GM plants and derived food and feeds are now fully available.

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INTENSIVE ANIMAL PRODUCTION – WHAT ABOUT ANIMAL HEALTH, ANIMAL WELFARE AND FOOD SAFETY?

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Introduction

For the last decades, there has been an ever since accelerating change in agriculture from an extensive, low-efficiency, diversified family farming structure towards an intensive, high-efficiency, specialised industry-like farming structure that supplies raw materials for the rapidly consolidating processing and distributing sectors of the food production chain.
According to WINDHORST (Proc. 18th IPVS Congress, 2004), this "industrialisation" of agricultural production resulted in:

- Sectoral concentration, i.e. a high share of a farm or a company in the production volume of one commodity,
- Regional concentration, i.e. a high share of a comparatively small region (i.e. one or a few counties) in the production volume of one commodity,
- Mass production of standardised commodities,
- Highly specialised farms, i.e. only one or a few commodities are produced,
- Capital intensive production and the use of the most recent technology,
- Vertical integration, i.e. the organisation of production or supply chains in one agribusiness company,
- Hierarchical management structures and decentralisation of management decisions.

All these characteristics of modern agriculture, especially of modern animal production, are the diametrical contrast to the nostalgic imaginations of the urban consumer about what agriculture should be: …the idyllic family farm with "happy" free roaming chickens, pigs and cows taken care of all day long from dawn to dusk by a happy farmer…
Thus, two strong standpoints are clashing:
1)international organisations (FAO, WHO, WTO), economists, animal scientists, and veterinarians are claiming that intensification and industrialisation of animal production is necessary to feed the world's growing population (WHO: food security is "safe, high-quality and affordable food for everybody"), and
2)urban well-to-do consumers, animal rightists, and food safety activists claim that intensification and industrialisation of animal production are the cause of systematic cruelty to farm animals and of an increase of food safety scandals such as BSE, Salmonella, dioxin, E. coli O57:H7 etc.

This paper tries to objectively evaluate the impact of the ongoing intensification and industrialisation of animal production on animal health, animal welfare and food safety of food of animal origin.

Perception and Reality

The Perception of Today's Food Production

In the last century, especially after World War II, a major goal of agriculture was to increase its productivity and efficiency for a low-cost (mostly subsidised) food supply as one of the preconditions for a growing affluence. As for animal production, this resulted in bigger herds and flocks and in an increasing specialisation - even within one animal species production such as specialised dairy cow operations for milk production and specialised calf rearing for veal production, or sow operations for producing weaner pigs and finisher operations for producing slaughter pigs etc. The focus then was on further developing the husbandry technology to maximise production. Animal health and animal well being were only taken into consideration, when the animals' performance was compromised (e.g. through so called "technology-derived" diseases).
This focusing on technology for maximising animal performance led indeed to an underestimation of the animals' needs. The result of this development is that many consumers and animal rights activists think that intensifying animal production is unavoidably coupled with a higher frequency of disease and with cruelty to animals. This perception was intensified when the recent, i.e. non-classical food safety scares such as BSE, Salmonella and E. coli infections, dioxin and other chemical residues as well as anti-microbial resistance emerged.
Both together, the animal health and welfare issues on the one hand and the "new" food safety issues on the other have led to the generally shared public perception that intensive (= "animal mass production") is ethically wrong and needs to be corrected to a more "natural" (for the animals' sake) and a more "organic" (for the consumers' sake) way of production.

As a result of this general feeling in the affluent societies, there has been a movement towards supporting alternative ("organic" or "biologic") production procedures, mostly in Europe and in North America. Today, about 2% to 5% of the agricultural production in the developed countries is "organic" or "biologic", but in contrast to earlier expectations of the supporters of this way of production, the percentage of "organic" products that are asked for by consumers does not grow any more (there is even a slight decrease of the market share of organic food), obviously due to the significantly higher prices. The perception of organic food is that it is "healthier", "animal friendlier" and its production is "more sustainable".

The following summarises the general perception of animal husbandry for food production:

- Intensive animal production leads to less healthy animals (permanent disease leads to an excessive use of antimicrobials), to breaches in animal welfare (animals cannot meet their species-specific demands) and to an increase of food safety risks (food is more and more adulterated with residues and pathogens).
- Organic farming leads to healthier and "happier" animals and produces safer food.

The Reality of Animal Health

In the small herd and flock family farming structure of the past, disease transmission from farm to farm was a permanent threat to the animals' health, and ecto- and endo-parasites were quasi unavoidable. The constant animal trading, mostly through animal markets and/or animal dealers led to a constant exchange of viruses, bacteria and parasites. The prevailing diseases were the highly contagious epidemic (notifiable) mono-pathogen diseases.

In the large herd and flock intensive farming structure, the disease transmission between farms is, due to the possibility to apply biosecurity measures, of minor importance. Ecto-and endo-parasites are, as well as the mono-pathogen epidemic diseases well under control (as long as the basic biosecurity rules are complied with). The prevailing diseases in large herds and flocks are the endemic, multi-pathogen and multi-factorial diseases that "take advantage" of the multiple animal passages that opportunistic pathogens need to produce ongoing disease in confined animal populations.

Thus, there is not more disease in the intensive animal production structure, but a different disease pattern than in the diversified family farming structure. However, it must be realised that the possibility to control and even to eradicate the diseases of the large populations are much better in the specialised and standardised production systems of today, since biosecurity and strategic disease prevention measures can be applied much easier in well-structured and well-managed production systems than in a non-structured, small scale and diversified farming system.

As for the "organic" and "natural" (out-door and free-roaming) production systems with no or minimal drug use: the animals seem to live more "species-specific", but to raise the animals without disease or even without certain pathogens is much more difficult than in intensive animal production systems with confined animals, and pathogens and parasites that are eradicated or under control in confinement animals husbandry systems become prevailing again under the "natural" conditions.

In essence, non of the two production systems has an "automatic" health advantage for the animals – both systems need targeted, appropriate animal health management measures that are tailored to the specific health risks of each system.

The Reality of Animal Welfare

As already said: it is obvious that the technology of modern animal husbandry systems needs to be corrected in terms of: instead of adapting the animals to any new, performance-enhancing technology, the technology must be adapted to the animals. This means the technology must provide an environment for the animals without pain, stress and anxiety. This environment must enable the animals to meet their demands to cope with their living conditions and to be able to express their species-specific behavioural needs with a reasonable possibility to move and groom themselves.

Providing the animals with the most natural environment is not necessarily meeting their demands, since it is too often forgotten that the breeds of today are not any longer the wild forms of their species. Many of our high-performance breeds need heating and ventilation for coping with the changing temperatures and climatic conditions of "natural" environments, that their "wild" ancestors were adapted to.

Out-door facilities e.g. for chickens provide free movement for the animals, but in many cases the mortality is significantly higher than in the same breed in confinement (predators, drastic temperature changes and other climatic influences).

Keeping sows in crates without any possibility to move and to turn within the crates is clearly compromising the animals' possibilities to express their natural need of movement. However, keeping sows after weaning only in groups without the possibility to hide from aggressive, dominant sows in the group, the non-dominant animals will suffer from being permanently attacked and even injured.

In essence, non of the two production systems has an "automatic" animal welfare advantage – both systems need targeted, appropriate equipment and management skills that are tailored to prevent the potential animal welfare breaches of each system.

The Reality of Food Safety

It is generally recognised by scientists and public health authorities that our food supply has never been as safe as today. But there is no argument about the fact that the food safety needs and can still be improved, but the perception of many a consumer that today's food safety is worsening, is simply incorrect.

However, it is important to analyse why a "new generation" of food safety incidents (BSE, Salmonella, E. coli O157:H7, chemical residues and the increase of antimicrobial resistance) has emerged and why the public perception of the food safety is the opposite of what the epidemiological numbers of food related disease cases indicate.

There is not only one reason for this striking paradox, but several:

1)today's diagnostic tools are much more sensitive than only some years ago, which means that even traces of food contaminants are detected, often way under the concentration that is able to do any harm to consumers,

2)the media make any food safety incident - even the cases where no real harm is involved - a scandal, and

3)the consolidation of the food processing industry vs. the multi-source farm supply means nowadays often a multiplication of the consequences compared to the small scale processing of the past decades.

All three reasons together have led to the wrong perception that our food safety system is increasingly failing.

Looking for reasons for the emergence of the recent food safety incidents, it is striking that a common feature of the "new generation" food safety incidents is that, although of various underlying causes (prions, bacteria, viruses, chemical residues etc.), they all have their origin in the so-called pre-harvest stage of the food production chain, which means that they come into existence prior the production stages, where the traditional food safety tools (e.g. meat inspection at slaughter) are applied.

As for the organic production procedures, there is no doubt that the risk of residues (drugs, chemicals etc.) is, of course lower than with production procedures that use drugs and chemicals. However, the risk of zoonotic pathogens contaminating the organic food products is definitively higher than in drug and vaccination controlled animal production systems.

In essence, non of the two production systems has an "automatic" food safety advantage – both systems need targeted, appropriate management skills and quality assurance programs that are tailored to the potential food safety breaches of each system.

The Codex Alimentarius has taken these developments into consideration and is strongly recommending to include the following principles into the current food safety systems regardless of the production system:
a) adding process optimisation (and auditing) to end product inspection,
b) enforcing the responsibility for food safety of anybody who produces food at any stage of the production chain,
c) founding all decisions on science-based risk assessment rather than on "gut feelings"
d) including the pre-harvest production stages into the food safety continuum, and
e) establishing the cascade: self-controls, neutral controls (audits) and state control of the control.
The European Union has turned these principles into a European legislation: the so-called "basis regulation for a new approach to food safety", the (EG) No. 178/2002.

The Future of the Food Supply System

Provided the affluence of the developed countries can be kept at the current level, and the threshold countries and more and more developing countries increase their living standard the following development of the global food supply system can be predicted:
1) a "mainstream" production = a low-cost food production system (mainly vertically integrated supply chains) to supply the growing urban centres with standardised, affordable, quality-defined (mostly branded), and safe food that is increasingly produced in optimised, quality assured supply chains and processed to so-called convenience food products ("ready to cook" or "ready to eat" products),
2) a "niche" production = higher-cost food production "pockets" to supply local and regional costumers asking for products with so-called subjective quality characteristics such as "organic" and "natural" production criteria that serve animal welfare, environmental protection and sustainability demands of certain consumer fractions,

The current dilemma of the "mainstream" food production is that it lacks the trust of the consumer despite the standardised and controlled production procedures at all stages of production. The reason for this lack of trust is the lack of transparency.
The current dilemma of the organic "niche" food production is that, although consumers trust the organic and natural products almost "blindly", it lacks the standardisation that is needed to produce repeatedly reliably safe food.

The way to cope with the lack of consumers' trust AND with the lack of standardisation is the establishment of specified quality management procedures (write quality handbooks and document your compliance with your own rules) and quality assurance procedures (apply quality management and have it neutrally audited and certified).

What about Intensive Animal Production?

In summary of the above, in contrast to its reputation, intensive animal production can be organised in a way that allows efficiently producing affordable and wholesome food from healthy animals AND complying with the societal demands for animal welfare and the highest standards of food safety.
It is not the production system that determines the compliance with the animal health, animal welfare and food safety standards, but how the used technology is adapted to the animals' needs and how the production system is managed and controlled (audited).

Antibiotics / Use and resistance

Oral Communications

PRINCIPLES FOR MONITORING OF ANTIMICROBIAL RESISTANCE AMONG FOOD ANIMALS

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INTRODUCTION

The introduction of antimicrobial agents in human medicine and animal husbandry has been one of the most significant achievements of the 20th century. The first antimicrobial agents were introduced in the 1930's, but shortly after resistance began to emerge and it has now become clear that antimicrobial resistance poses a threat to human and animal health that should be taken seriously. Modern food animal production depends on the use of large amounts of antibiotics for disease control, which provides favourable conditions for selection, spread and persistence of antimicrobial-resistant bacteria capable of causing infections in animals and humans. Food animals and food of animal origin is traded worldwide. Thus, the occurrence of antimicrobial resistance in one country is today a problem for all countries, which emphasise the need for global initiatives and establishment of monitoring systems for determining resistance in all countries.

MONITORING OF ANTIMICROBIAL RESISTANCE

Points	Options to consider
Purpose	General trends, Emerging resistance
Reservoir of interest	Herd, Slaughterhouse, Country/region
Bacterial species	Indicator, Zoonotic, Pathogens
Sampling strategy	faeces, skin, herd, slaughterhouse, retail
Laboratories	Centralised, decentralised
Isolation procedure	Enrichment, CFU, Single bacteria
Susceptibility testing	MIC, Disk, Genes
Recording	Inhibition zones or MIC, Categories
Data handling & availability	Paper reports, Internet Availability of bacteria and results

Monitoring of antimicrobial resistance is a requirement to assess the magnitude of the problem. When establishing a monitoring programme several factors have to be taken into consideration including decision on bacterial species to be included, sampling strategies, isolation procedures, susceptibility testing methods, and data recording, computing and reporting. It is easiest to collect data on resistance in pathogenic bacteria received at laboratories for susceptibility testing. However, results obtained from such isolates will be greatly biased because requisition varies among veterinarians, some infections are more likely to generate isolates and isolates from some infections are more likely to be tested for susceptibility. Furthermore, isolates are in several cases collected after initial treatment and will often include several isolates from the same herds. Reporting of data from these types of collections has to be done with great caution and will be necessary to consider the value of each individual sample. A more optimal method is the collection of isolates from randomly selected animals, based on epidemiological considerations and aimed at covering the target population. Data can be collected from different regional laboratories and compiled centrally. However, differences in susceptibility testing methods might include testing biases. Careful standardisation and inter-calibration between laboratories can make such programmes worthwhile. Data recording, computing and reporting are essential for an appropriate monitoring system. Thus, the database has to include information on the sample population, origin down to specific patient or herd, time of isolation, testing procedure. Furthermore, to enable later studies on the emergence of resistance it is advisable to store the isolates centrally. Thoughtfully designed, longitudinal monitoring networks can provide invaluable insight into where resistance is emerging or increasing and which species pose the main problems for animal and human health. In addition, combing results from several national networks worldwide can provide information on how and where new resistant clones and genes are emerging and spreading.

Purpose of a monitoring system. A monitoring system should always be designed according to its purpose. The data collected during monitoring should always be used otherwise it is just a collection of data for no use or purpose. The purpose of monitoring can be to guide empheric treatment strategies for animals and humans, to study trends in resistance and associations between usage of antimicrobial agents and resistance, to detect emerging resistance, to detect outbreaks with resistant clones, to guide policy and to study the effects of interventions and to provide data for the education of the public, farmers, veterinarians, etc. Since most monitoring systems are based on collected and identified isolates the sensitivity of most systems in detecting emerging resistance is in general low.

Choice of bacteria to include. Most systems are based on: animal pathogens, zoonotic bacteria and indicator bacteria. Animal pathogens are included because it is important to observe trends in pathogenic organisms. Indicator bacteria are included because they can be isolated from healthy individual animals and thus, give a more true value of the occurrence of resistance in the entire animal population than pathogenic isolates. Most programmes use *Escherichia coli* to represent Gram-negative bacteria and *Enterococcus faecalis/faecium* to represent Gram-positive. Zoonotic bacteria are included because they develop resistance in the animal reservoir

and transfer to and cause infections in man. The most commonly included zoonotic bacteria are *Salmonella*, *Campylobacter coli, Campylobacter jejuni* and *Yersinia enterocolitica*.

Sampling strategies. Great care should be applied to decide on the sampling sites and strategies. Thus, a decision should be made whether the samples should represent individual herds or entire regions or countries and the number of samples taken adjusted to ensure an appropriate number of isolates to enable epidemiological valid comparisons between reservoirs or over time. If samples are taken to represent entire regions, care should be taken to ensure that the monitoring is not based on several isolates originating from a few animals or farms, but are taken randomly among the sample population. Isolates originating from the same farm might be one single clone, which can greatly bias the outcome of the monitoring.

Isolation of the bacteria. Different methodologies for isolation of the bacteria of interest are available. One possibility is to isolate the bacterium from e.g. a faecal sample and then test it for resistance to a panel of antimicrobial agents. This will provide data on several antimicrobial agents at the same time and a randomly well-characterised selected isolate that can be stored for later research purposes. This method has, however, a relatively low sensitivity. Another option is to perform selective enrichment where a larger quantity of e.g. faeces is placed in an enrichment medium supplemented with the antimicrobial agents of interest. The advantage of this method is that it will enable a more sensitive detection of emerging resistance. It will, however, only provide information on a single antimicrobial agent at a time.

Susceptibility testing methods and antimicrobial agents to include. Definition of a bacterial isolate as resistant or susceptible ultimately depends on clinical success or failure of treatment. However, to guide therapy different pheno- and genotypic i*n vitro* methods are used, which both require standardisation and quality control. Antimicrobial agents to be included in a monitoring programme should both fulfil the need for important information and also selected to ensure the highest possible sensitivity in detecting the presence of resistance mechanisms.

Data handling and reporting. Most diagnostic laboratories only report susceptibility to a given antimicrobial agents as susceptible, intermediate resistant or resistant. However, for the purpose of a monitoring programme it would be more optimal if data could be reported as inhibition zones or MIC. This would not only make it possible to compare data over time if breakpoints are changed, but also make it possible to look at the population distributions of the data from different laboratories and if necessary introduce the same or different clinical breakpoints or epidemiological cut-off values. The data obtained from a monitoring programme should always be reported as rapidly as possible to as wide an audience as feasible. Today most monitoring data are published once annually. However, the use of the Internet should make it possible to publish data more rapidly on a web-page in the future.

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP)

The Danish monitoring for antimicrobial resistance was established in 1995 and is based on the examination of representative bacterial isolates from both healthy and diseased animals. Pathogenic, zoonotic and indicator isolates are sampled. All major food animal sources are covered and the programme is coordinated with a similar sampling of data from bacteria causing infections in humans. In addition, information on the usage of antimicrobial agents is collected. The objectives of DANMAP are to:

- Monitor the usage of antimicrobial agents for food animals and humans
- Monitor the occurrence of resistance in bacteria isolated from food animals, food of animal origin and humans
- Study associations between usage and resistance and identify their trends
- Identify routes of transmission and areas for further research studies

All isolates included in the Danish monitoring system are routinely stored, making it possible to study the occurrence of resistance among historical isolates at a later time and enabling studies into the mechanism of resistance among the different resistant bacteria.

Example of data obtained through the DANMAP programme. Since the ban of avoparcin in 1995 the occurrence of vancomycin resistance has decreased significantly among enterococcal isolates from broilers, whereas no significant change occurred in pigs. It was shown that all VRE isolated from pigs in Denmark belonged to the same clone and that the genes encoding resistance to macrolides (*ermB*) and glycopeptides (*vanA*) were located on the same mobile DNA-element. The consumption of tylosin for growth promotion decreased substantially during 1998. During 1999 and 2000 a significant decrease in the occurrence of VRE among *E. faecium* isolates from pigs have been observed. These findings suggest that the persistence of VRE among the pig population was caused by the continued use of macrolides, mainly tylosin, for growth promotion and therapy. Similarly the occurrence of resistance to macrolides has closely followed the consumption of tylosin for growth promotion and therapy.

Pan-European (ARBAO-II)

The EU is funding ARBAO-II for the period 2003-2005. ARBAO-II is a concerted action involving 19 laboratories in 18 European countries. This concerted action has created a network of national veterinary reference laboratories in Europe and established a surveillance system for monitoring the occurrence and emergence of antibiotic resistance among bacteria from food animals. An external quality control for the capability of laboratories to perform susceptibility testing of bacteria correctly is performed. Different bacterial strains with known susceptibility patterns are sent four times each

year to the different laboratories for testing and the results entered into a central database. Each year the data generated in the individual laboratories are collected centrally and a report on the occurrence of antibiotic resistance among the different bacterial species isolated from food animals generated and published. The data from 2002 are available at: http://www.dfvf.dk/

DISCUSSION

Today national monitoring programmes have been implemented in a number of countries worldwide. Most of these programmes focus on pathogenic bacteria or salmonella, but some of them also report data on resistance in indicator bacteria isolated from healthy animals. However, none of the programmes aim specific at detecting emerging resistance using selective enrichment. In addition, the different programmes differ in their methodology used and antimicrobial agents tested fore. The different programmes are not coordinated and no exchange of data takes place. Furthermore, there is currently no central evaluation of the data.

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MONITORING OF ANTIMICROBIAL RESISTANCE IN FOOD PRODUCTION ANIMALS IN EUROPE. HOW TO BUILD A COHERENT SYSTEM

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Introduction

The development of antimicrobial resistance among bacteria from food animals is considered a major public health problem in the European Union. The Zoonoses Directive (2003/99/EC), adopted on 29 September 2003, requires that EU Member States implement a monitoring programme that provides comparable data on the occurrence of antimicrobial resistance in zoonotic agents and other public health threatening agents. A proposal for a Monitoring Programme attempting to specify Article 7 of the Zoonoses Directive is drafted by the Community Reference Laboratory for the Epidemiology of Zoonoses (CRL-E). This draft will be discussed at the annual meeting of the National Salmonella Reference Laboratories in Berlin at the Federal Institute for Risk Assessment (BfR) on 21 and 22 October 2004. Subsequently a final proposal will be forwarded to the Commission to draft a decision on this issue.

There are several factors in monitoring programmes that affect the quality and comparability of the data. Next to sampling strategies, microbial test methodologies used are important factors . Isolation and identification methods used can be standardised, for susceptibility tests this is more complicated because historically, different guidelines are used in different European countries. The optimum goal is to harmonise the quality of the susceptibility data by controlling this data with ATCC quality control strains. Community and National Reference Laboratories (CRLs and NRLs) with adequate expertise in antimicrobial resistance will have to be designated. Coordination of surveillance activities will be done by the CRLs by building an active network of all NRLs. External and Internal Quality Assurance Systems for susceptibility tests need to be developed and stimulated by this network.

In Europe the existing active network of national reference laboratories that monitor antimicrobial resistance is based on two EU-projects. The first project was ARBAO (FAIR5- PL97-3654), which report was published in 2001 (1). The second project, a EU-wide External Quality Assurance Programme (EQAS) has started in 2003 (FP5-EU-project ARBAO-II, QLK2-CT-2002-01146). Both projects are the basis for the coherent system to be built. ARBAO-I was a concerted action focussed at describing well-founded recommendations for EU-wide monitoring of resistance. For standardisation of susceptibility tests three option were given: *i.* no standardisation and as a result the data could only be used as a main alert statistic; *ii.* intercalibration of the results by organising external quality assurance systems; *iii.* a standard method to be used, which was considered to be a future perspective. Based on the recommendations of ARBAO, ARBAO-II started an External Quality Assurance System (EQAS) to harmonise the quality of susceptibility test results in Europe.

Method

In 2003 eighteen veterinary reference laboratories participated. Moreover, 13 regional veterinary laboratories in 4 countries participated on equal terms as the veterinary reference laboratories and these numbers are expanding in 2004. Four laboratories (DFVF Denmark AFSSA France, VLA United Kingdom and CIDC Netherlands) act as reference laboratories. These laboratories select reference panels of isolates of *Salmonella*/*E. coli, Pasteurella* spp../*Actinobacillus pleuropneumoniae, Campylobacter* spp., and streptococci/staphylococci/enterococci, respectively. MICs of relevant antimicrobials are determined with NCCLS methods and always confirmed by at least one other laboratory. Four times a year, panels of strains are distributed to all participants for susceptibility testing. The participants use their own routine methods for susceptibility testing and download their results electronically on a web page. They are directly informed of the numbers of deviations compared to the reference results. Deviations are classified as minor, major or very major. Minor deviations are defined as an intermediate result that was determined as sensitive, resistant or vice versa (i.e. I ↔ S or I ↔ R). When a susceptible strain is classified as resistant it is regarded as a major deviation (S ↔ R). When a resistant strain is classified susceptible it is regarded as a very major deviation (R ↔ S). Annually, a two-days meeting is organised by the coordinating laboratory (DFVF). At this meeting, all results are presented and discussed.

Results

A report of the 2003 EQAS results was published in spring 2004 (2). In Figure 1 the results of the EQAS of 2003 for *Salmonella, E. coli,* streptococci and staphylococci demonstrate that depending on the bacterial species involved a variety of deviations from the expected results is obtained in the participating laboratories. It also demonstrates that for certain more fastidious bacterial species like streptococci, the numbers of deviations are substantially higher than for the *Enterobacteriaceae* and staphylococci. These results facilitate the introduction of threshold levels for the accuracy of laboratories that supply summary resistance data to a central database (3).

Conclusion

Since approximately 1997 the core laboratories of ARBAO-II form an active network of veterinary reference laboratories that work on antimicrobial resistance. The focus of the cooperation in this network has constantly been aimed at stimulating, improving and implementing resistance monitoring in food animals in the EU. As a result of Directive 2003/99/EC CRLs will be designated for antimicrobial resistance monitoring. Because of the complexity of antimicrobial resistance in the different bacterial species listed in the Directive, the

ARBAO-II group strongly advises to use the expertise in this network as the motor for the European monitoring.
The CRLs to be appointed need adequate expertise on antimicrobial resistance to be able to conduct this task properly and to control and stimulate the implementation of the final monitoring programme after adoption by the Commission.

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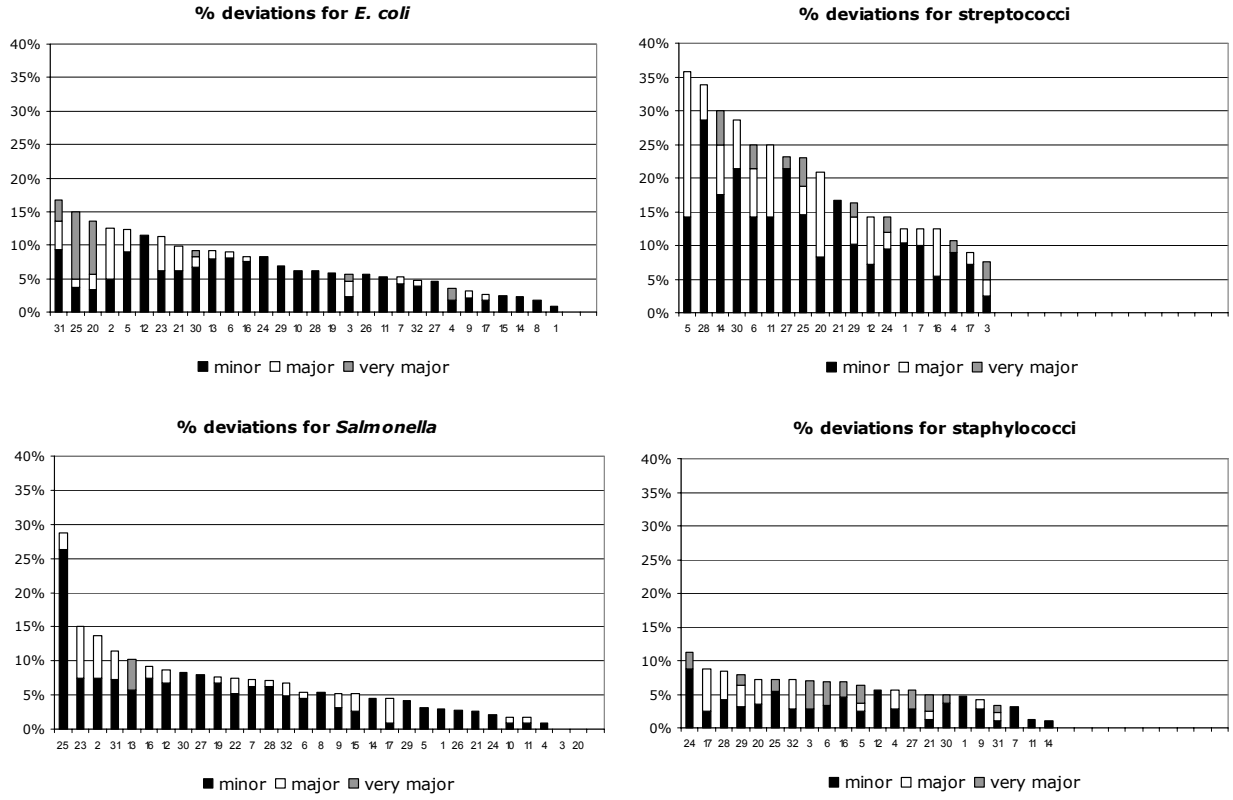
Figure 1. The percentage of minor, major and very major deviations from the expected susceptibility test results for E. coli, Salmonella, streptococci and staphylococci for each laboratory participating in the External Quality Assurance System of the ARBAO-II project in 2003, ranked in descending order.



OPTIMISING USE OF ANTIMICROBIALS FOR ANIMALS – SWEDISH EXPERIENCES

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The controversy concerning possible effects of the use of antimicrobials for use in food producing animals on the selection of resistant bacteria goes back to the 50s. In 1969, a Brittish committee recommended restrictions in the use of antimicrobial growth promoters (AGPs) in order to mitigate the risk of resistance (Swann_Committee 1969). Today, it is clear that emergence of antibiotic resistance in bacteria threatens our possibilities to successfully treat infectious diseases in humans and animals. Most experts and policy makers agree that to contain the problem, unnecessary use of antimicrobials must be curtailed (eg. SSC, 1999; WHO, 1997, FAO/OIE/WHO 2003).

In Sweden, the intensive debate from the 60s never ceased. In the beginning of the 80s, a broad discussion on use of antimicrobials and practices in animal production prompted the Swedish Farmers Union (LRF) to adopt a policy on use of antimicrobials, and to write to the Government and ask for a ban on growth promoting antimicrobials. In 1986, the use of antimicrobials for animals was restricted to veterinary purposes. This means that the use of antimicrobials for growth promotion was banned. All antimicrobials for animals are now classified as veterinary medicines and are only available on veterinary prescription.

The changes in 1986 had little or no impact on dairy, beef, calves, sheep or layers as these production sectors never or hardly used AGPs. More affected were the chicken and swine industry. Antimicrobials given at growth promoting doses prevent certain intestinal diseases. The ban was swiftly implemented, and some health disturbances had to be tackled. Other preventive strategies had to be found.

Broiler production

Before 1986, almost all chicken feed contained both AGPs and a coccidiostat. The chicken producers identified the occurrence of clinical or subclinical necrotic enteritis as the main problem to tackle subsequent to the ban. It was agreed that a transition period would be necessary and that the veterinarians would prescribe virginiamycin as prophylaxis during this period. Field experience and more formal research confirmed the construction and climate of stables, hygiene, management and feed composition all contributed to the disease. Further, it was found that coccidiostats of the ionophore type also prevent necrotic enteritis (Elwinger *et al.* 1992).

Already in 1988, all prophylactic medications were abandoned. Strong emphasis was placed on improving animal environment, measures that could be foreseen to prevent other diseases as well. A special bonus was given for good animal management and care, which also led to improvements in the total level of quality of the production. The most important changes related to feed involved a reduction of protein content, a higher fibre content and supplementation with enzymes. Ionophores are used as coccidiostats for all conventionally reared chickens. Today, outbreaks of necrotic enteritis are rarely seen. Prescription data show that during 1996-2000, less than 0.05% of the chickens were medicated for this disease.

Few other health problems are observed in Swedish broilers. A high level of biosecurity is applied to maintain a salmonella free status, and this also helps to control other infectious diseases. Through bioscreening measures, the production has remained free from mycoplasmosis and most other infectious diseases. Consequently, the overall use of veterinary antimicrobials in broiler production is very low (SVARM 2000)

Pig production

Before 1986, practically all piglets were given antibacterial feed additives (olaquindox or carbadox), from weaning until delivery to the finishing units at the age of 10-12 weeks. Slaughter pigs were, to a lesser extent, given antibacterial feed additives (avoparcin or virginiamycin) until slaughter.

The most notable problems that were observed arose in weaner pigs. The Swedish Veterinary Association adopted a policy for prescription of medicated feed with particular emphasis on weaners. According to this policy, prescription of antimicrobials for mixing into feed or water should be coupled with a number of other measures such as a thorough herd investigation targeting etiology and predisposing factors, and written recommendations on changes in management, feed, hygiene *etc*. For a number of years, olaquindox was prescribed to problem herds. In the beginning of the 90s, this use was gradually replaced by zinc oxide. Zinc oxide is presently licensed for sale as a pharmaceutical subject to veterinary prescription and its use has declined by more than 90% since the mid 90s. Numerous measures have been, and are continuously, undertaken to optimise rearing and production systems and to employ available techniques (e.g. sectioning of buildings, age segregation, planned production). In the late 90s, a strategy for education of farmers and veterinarians, managed by the Swedish Animal Health Services, has been agreed and successfully implemented.

The ban also stimulated a development towards new rearing systems. Today, most of the pigs are reared in age-segregated systems aiming to minimise the spread of infectious diseases. The major indications for use of antimicrobials in pig production are enteric and respiratory problems. The former are mainly weaning diarrhoea and swine dysentery. Strategies for eradication of swine dysentery are applied in some problem herds. In 2002, the use of antimicrobials for medication of pigs via feed or water had decreased by 66% since 1988 (two years after the ban) and by 30% since the mid 90s (SVARM 2002).

Use of antimicrobials – the overall figures

In Sweden, statistics on sales of antimicrobials for animals have been available since 1980 (for a review see SVARM 2000).

Before 1986, the average total usage for animals per year was 45 metric tons. Between 1988 and 1994, the sales were stably around 30 tons. From 1995, a steady decline in total sales has been recorded. In 2003, the figure was 16 tons, representing a decrease since 1980-84 by 64%. Today, few antimicrobials for administration to groups of animals via food or water are available. The proportion of the total sales of antimicrobials of drugs suitable for in-feed or water medication has declined steadily over the 90s and was in 2004 only 8% of that in 1980 (SVARM 2003), while use for mediation of individual animals has remained relatively stable over two decades.

Prevalence of resistance among animal bacteria is low

Data from the Swedish Veterinary Antibiotic Resistance Monitoring programme (SVARM), indicate lower prevalence of resistance to AGPs, but also to most therapeuticals, among bacteria from Swedish animals compared to materials collected and tested in similar ways in other European countries (SVARM 2003). Multiresistant *Salmonella* strains are rarely reported from food producing animals and the prevalence resistance to, for example, quinolones among *Campylobacter jejuni* from broilers is very low. The latter is reflected in a very low prevalence of resistance among *Campylobacter jejuni* isolated from humans infected domestically, in contrast to much higher prevalence in isolates from infections acquired abroad. Also, the occurrence of resistance to therapeutic antimicrobials among animal pathogens is lower than in many other countries.

Conclusions

To conclude, the ban of growth promoting antimicrobials in Sweden 1986 was initially associated with some health problems in piglets, and to a lesser extent in chickens. Most problems have today been solved. The Swedish experience shows that changes in production systems towards health-orientated systems are necessary in order to adjust to animal production without AGPs. Infectious diseases are controlled though biosecurity and control programmes, and efforts are continuously made to optimise management and feeding. Antimicrobials are used when needed, but crucial in the long-term strategy is to minimise the occurrence of diseases, thereby reducing the need for antimicrobials. Monitoring of resistance and use of antimicrobials are other important tools, providing guidance on the need for policy changes and other interventions. Taken together, the overall strategy appears to be effective in containing resistance to antimicrobials, and is also beneficial for animal health and animal welfare.

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ANTIMICROBIAL RESISTANCE IN PATHOGENIC *ESCHERICHIA COLI* FROM SWINE RESAPATH NETWORK – RESULTS 2002

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Introduction

The emergence of antimicrobial resistance was observed for the first time in 1947 with *Staphylococcus* and penicillin. Nowadays, antimicrobial resistances exist for the main pathogenic bacteria that allows infections to progress in many people [2, 9, 10]. In veterinary medicine, the same phenomenon is observed with three risks associated: failures of animals treatments, selection of antimicrobial resistant zoonotic bacteria and creation of a reservoir of resistance genes [11, 13].

Because of the rapid development and spread of antimicrobial resistance, a lot of network exist in the world to follow this evolution in human and veterinary medicine [1, 8, 9, 10, 12, 14].

In France, the resistance monitoring of bovine pathogens, which has been existing since twenty years, was extended to poultry and pig production in 2001 to give a single network: RESAPATH. It is managed by the French Agency for Food Safety (AFSSA) in Lyon and Ploufragan [5, 7].

Materials and Methods

RESAPATH is a multicentric network. Antimicrobial susceptibility data are collected from voluntary public and private veterinary diagnostic laboratories.

The *in vitro* antimicrobial susceptibility test used by all laboratories involved in this network is the disk diffusion method [3, 6]. Antibiotic disks (6 mm diameter) are placed on Mueller-Hinton agar previously inoculated with bacterial suspension. Results of this method are inhibition zone diameters obtained after an incubation at 37°C for 18 to 24 hours. The size of inhibition zones is inversely correlated with the minimum inhibitory concentration (MIC) for a particular bacterium / antimicrobial combination.

Inhibition zone diameters are registered by RESAPATH then interpreted in susceptible, intermediate or resistant category. For antimicrobials used both in human and veterinary medicine, this classification is established with CA-SFM breakpoints [4]. For antimicrobials only used in animals, breakpoints are given by pharmaceutical laboratories.

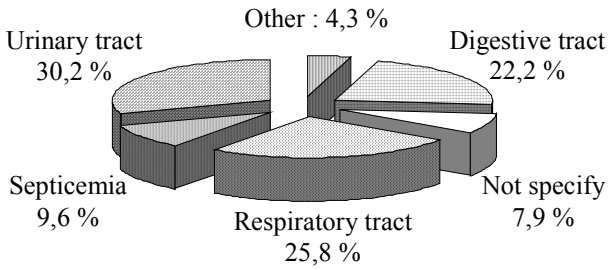
Quality control procedures are necessary to assure reproductibility and comparability of results. A regular internal quality control has to be realized by each laboratory with reference strains recommended by CA-SFM [4]. External quality control was also organized by RESAPATH managers.

Bacteria followed by RESAPATH are isolated from diseased animals : *Escherichia coli*, *Salmonella*, *Streptococcus*, *Staphylococcus* and *Pasteurellaceae*. For each isolate, epidemiological data are also recorded : species and localisation of diseased animal, type of production, pathology and type of sample.

Results

In 2002, 2664 antimicrobial susceptibility data were collected from 17 laboratories. The majority of these results concerned fattening pigs (40.7 %) followed by piglets (30.8 %) and sows (28.5 %). Seventy eight percent of antimicrobial susceptibility tests were performed for bacteria isolated in urinary, respiratory and digestive tracts (figure 1).

Figure 1 : Repartition of samples associated with antimicrobial susceptibility results recorded by RESAPATH in 2002



E. coli was the main swine pathogenic bacteria associated with antimicrobial susceptibility results (58.5 %, table 1).

Table 1 : Repartition of bacteria associated with antimicrobial susceptibility results recorded by RESAPATH in 2002

Bacteria	Number of strains	Percentages
*E. coli**	1300	52.6
Pasteurella multocida	355	14.4
Streptococcus suis	209	8.4
A. pleuropneumoniae	159	6.4
E. coli K88	146	5.9
Staphylococcus	119	4.8
Salmonella	75	3.0
Haemophilus parasuis	56	2.3
*Streptococcus**	36	1.5
Actinobacillus suis	18	0.7

** different from serovar K88 or not serotyped with K88 reagent*
*** different from S. suis*

The lowest percentages of *E. coli* susceptible strains were obtained for amoxicillin, spectinomycin, tetracycline and trimethoprim/sulfonamide with respectively 46.8 %, 60.9 %, 12.1 % and 33.5 %. The percentages of susceptible strains to the 12 other studied antimicrobials were between 74.8 % and 99.6 % (table 2).

Table 2 : Percentages of E. coli susceptible strains for 16 antimicrobials

Antimicrobial	Number of tested strains	Percentage of susceptible strains
Amoxicillin	1356	46.8
Amox.+ Clav.*	863	81.6
Cephalexin	759	96.0
Ceftiofur	1440	99.2
Colistin	1263	99.6
Neomycin	1241	88.2
Gentamicin	1262	93.7
Apramycin	1144	95.5
Spectinomycin	1239	60.9
Florfenicol	966	96.3
Tetracycline	1432	12.1
Trim.+Sulf.**	1442	33.5
Flumequine	1351	74.8
Oxolinic acid	1346	80.5
Enrofloxacin	1442	92.4
Marbofloxacin	1337	94.6

** Amoxicillin + clavulanic acid*
*** trimethoprim-sulfonamide*

Discussion

Excepting four antimicrobials (ceftiofur, florfenicol, enrofloxacin and marbofloxacin), the number of *E. coli* susceptible strains was calculated with breakpoints used in human medicine [4]. The therapeutic impact of these percentages depends on pharmacokinetic parameters of the antimicrobials in animals.

Nevertheless, these data show the ratio of bacteria with one or more resistance mechanisms (epidemiological aspect). RESAPATH allows the monitoring of resistance to antimicrobials used in veterinary medicine and the detection of potential emergence of new resistance phenotypes. Thus, the resistance to colistin or ceftiofur for a few *E. coli* strains from animal origin was confirmed by AFSSA. A particular surveillance is focused on the evolution of these resistances and their molecular mechanisms.

Reliability of antimicrobial resistance monitoring and successful therapeutic treatments in veterinary medicine depend on quality of *in vitro* antimicrobial susceptibility results and their interpretation. Therefore, the RESAPATH coordinators work on standardisation of antimicrobial susceptibility tests with the collaboration of some veterinary laboratory managers. Moreover, a sub-committee associated with the CA-SFM [4] works on determination of interpretative criteria for antimicrobials used both in human and veterinary medicine and those used only in animals.

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INFLUENCE OF ANTIMICROBIAL TREATMENTS ON OCCURRENCE OF ANTIMICROBIAL RESISTANCE IN *Escherichia coli* FROM FAECAL FLORA OF PIGS

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Introduction

Antibiotic use in food-producing animals is considered as a risk factor for emergence of resistant bacteria both in treated animals and in human beings. However, little is known about relation between therapeutic schemes and occurence of resistance in herds where antibiotics are used.

The purpose of this investigation was to determine the effect of antimicrobial use on resistance in commercial swine facilities. *Escherichia coli* strains from faecal flora of pigs were used as indicator bacteria for evaluation of resistance.

Material and Methods

Sixteen farrow-to-finish pig herds from west of France were included in the study. Depending on their antimicrobial use, they were classified as either low-users (LU) , medium-users (MU) or high-users (HU) if number of collective treatments applied to animals during the study period was 2 or less, 3-4 or >4 respectively. Numbers of farms in different groups were 6 (LU), 4 (MU) and 6 (HU). In a second step, herds were classified as users (U) or non-users (NU) depending on whether a specific antimicrobial class was administered to pigs or not.

In each farm, 5 sows and 3 piglets from each sow were randomly selected for faecal sampling. Sows were sampled before farrowing and then 7, 30 and 60 days after farrowing and piglets at 7, 30, 60 and 150 days of age. Four indicator *Escherichia coli* strains were isolated from each faecal sample and tested for antimicrobial susceptibililty by standard disk diffusion method. Isolates determined to be intermediate were classified together with resistant ones. For each sampling time and animal type, percentages of resistant strains were compared using chi square test in order to assess effect of antimicrobial use.

Results

Among antimicrobial tested either no or few resistant strains were evidenced for the following : ceftiofur, cefquinome, florfenicol and colistin. Antimicrobials to which resistance was observed either transiently or continually during the study period were assessed by statistical analysis and results are illustrated in Tables 1 and 2.

Evolution of resistance with time was similar for all antimicrobials in pigs : percentage of resistant isolates increased during post-weaning period and decreased thereafter. Consequently lowest percentages were observed at the end of the fattening period. For sows, percentage of resistant strains were higher during lactating period (days 7 and 30). This evolution war more evident for amoxicillin and gentamicin than for trimethoprim-sulfamethoxazol and tetracycline. High variability of percentages of resistant strains was observed for amoxicillin, gentamicin and trimethoprim-sulfamethoxazol depending both on herds and on sampling times. Results were more constant for tetracyline to which a high percentage of strains exhibited resistance.

Table 1 Percentages of resistant *Escherichia coli* strains isolated from sows and pigs in herds exhibiting different levels of antimicrobial use

			*N**	Amoxicillin	Gentamicin	Trimethoprim-sulfamethoxazol	Tétracycline
Sows		LU	*96*	**16,7**	0	47,9	**87,5**
	Day 0	MU	*76*	**34,2**	1,3	55,3	**73,7**
		HU	*104*	**26**	7,7	55,8	**86,5**
		LU	*92*	**27,2**	**1,1**	51,1	**87**
	Day 7	MU	*73*	**58,9**	**4,1**	65,8	**93,2**
		HU	*104*	**38,5**	**18,3**	57,7	**79,8**
		LU	*96*	**27,1**	**0**	**39,6**	72,9
	Day 30	MU	*73*	**50,7**	**1,4**	**74**	74
		HU	*99*	**50,5**	**23,2**	**57,6**	82,8
		LU	*92*	**19,6**	3,3	**43,5**	**90,2**
	Day 60	MU	*46*	**28,3**	0	**39,1**	**63**
		HU	*96*	**35,4**	8,3	**63,5**	**87,5**
Pigs		LU	*268*	**23,5**	**0**	**36,9**	**67,2**
	Day 7	MU	*209*	**54,1**	**1,4**	**58,4**	**76,1**
		HU	*276*	**44,9**	**19,9**	**57,2**	**83,4**
		LU	*263*	**19,8**	**6,1**	**36,1**	**83,3**
	Day 30	MU	*204*	**48,5**	**0,5**	**58,8**	**88,7**
		HU	*239*	**66,1**	**28**	**69,9**	**91,2**
		LU	*259*	**26,3**	**7,3**	**34,7**	**84,6**
	Day 60	MU	*195*	**70,3**	**21,5**	**86,4**	**99**
		HU	*244*	**54,5**	**31,6**	**82,4**	**93,9**
		LU	*202*	**8,4**	1	**18,8**	**80,2**
	Day 150	MU	*94*	**26,6**	0	**46,8**	**91,5**
		HU	*203*	**34**	0,5	**63,5**	**87,7**

** number of E coli colonies tested*
Bold type indicate a significant effect of level of antimicrobial use ($p<0.05$)

Effect of level of antimicrobial use on percentage of resistant *E coli* was more noticeable in pigs than in sows. Indeed, for the first animal type, this effect was significant in all cases except for gentamicin at the end of the fattening period.

In sows, use of an antimicrobial class was not frequently associated with a significant increase of percentage of resistance to a member of this class except for aminoglycosides on gentamicin resistance. This association was observed in pigs for all antimicrobials except tetracycline.

Table 2 Percentages of resistant *Escherichia coli* strains isolated from sows and pigs in herds using (U) or not using (NU) the class of tested antimicrobials

			Amoxicillin	Gentamicin	Trimethoprim-sulfamethoxazol	Tétracycline
Sows	Day 0	U	25.1	**7.6**	53.7	84.6
		NU	22.7	**0.4**	53.6	78.4
	Day 7	U	40.4	**18.2**	56.2	82.3
		NU	30.7	**1.9**	57.8	84.9
	Day 30	U	40.2	**23.2**	**64.5**	81
		NU	39.1	**0.4**	**51.7**	73.5
	Day 60	U	**33.9**	**8.3**	56.5	**82.4**
		NU	**18.5**	**2.3**	48.9	**84.5**
Pigs	Day 7	U	**43.4**	**19.8**	54.5	**72.9**
		NU	**33.6**	**1.1**	49.1	**79.1**
	Day 30	U	**47.9**	**28**	**64.6**	87
		NU	**39.3**	**2.9**	**53.9**	86.2
	Day 60	U	**56.9**	**31.5**	**88.5**	91.9
		NU	**42.5**	**10.7**	**59.1**	92.2
	Day 150	U	**29.8**	0.4	**69.8**	89.6
		NU	**15.9**	0.4	**39.6**	84.2

Bold type indicate a significant effect of level of antimicrobial use ($p<0.05$)
Amoxicillin : U : β-lactam-user herds
Gentamicin : U : aminoglycoside-user herds
Trimethoprim-sulfa : U: trimethoprim-sulfamide-user herds
Tetracycline : U : tetracycline-user herds

Discussion

Farms included in this study were selected for their use of antimicrobials similar to what is currently observed in french swine herds.

Percentages of resistant *E coli* strains exhibited variability depending on antimicrobial class with lowest levels of resistance observed for gentamicin. Gentamicin-resistant strains were less frequent here than observed in pigs in the USA where up to 92% of strains were resistant to this antimicrobial (4). On the opposite, percentages of resistance to tetracycline appeared to be very high. Both high prevalence and persistance of tetracycline-resistant strains have been previously demonstrated elsewhere (3,4).

Kinetic evolution of resistance was more or less noticeable depending on tested antimicrobial (decrease during fattening period was not very important for tetracycline) and has been previously reported with pathogenic bacterial strains (1). This variability in levels of resistance with time may be due to more frequent administration of antimicrobial treatments around farrowing for sows and during post-weaning period for pigs. However similar evolution has been observed in LU herds without use of antimicrobials. Such a phenomenom could be linked to other factors that have been shown to interfere with resistance such as thermic stress or overcrowding (5).

Evaluation of impact of level of antimicrobial use on occurrence of resistant *E coli* strains in faecal flora suggested that the more antimicrobials were used in swine herds the more frequently resistant strains were selected. This effect was observed for antibiotics to which resistance in low-user herds was rather low whereas could not be evidenced for tetracycline. Moreover, relation between level of use and resistance was more noticeable in pigs than in sows, the later being less frequently treated than their progeny.

Effect of use of a given antibiotic on occurrence of resistance to this molecule and others from the same class was studied. A relation was observed between use of aminoglycosides and resistance to gentamicin both in sows and pigs although these antimicrobials were mainly administered to pigs in selected herds. Resistance to amoxicillin in pigs was associated with use of β-lactams, this class of antimicrobials being usually administered to piglets during either lactation or first part of post-weaning period. On the other hand, resistance to tetracycline was high and unrelated to the use of this class of antimicrobials.

Conclusion

From this study level of use of antimicrobials was evidenced as a factor influencing prevalence of resistant *E coli* in faecal flora of pigs for some antimicrobials. However, further work is needed in order to describe more precisely effect of different antimicrobial treatments (molecule dose, administration route) on resistance.

Level of antimicrobial use has also to be more precisely studied using quantitative methods although measurement of drug consumption in veterinary medicine is difficult (2).

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STYRIAN RESISTANCE MONITORING PROGRAMME (REMOST) – THREE YEARS TREND IN ANTIMICROBIAL RESISTANCE

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Introduction

The EU Council passed a resolution on antibiotic resistance entitled "A strategy against the microbial threat" on 8 June 1999. In the same year, the Department of Veterinary Administration (DVA) in Styria established a Resistance Monitoring Programme (REMOST) (KÖFER et al., 2002) modelled on the trend-setting Danish activities (AARESTRUP et al., 1998). In the REMOST programme the resistance behaviour of zoonotic pathogens (*Salmonella* spp., *Campylobacter* spp.) and indicator bacteria (*Enterococcus* spp., *E. coli)* isolated from slaughter pigs, cattle and broilers is tested on a continuous basis. Additionally, indicator bacteria in bulk milk samples from cows are also tested. The test results are published on an annual basis and are fed into a central database, which is linked to a geographical information system named VETGIS© Styria (FUCHS et al., 2001).

Materials and Methods

The REMOST programme consists of a sampling system, which indicates where, how and when samples are to be taken, an analysis system for the continuous analysis of data and a catalogue of measures based on these modules. Isolation of the bacterial strains is done by streaking the material to be tested (faeces, meat, milk) on different agar media: *E. coli* (Coli IDAgar, Biomerieux No. 42017), *Enterococcus faecalis/faecium* (CATC medium, ÖNORM DIN 10106), *Salmonella enterica* (MSRV method), *Campylobacter jejuni/coli* (mCCDA). After biochemical verification of suspect colonies, the resistance behaviour is tested using the SENSITITRE® system, a commercially available MIC technique using dehydrated antimicrobials in microtitre wells. The wells were inoculated according to NCCLS guidelines using breakpoints recommended by NCCLS or DANMAP. During the investigation period (2001 – 2003) a total of 537 *Salmonella* spp., 1290 *Campylobacter* spp., 1294 *E. coli,* 1340 *Enterococcus* spp. from faecal specimen and 761 *Enterococcus faecalis,* 184 *E. coli* strains isolated from bulk milk samples were tested against 12 to 16 antibiotics.

Results and Discussion

Faecal isolates of *Salmonella* spp. showed high resistance rates to streptomycin (62-73%) and tetracycline (19-50%). The quinolones nalidixic acid and ciprofloxacin produced different results. While the resistance rates of *Salmonella* spp. to nalidixic acid were in the 27-56% range not a single Salmonella isolate showed resistance to ciprofloxacin (Tab. 1).

Campylobacter spp. displayed considerably higher resistance rates than *Salmonella* spp. The situation for *C. jejuni* is of particular significance in this respect, since this pathogen is involved in approx. 90 % of Campylobacter induced human illnesses. As expected, *Campylobacter* spp. isolated from broilers showed higher resistance rates (CIP, ERY, TET) than strains from cattle (Fig. 1, 2).

The analysis of the resistance behaviour of *E. coli* from broilers and pig samples produced high resistance rates to streptomycin, tetracycline and spectinomycin (Fig. 3). The situation for isolates from cattle faeces and beef surfaces, in contrast, was much more favourable..

Tab. 1: Occurrence of resistance among *Salmonella* spp. from broilers, faeces

	2001			2002			2003		
	n	**% res**	**CI 95**	**n**	**% res**	**CI 95**	**n**	**% res**	**CI 95**
AMP	74	16.2	[9.6, 26.3]	104	31.7	[23.6, 41.2]	48	29.2	[18.3, 43.3]
AUG	74	6.8	[3, 14.9]	104	6.7	[3.3, 13.3]	48	8.3	[3.4, 19.6]
CHL	74	6.8	[3, 14.9]	104	17.3	[11.3, 25.7]	48	12.5	[5.9, 24.8]
GEN	74	0.0	[0, 4.8]	104	1.0	[0.2, 5.2]	48	4.2	[1.3, 14]
NAL	74	27.0	[18.2, 38.1]	104	40.4	[31.5, 50]	48	56.3	[42.2, 69.3]
NEO	74	4.1	[1.5, 11.2]	104	15.4	[9.7, 23.6]	48	18.8	[10.2, 32]
SPE	74	5.4	[2.2, 13.1]	104	7.7	[4, 14.5]	48	16.7	[8.8, 29.7]
STR	74	62.2	[50.7, 72.4]	104	69.2	[59.8, 77.3]	48	72.9	[58.9, 83.4]
TET	74	18.9	[11.6, 29.3]	104	38.5	[29.7, 48.1]	48	47.9	[34.4, 61.7]
TMP	74	10.8	[5.6, 19.9]	104	5.8	[2.7, 12]	48	10.4	[4.6, 22.2]

CIP, COL, FFN, XNL < 3% resistant

Legend: AUG… amoxicillin+clavulanic acid, AMP … ampicillin, XNL … ceftiofur, CHL … chloramphenicol, CIP … ciprofloxacin, COL … colistin, FFN … florfenicol, GEN … gentamicin, NAL … nalidixic acid, NEO … neomycin, SPE … spectinomycin, STR … streptomycin, TET … tetracycline, TMP … trimethoprim

Fig. 1: Resistances among *C. jejuni* from broilers (n=327)

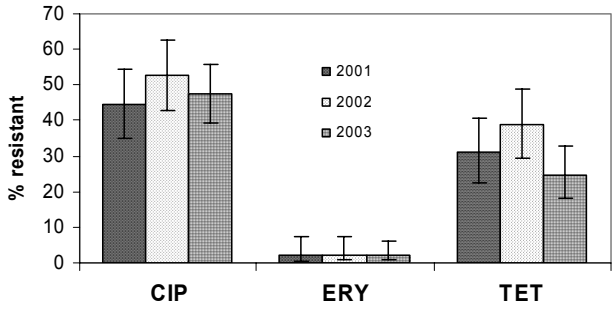


Fig. 2: Resistances among *C. jejuni* from cattle (n=228)

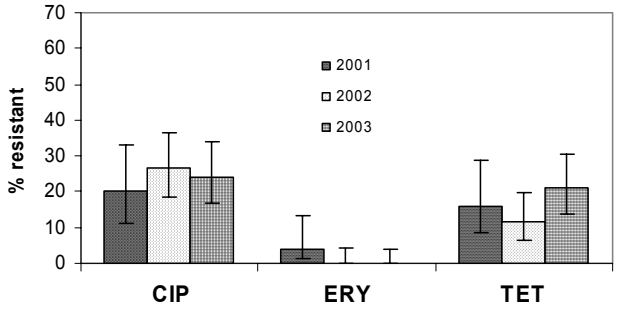


Fig. 3: Resistances among *E. coli* from pigs (n=428)

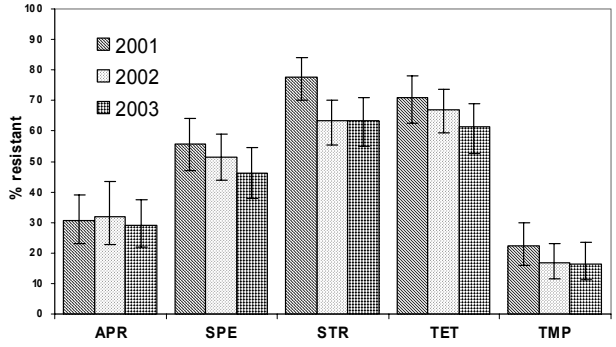
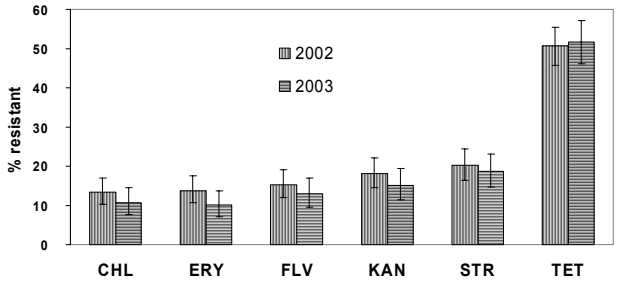


Fig. 4: Resistances among *E. faecalis*, bulk milk (n=761)



The resistance rates of *Enterococcus* spp. isolated from cattle faeces were also considerably below those obtained for poultry and pigs, as in the case of *E. coli*. The bacterial strains obtained from cattle revealed higher levels of resistance only to flavomycin, tetracycline and bacitracin. The poultry isolates showed very high rates of resistance to bacitracin, erythromycin, tetracycline and virginiamycin.

Enterococcus spp. isolates from cattle faeces also displayed a high level of resistance to flavomycin, whereas isolates from bulk milk samples showed a high level of resistance only to tetracycline (Fig. 4).

The results of our investigation of the resistance behaviour of indicator bacteria and zoonotic pathogens are comparable with data from other countries, like Denmark (DANMAP, 2002), Sweden (SVARM, 2003) or Norway (NORM/NORM-VET, 2002). In addition to the monitoring of antimicrobial resistance it will be necessary to collect valid data about the consumption of antibiotics and chemotherapeutics in livestock husbandry. The prudent use of antimicrobials in the production of food of animal origin according to the principles of Good Veterinary Practice (VAN MIERT, 1993) provides the basis for optimising veterinarian support in the management of farm animals.

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PIG REARING METHODS AND RATE OF ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI*

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Introduction

The antimicrobial resistance question trends to be more and more considered as a global ecological problem. The normal intestinal microfloras of animals represent a reservoir of antibioresistant strains and/or antibioresistance genes, that can be transmitted to humans. *Escherichia coli* (*E. coli)* are common inhabitants of the intestinal tracts which show a great ability to gain resistance under antimicrobial use (**5**, **6**, **9**). Not only pathogenic to animals and humans, they can also be potential donors of antibioresistance determinants to be transferred to other pathogenic bacteria.

The aim of this study was to assess antimicrobial resistance rates in the *E. coli* population of healthy French pigs that were reared according to different methods.

Material and Methods

Four classes of pig farms with various rearing methods were constituted as follows:

Class	Age at slaughter	Ground surface / animal	Type of areas surface / animal	Antimicrobial use constraints
1	182 days	> 1.2 m^2	straw or outdoor	No preventive antibiotherapy and maximum 2 treatments / animal in case of disease
2	182 days	> 1.2 m^2	straw or outdoor	No antimicrobial growth promoters
3	-	0.65 to 1.1 m^2	slatted floor	Low health expenses
4	-	0.65 m^2	slatted floor	High health expenses

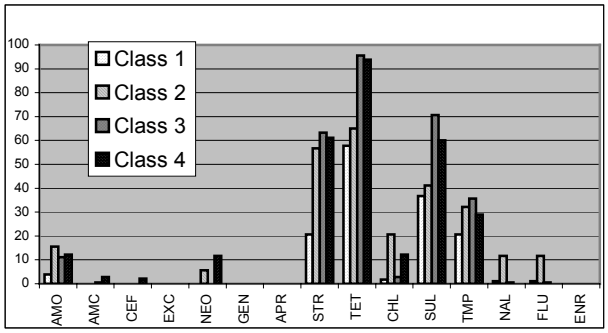
In each class, 6 pig farms belonging to at least 4 different commercial organizations were included in the study. Altogether, 24 farms were sampled. Sampling was carried out between april 2001 and april 2002.

In each farm, 30 finishing pigs were randomly selected and fecal material was collected via rectal swabs. Altogether 720 fecal samples were collected. The cotton-tipped 30 swabs from each farm were cut and pooled, thus forming a sole composite suspension which was plated onto Mac Conkey agar. From each plate, 30 colonies identified as being *E. coli* after biochemical trias, were tested for antimicrobial susceptibility using disk diffusion method on Müller-Hinton agar (BA 20, program 116). Interpretation of zone diameters and classification into susceptible or resistant categories were performed according to the guidelines of the Antibiogram comity of the French Society of Microbiology. Intermediate responses were included in the resistant group. The fifteen next antimicrobial compounds were included:

Name	Symbol	Breakpoint diameters after an overnight incubation at 37°C (mm)	Corresponding breakpoint concentrations (mg/L)
Amoxicillin	AMX	14-21	4-16
Amoxicillin and clavulanic acid	AMC	14-21	4-16
Cefalexin	CN	12-18	8-32
Ceftiofur	XNL	21-24	2-8
Neomycin	N	15-17	8-16
Gentamycin	GM	14-16	4-8
Apramycin	APR	11-14	16-32
Streptomycin	S	13-15	8-16
Tetracycline	TE	17-19	4-8
Chloramphenicol	C	19-23	8-16
Sulfonamides	SSS	12-17	64-256
Trimethoprime	TMP	12-16	4-8
Nalidixic Acid	NA	15-20	8-16
Flumequin	UB	21-25	4-8
Enrofloxacin	ENR	18-22	0.5-1

Results

Rates of resistant *E. coli* in the four classes:



Discussion

Resistance rates found in the standard classes 3 and 4 are in agreement with these found by the National Surveillance Plan performed in France in 2000 (**4**). High resistance rates were observed for the most commonly used antimicrobial compounds (tetracycline 95%, sulfonamides 65%, trimethoprim 32%). Some antibiotics which had been used extensively in the past caused significant resistance rates to persist (streptomycin 62%, chloramphenicol 7%). But little or no resistance was observed for some compounds used in human medicine, like the cephalosporins, the aminoglycosides and the quinolones families. With a percentage of 12%, few isolates were resistant to amoxicillin comparing with its use, and less than 2% were resistant to its association with clavulanic acid.

Classes 3 and 4 displayed strictly the same profile, so proving that previous health expenses is not a good indicator to estimate antimicrobial exposure, on one hand because they include drugs other than antimicrobials (vaccines, antiparasitic drugs…) and on the other hand because they were calculated from previous batches.

In the class 1 antimicrobial use is really exceptional and no pig included in the study received any antimicrobial treatment. So significantly lower resistance rates in this class is quite normal. The rates of 58% for tetracycline, 37% for sulfonamides and 21% for the trimethoprim and the streptomycin, were never more important than could be expected. These results confirm the well known fact that the selection of resistant fecal coliforms by antibiotic use, may not be rapidely reversed by long-term withdrawal (**7**). Contarily to the pre-antibiotic era *E. coli* collections that were sensible to all compounds (**5**), genetic resistance determinants are nowadays widespread among enteric bacteria, even in the absence of antibiotic use. The resistance genes persistence can be the consequence of the gene association with other genetic elements which help to conserve it (**2**, **3**, **11**).

The class 2 where rearing conditions were less intensive, showed resistance rates lower than the standard classes 3 and 4 for tetracycline and sulfonamides, which are really less used in this class. Several authors (**7**, **10**) ever reported such result in concreted pens compared with pigs on pasture: comparable or inferior resistance rates, depending on the antimicrobial families. But here the overall rates were not statistically different from these of the standard classes. This result suggests that an increased number in resistant strains from the intestine is mainly the result of antimicrobial exposure, that was quite the same between the classes 2 and 3. In the same way, several other researchers noted that herds with low antimicrobial use obtained resistance patterns approaching those of herds continuously exposed to antibiotics (**1**, **7**, **8**).

Conclusion

These results allow to suppose that less intensive pigs rearing conditions are not sufficient to reduce resistant strains rates significantly. Only a strict antimicrobial use limitation is effective in preserving the sensibility of most of the strains.

As the debate continues over the public health impact of agricultural use of antimicrobial medicines, veterinary and pig production organizations are waiting for information to achieve intended animal health goals while minimizing resistance problems.

These data are the result of a national approach to link usages and antimicrobial resistance rates, in order to clarify how to use antibiotics carefully.

Acknowledgements

The authors gratefully acknowledge the production companies and the farmers for their cooperation in the project, E. Chaslus-Dancla, J. F. Guillou, J. Pourquié for their scientific support. This study was supported financially by the Food safety Department of the French Ministry of Agriculture, Food Fisheries and Rural Affairs (program AQS R99/06).

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PERSISTENCE OF *MYCOPLASMA SYNOVIAE* IN HENS AFTER TWO ENROFLOXACIN TREATMENTS

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Introduction

Mycoplasma synoviae (MS) infection most frequently occurs as a subclinical upper respiratory infection and is sometimes responsible for infectious synovitis in chickens and turkey (1). The economic consequences may be important because of decreased egg production, retarded growth, reduced weight gains, and condemnation at slaughter due arthritis lesions. MS may be transmitted either vertically, through the eggs, or laterally, by direct contact or via the environment (1, 2). MS is susceptible to several antibiotics *in vitro*, including tetracyclines, macrolides (except erythromycin) and fluoroquinolones (3, 4). However, even if antibiotic treatments decrease the symptoms, they do not eliminate MS infections (1). These therapeutic failures may be due to the development of resistant strains, as shown *in vitro* (5).

In the present study, we report the effect of two enrofloxacin treatments on the persistence of *M. synoviae* in experimentally infected hens and on the emergence of resistant mycoplasmas.

Material and Methods

On day 0, thirty eleven-week-old mycoplasma-free hens were infected by aerosol with 500 mL of a culture of the MS 317 strain containing approximately 10^6 colony forming units (CFU) per milliliter. This MS strain was susceptible to enrofloxacin (minimum inhibitory concentration (MIC)= 0.25 µg/mL) (3).

Hens were randomly assigned to two separate animal rooms, containing 20 and 10 birds.

Three weeks after infection, the first medication with enrofloxacin (Baytril 10%) at the therapeutic dose (TD= 10 mg/kg of body weight) was given for five consecutive days (from day 23 to day 28) in the drinking water of the first animal room. A second treatment was administered one week after the end of the first medication (from day 36 to day 41). Hens of the second animal room were kept untreated.

Before and after each treatment, five hens of the first animal room were sacrificed and examined postmortem for gross lesions. Tracheal swabs were collected and cultured for MS recovery. Five infected untreated hens were also sacrificed after each treatment.

All tracheal swabs were placed in 2 mL of transport medium (2% buffered peptone water containing glycerin (1.2% v/v), amphotericin B: 2.5 µg/mL, ampicillin: 2 units/mL and colistin: 7.5 µg/mL). Mycoplasmas were directly cultured from tracheal swabs by diluting 100 µL of transport medium from each swab in 900 µL of FM4 broth (6). Serial ten-fold dilutions up to 10^{-4} were prepared and incubated at 37°C until the culture developed an acid color change or up to 30 days. When a color change of the broth medium was observed, the uncloned cultures were aliquoted and stored at -70°C before MIC determination.

As these cultures could contain mixtures of wild and mutant cells, some cultures, obtained before or after the first treatment or after the second treatment, were cloned, aliquoted and stored at -70°C.

The enrofloxacin MIC of the different positive cultures and of the clones were determined by the metabolic inhibition method performed with 96-well microtiter plates in Frey broth medium (7) as previously described (8). Enrofloxacin concentrations ranged from 0.03 to 32 µg/ml.

The SAS software was used to compare MIC results with the Student-Newman-Keuls test. Differences were estimated significant when $p< 0.05$.

Results

After infection and before the first treatment, the mycoplasmal infection was confirmed by culture. One hundred percent of the hens were positive at the beginning of the first treatment at the therapeutic dose, and 100% of the birds were still positive at the end of the two successive treatments (Table 1). Furthermore, no difference in the number of mycoplasmas was observed between the cultures from tracheal swabs collected before and after the treatments, or between the cultures from treated and untreated birds (data not shown).

The results of MIC determination did not show any significant difference between the susceptibility to enrofloxacin of the MS uncloned cultures recovered after the first treatment or before the second treatment and the wild-type strain (Table 1). However, a significant increase of the resistance level to enrofloxacin of the reisolated mycoplasma cultures obtained from the treated birds was observed after the second treatment ($p< 0.05$).

Table 1: re-isolation of M. synoviae *and susceptibility levels of the MS uncloned cultures before and after two successive treatments with enrofloxacin.*

	Percentage of positive hens		MIC_{ENRO}[c] for MS cultures (µg/mL)	
	Treated	Untreated	Treated	Untreated
Before T1[a] (Day 23)	100	ND	0.25-0.5[d] 0.46[e]	ND
After T1 (Day 28)	100	100	0.25-1 0.50	0.25-0.5 0.46
Before T2[b] (Day 36)	100	ND	0.25-0.5 0.42	ND
After T2 (Day 41)	100	100	1-2 1.18*	0.25-1 0.53

[a]: first treatment; [b]: second treatment
[c]: minimum inhibitory concentration of enrofloxacin
[d]: MIC range; [e]: MIC geometric mean
*: significantly different from the five other groups of cultures ($p< 0.05$).

Enrofloxacin MICs were also determined on clones of some MS cultures obtained before and after the first and second treatment (Table 2).

As for the uncloned suspensions from the positive cultures, these results clearly point out an increase in the MICs of *M. synoviae* after the second treatment for most

of the clones, with values ranging from 0.5 to 4 µg/mL of enrofloxacin.

Table 2: Susceptibility levels of clones of M. synoviae *before and after two successive treatments with enrofloxacin.*

	Number of clones isolated	MIC_{ENRO}[c] (µg/mL)
Before T1[a]	5	0.25 0.25 0.5 0.5 0.25
After T1 and before T2[b]	5	0.5 0.5 0.5 0.5 0.25
After T2	6	1 0.5 1 4 2 1-2

[a]: first treatment; [b]: second treatment
[c]: minimum inhibitory concentration of enrofloxacin

Discussion-Conclusion

Two successive treatments at the therapeutic dose of enrofloxacin did not have any influence on the MS recovery from tracheal swabs, with 100% of the hens still positive after the second treatment. This persistence phenomenon has already been described for *M. gallisepticum* (MG) in chickens (9), but successive treatments at the therapeutic dose reduced the percentage of MG-infected birds. Hypotheses to explain the mycoplasma persistence in treated birds include possible development of resistance mechanisms, survival on materials in the animal room environment and subsequent natural re-infection of birds, the ability to invade host cells for long periods and persist inside the cell in a fluoroquinolone-insensitive state, as described for *M. penetrans* (10). Mycoplasmas might also persist in tissues or subcellular fractions where enrofloxacin cannot diffuse.

The development of resistance in *M. synoviae* and *M. gallisepticum* has already been described *in vitro* (5). However, this is the first time that such an increase of resistance is described after several *in vivo* treatments at the therapeutic dose: despite the re-isolation of mycoplasmas, no significant MIC change was previously observed in *M. gallisepticum* clones after several treatments (9).

Further studies should be conducted to determine if the increase of resistance can be attributed to the development of resistance mechanisms, especially in the genes coding for DNA-gyrase or topoisomerase IV, as described for mutants of *M. gallisepticum* selected *in vitro* (11, 12)

In conclusion, results showed that, under these experimental conditions, oral enrofloxacin treatments were not effective for the eradication of *M. synoviae*. This persistence could be associated to a decrease of the susceptibility level of some reisolated clones.

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Antibiotics / Use and resistance

Posters

RESISTANCE OF CAMPYLOBACTER SPP. TO QUINOLONES IN FOOD PRODUCING ANIMALS IN STYRIA (AUSTRIA)

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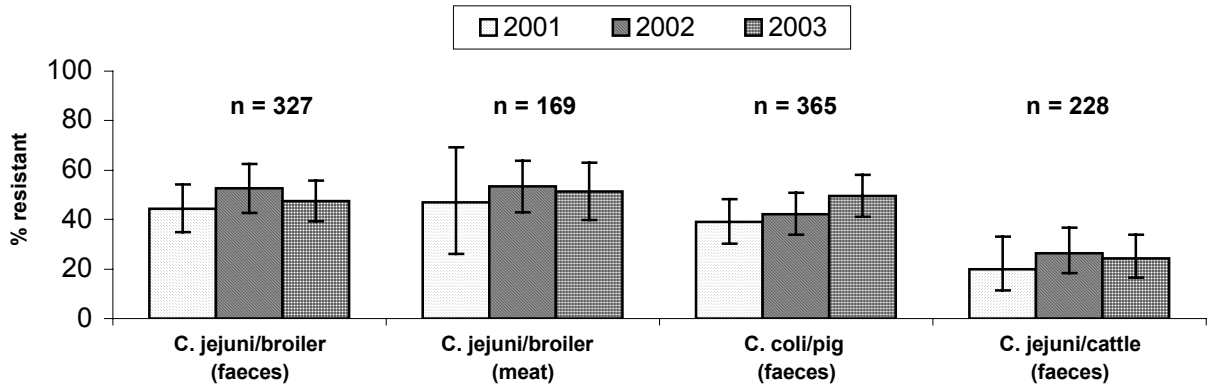
Introduction

Campylobacter, especially *C. jejuni*, is one of the most common bacterial causes of diarrhoeal illness in humans (1). Feierl et al. (2) reported 3,771 cases of illness caused by *C. jejuni* in Austria in 2003, corresponding to an incidence of 46.5 per 100,000 inhabitants. The resistance of *Campylobacter* to quinolones is increasingly limiting their therapeutic use. The rise in human infections caused by *C. jejuni* and *coli* shows the significance of growing quinolone resistance in *Campylobacter* (3). The goal of our study was to investigate the extent of resistance of Campylobacter to quinolones.

Material and Methods

Campylobacter jejuni was isolated from faeces of broilers (n=327), faeces of cattle (n=228) and from the surface of broiler meat (n=169); *Campylobacter coli* was isolated from pig bowels (n=365). Isolation of *Campylobacter* spp. is done by streaking the faeces on mCCDA agar. After biochemical verification of suspect colonies, they were tested for resistance to ciprofloxacin and nalidixic acid using the SENSITITRE® system, a commercially available MIC technique. The breakpoints used are recommended by NCCLS.

Figure 1: Resistances to ciprofloxacin of *Campylobacter jejuni and coli* isolated from faeces and meat (2001 – 2003)



Results and Discussion

Our study shows a stable quinolone resistance of Campylobacter for the years 2001 to 2003. Approximately 50 per cent of the *C. jejuni* strains isolated from poultry show resistance to ciprofloxacin and about 44% to nalidixic acid (fig. 1). Although these results lie within the range reported in other European countries, quinolone resistance in Campylobacter remains high (4). The cause for these high resistance rates can be found in the intensive use of quinolones in the treatment of Salmonella infections. European legislation requires that only Salmonella free poultry herds may be slaughtered. Quinolone resistance in Campylobacter is as familiar in pig production as it is in poultry production. Because of their effectiveness against E. coli and other infective gram-negative bacteria, quinolones are also widely used in the therapy of pig diseases. On the other hand, quinolones are used less in the therapy of cattle diseases, which is reflected in the significantly lower extent of quinolone resistance in *C. jejuni* isolated from cattle faeces. The development of bacterial resistance to quinolones is caused by multiple step mutations of two essential bacterial enzymes, DNA gyrase and DNA topoisomerase IV. The level of resistance depends on the frequency and intensity of bacterial contact with quinolones (5). Because of the importance of quinolones in the treatment of human bacterial infections Austrian regulations are aimed at minimising the therapeutic use of quinolones in animal production. Quinolones are thus acknowledged as "last resort antibiotics" and must not be administered to animals without prior examination and evidence of their absolute necessity. This means that it must be proved that therapeutic success cannot be achieved by any other approved antibiotic.

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PREVALENCE OF MULTIDRUG-RESISTANT (MDR) *SALMONELLA* ON BOVINE DAIRY HERDS IN FRANCE

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Introduction

Multidrug resistant (MDR) salmonellae have been isolated from bovine herds since the 80's, then from other species, and finally have been found to cause human food-borne disease in numerous developed countries. Among MDR salmonellae, one particular serotype -namely *Salmonella* Typhimurium (STm) DT (definitive type) 104- was the most prevalent (1,2). The chromosomal structure responsible for the resistance to at least five antibiotics simultaneously has been found in strains belonging to other serotypes (3). Therefore, the study of MDR salmonellae can not be focused on STm DT104 only. Moreover, no precise epidemiological study has been realised in France in order to appreciate the prevalence of such strains in bovine dairy herds. In the global frame of Quantitative Risk Analysis (QRA) on food-borne diseases due to MDR *Salmonella*, a 2 year-study was conducted in order to appreciate, in first intention, the prevalence of these bacteria on bovine dairy herds located in Western France. These data are essential for risk assessment of human infection by MDR *Salmonella* of bovine origin.

Material and Methods

Hypothesis :

- prevalence of contaminated herds = 10%
- overall precision expected = 3%Dairy herds
- 489 bovine dairy herds (>20 cattle).
- from 3 "départements" in Western France.
- simple random sampling (5%) from exhaustive database.
- all producers were voluntary.
- 2 sampling periods in 2001-2003. (November to April)

Sample collection

- first visit (V1) : manure or dung sample (60 ml).
- second visit (V2) for V1 positive herds : bulk milk, water and environmental swabs sampling.
- pools of individual fecal sampling (1-6/pool) according to calving status and birth date.Sample testing
- Rappaport-Vassiliadis culture following enrichment in EPT (dilution 1/5).
- XLT4 agar isolation plates, biochemical confirmation.
- serotyping and antimicrobial susceptibility testing.

*MDR = resistant or intermediate to, at least, two antimicrobials belonging to different families, according to the CASFM recommendations.*Epidemiological data

- herd structure and animal husbandry questionnaire at V1 (herd level exposure).
- animal level prevalence estimated by Bayesian approach (WinBUGS).

Results

Sample description

- Herd sampling representative of the dairy herds density.
- Predominant breed : Prim'Hostein (60,5%), Normande (32,2%).
- median annual milk production / cow = 6991 litres (range : 2100 – 10300).
- Average number of dairy cows sampled / herd : 42 (CI95%: 29-56, range : 21-87).

Herd prevalence - 35/489 bovine dairy herds contaminated by *Salmonella* in manure or dung, - 9 contaminated herds showed MDR strains.

- weighted prevalence for the 3 "departments": p*Salmonella*=8.1% and pMDR=1.9%.
- 11 *Salmonella* serotypes identified : *S*. Montevideo (26%), *S*. Typhimurium (14%)….
- *Salmonella* have been isolated in V2 sampling in 14/35 herds contaminated at V1. Strains isolated at V2 belonged to the serotype initially recovered in the herd, and exhibited the same antibiotic resistance phenotype. - Data analysis stratified by month of sampling date and "département" revealed more MDR strains isolation at the end of winter (*Fisher test, p=0.04*).

salmonellosis history was a predictive risk factor for detection of MDR *Salmonella* contamination in manure or dung

Discussion

These results are in agreement with the initial hypothesis. Prevalences are probably underestimated because there were probable false negatives for manure or dung contamination (sensitivity not 100%) and shedding without faecal excretion of *Salmonella* is possible. The end of winter is a risky period for herd contamination by MDR *Salmonella*. This observation suggested that a selection pressure linked to antimicrobial use during winter could lead to MDR strains emergence. Further works are needed to confirm the impact of antimicrobial use on MDR *Salmonella* herd contamination.

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PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER COLI* ISOLATED FROM FATTENING PIGS IN FRANCE

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Introduction

Campylobacter are a leading cause of human diarrhea[1]. The usual source of infection is contaminated food, particularly poultry but pork has also been described[2]. Increasing resistance to antimicrobial drugs has been documented in human and animal strains of this zoonotic pathogen[3] and the veterinary use of antimicrobial drugs has been suggested to be largely responsible for this resistance. A study was carried out to investigate the occurrence and antimicrobial resistance of *Campylobacter* isolated from French fattening pigs.

Materials and methods

Thermophilic *Campylobacter* were isolated from stomach samples obtained at slaughter from 240 fattening pigs originating from 24 different farms in the period March 1998-June 1999. The isolates were characterised at the species level by multiplex PCR. The agar dilution method was used for MIC determinations[4]. GyrA mutations were detected by MAMA-PCR and CmeB expression analysed by Western Blot as described previously[4].

Results

Half of the pigs (121 out of 240 animals) were found to be positive for *Campylobacter* but considerable variation was observed between farms (4 farms free of *Campylobacter,* and for the other farms 2 to 9 positive pigs among the 10 animals sampled). Isolates all belong to the *C. coli* species. Resistance to tetracycline and erythromycin was high (79 and 55 % respectively) and for nalidixic acid, enrofloxacin and ampicillin, resistance was observed in 34, 15 and 20 % of the isolates respectively (Table 1). More than one third of the strains was resistant to at least three antimicrobial drugs. A biphasic distribution was observed for tetracycline resistant isolates (MIC of 32 µg/ml and 256 µg/ml)(Table 1). Two groups of erythromycin-resistant strains could also be distinguished according to their level of resistance (MIC of 8-16 µg/ml or MIC≥256 µg/ml for 10% of the isolates) (Table 1). One-third of the pigs whose isolates were included in the MIC determinations were found to harbour more than one isolate (exhibiting different susceptibility patterns). A Thr86Ile modification in GyrA was observed in the 16 enrofloxacin-resistant strains analysed. These isolates were also resistant to erythromycin, the other therapeutic molecule used to treat human campylobacteriosis. The multiresistant strains analysed expressed the multidrug transporter CmeB at a high level.

Table 1. Distribution of MICs and resistance (%R) for five antimicrobial drugs (Ap, ampicillin; Nal, nalidixic acid; Enr, enrofloxacin; Ery, erythromycin; tet, tetracycline) of the C. coli strains (n=131) isolated from pigs at slaughter. Vertical lines indicate the breakpoints used.

	No. of isolates with a MIC (µg/ml) of								%R
	≤ 0.25	0.5-2	4	8	16	32	64	≥128	
Ap		9	17	60	19	24	2		20
Nal		1	9	44	32	9	18	17	34
Enr	96	15	1	7	12				15
Ery	1	40	18	15	50			7	55
Tet	10	15	1	1	5	74	2	23	79

Discussion

Results indicated a high prevalence of *C. coli* in the stomach of the French pigs examined. A high prevalence of *Campylobacter* in pigs was also described in other european studies, going from 63 to 100%. The predominance of *C. coli* is also in accordance with previous reports. In addition, a high proportion of the strains was resistant to antimicrobial drugs, particularly to tetracycline and erythromycin, or were multiresistant. Tetracycline is extensively used in pig production and macrolides (especially tylosin) were permitted as growth promoters for pigs in Europe until 1999. This could explain the high rates of resistance observed. Increasing quinolone resistance is compromising the use of this criteria for routine clinical laboratory identification of thermophilic *Campylobacter*.

Conclusion

Increasing antimicrobial resistance in *Campylobacter* is raising concern not only for human therapy but also for horizontal transmission of this resistance in the natural ecosystem of this bacterium.

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ANTIBIOTIC RESIDUES IN RAW MILK IN MEXICO CITY

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Introduction

Mastitis is the most frequent cause of antibacterial use on dairy farms and contributes to a substantial portion of total drug and veterinary costs incurred by the dairy industry. 3 commercially assays are available on the market: Delvotest, Penzyme MilkTest and SNAP beta-lactam.

The objective of this study wase to investigate the: presence of antibiotic residues in milk produced and commercialized in south of Mexico City.

Material and Methods

We used The Delvo- test SP ® is based on biological detection of antibiotics in the milk is suited for raw milk processing factories.

Test sensitivity is for ß-lactams, Sulphonamides, Tetracyclines, Macrolides, Aminoglycosides, Lincosamides and others. Sensitivity of the Delvo-SP assay is > 90%, (1). Kang, 2001(3) indicate that the Delvotest SP assay may provide a suitable means for the detection of drug residues.

Sample population: 22 farms they produce raw milk for to sell cheeses factory. 264 Milk samples were collected at the farm afternoon after the milking. We take samples one time a week along 3 months.

Results

We founded antibiotic residues in 77% of the samples analyzed, in 17 farms This reflected hazard for public health and the importance that companies of milk manufacturing take a quality system that include HACCP.

Discussion

Problems arise when the test is used on milk samples of a single cow. Buffers, cells, bacteria, cell contents, and disinfection without using antibiotics may give false positive and false negative results.

Hassig, 2003, (2) reports an error up to 44.8% was detected when test is used on single cow milk samples. The use on single cow milk samples to prove absence of antibiotics is not recommended.

Conclusion

The most frequent reasons suggested by farmers for their test failures were not withholding milk for the full withdrawal period (95 per cent) and accidental transfer of milk (5 per cent). In some farms lactating and dry cow intramammary antibiotic preparations were held responsible

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ANTIBIOTIC RESISTANCE OF ZOONOTIC BACTERIA ISOLATED FROM FRENCH HEALTHY CATTLE AT SLAUGHTER

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Introduction

Survey of antibiotic resistance of bacteria collected in healthy animals is useful to evaluate the prevalence of resistant bacteria in normal flora and the evolution of this resistance over years. It could also be a tool to appreciate veterinary use of antibiotics and risk factors spread for public health.

French surveillance programmes of resistance in commensal bacteria (*Escherichia coli* and *Enterococcus*) and zoonotic bacteria (*Salmonella* and *Campylobacter*) have been carried out in poultry and swine since 1999 [1]. In 2002, this surveillance programme of resistance was set up in cattle.

Materials and Methods

This survey was performed in three main types of French bovine production : calves, steers and culling cows. Isolation of zoonotic bacteria was performed on 600 faecal samples collected each year in 2002 and 2003 in nine French slaughterhouses representative of the bovine production. All isolated strains were identified : *Salmonella* were serotyped according to the Kauffmann-White scheme. Thermotolerant *Campylobacter* were isolated and characterization of *Campylobacter jejuni* and *coli* was realized by PCR.

Antimicrobial susceptibility testing for *Salmonella* was carried out by disk diffusion method according to the guidelines of the Antibiogram Commitee of the French Society of Microbiology [2]. The following antibiotics were tested : AMX, AMC, CAZ, CF, FOX, CFM, S, K, Apra, GM, TET, C, TMP, SXT, NAL, OA, ENR. *Campylobacter* isolates were screened for resistance to AM, GM, ERY, ENR, TET, NAL by the dilution method according to NCCLS guidelines.

Detection of resistance genes in *Salmonella* was performed by PCR.

Results

The prevalences of *Salmonella* isolated in 2002 and 2003 were quite similar (6/600 (1%) in 2002 and 9/600 (1.5%) in 2003). In 2002, all of the *Salmonella* were susceptible to the antimicrobials tested. In 2003, most *Salmonella* were susceptible excepting two *Salmonella* Typhimurium with the following multidrug resistance profiles : AMX/AMC/S/TET/C and AMX/AMC/S/Apra/GM/TET/C/SXT/TMP.

Both *Salmonella* Typhimurium showed a decreased susceptibility to cefalotin.

PCRs performed on these two strains assessed the presence of the multidrug resistance region (MDR) and *Salmonella* genomic island 1 (SGI1) described in multidrug resistant *Salmonella* Typhimurium DT 104 [3]. Moreover, additional resistances to gentamicin and trimethoprim were observed in one of the two strains harboring SGI1. Characterization of genes conferring this resistance is in progress to assess localization of these genes on MDR.

Campylobacter was isolated in 60/600 (10%) and 130/600 (21.6%) of faecal samples in 2002 and 2003 respectively. *C. jejuni* was the predominant species (49/60 in 2002 and 100/130 in 2003). This species was mostly isolated from calves (37/60 (75%) and 68/100 (68%) in 2002 and 2003) whereas the frequency of this species was lower in culling cows (5/49 (10%) and 17/100 (17%)), and in steers (7/49 (14.3%) and 15/100 (15%)).

Campylobacter coli was mainly isolated from calves (10/60 in 2002 and 30/130 in 2003).

The percentages of *Campylobacter* resistant strains are shown in Table 1.

	2002		2003	
	C. jejuni, (n=48)	*C. coli*, (n=10)	*C. jejuni*, (n=85)	*C. coli*, (n=30)
AM	20	0	12	16.6
GM	0	10	0	3.3
ERY	16	10	4.7	36.6
TET	56	80	49.4	96.6
NAL	35	20	28.2	43.3
ENR	23	30	27	43.3

Distribution of MICs showed that most of *C. jejuni* strains were sensitive or intermediately sensitive to ampicillin, erythromycin and enrofloxacin.

Discussion

The prevalence of *Salmonella* isolated from healthy animals in 2002 and 2003 was very low. However, isolation of multidrug resistant *S.* Typhimurium harboring SGI1 is particularly interesting. Indeed, SGI1 and variants were described in other *Salmonella* serovars [4, 5] suggesting an horizontal transfer of this element and its contribution to the rapid dissemination of multidrug-resistant strains of *Salmonella* serotypes.

A decrease in the percentage of resistance to erythromycin and ampicillin was observed for *C. jejuni*. However, distribution of MICS showed that most strains were sensitive or intermediately sensitive to these antibiotics. Surveillance of resistance of *Campylobacter* is important to monitor over time to appreciate evolution of resistance. Moreover, use of antimicrobials in cattle should be analysed to evaluate the influence on resistant *Campylobacter*.

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PHENOTYPICAL EXPRESSION OF *Staphylococcus aureus* VIRULENCE FACTORS ISOLATED FROM DAIRY COWS WITH SUBCLINICAL MASTITIS

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Introduction

Staphylococcus aureus is an important economic and clinical pathogen of dairy cows (Lammers *et al*.,1999; Joo *et al.*, 2001). The prevalence for subclinical and clinical mastitis in cows is related to the level of infection in the herd and the management and hygiene conditions of the animals (Kaphur *et al*., 1995), associated to the genetic variability as well as the agent's pathogenecity (Lammers *et al*.,2001; Schuberth *et al.*,2001; Begoña e Iturralde, 2001). The objective of this study was to identify the phenotypical expression of *S. aureus* virulence factors in dairy production cows with subclinical mastitis in family production units.

Material and methods

288 milk samples were taken from 11 family dairy production units in the Toluca Valley by a randomly stratified sampling method from cows in different milk production stages (Farver *et al.,*1985; Magalhaes *et al*., 1990). Milk was cultured on Vogel Jonson and Baird Parker with potassium telurite (3.5%) agar plates for 24 hrs at 37ºC. *API Staph* (Biomeriux Vitek, Inc.) system was used for final identification of the isolations. The phenotypical characterization of *S.aureus* virulence was made by using protein A, latex agglutination, Staphylase test (Oxoid, USA); and coagulase tubes with rabbit, bovine and human plasma incubated at 37ºC. The human, bovine and canine biotypes were characterized on brain and heart agar with crystal violet (1:10000) (Cottral, 1986) The type of haemolysin (α, β, γ, δ) was obtained from blood agar containing erythrocytes from different species at 10% CO_2. Capsule was observed in soft agar culture tubes (0.035% brain and heart infusion supplemented with milk sera 4%). Pigment production was determined on dextrose manitol (10%) agar (Velázquez *et al*., 1994). *In vitro* sensitivity to antibiotics was made by Kirby-Bauer's method, using penicillin 10U, ampicillin 30*m*g, cephalotin 10*m*g and oxacyllin 1*m*g unidiscs (Beckton Dickinson USA). β- lactamase test was confirmed by the iodinemetric assay (Dike, 1979). The results were evaluated by using the estimation of proportion test and ANOVA to evaluate the inhibition ring for antibiotics (Thrusfield, 1990).

Results

38 isolations were obtained, with an infection rate of 13.2% ($p<0.01$). The biotypes observed were; 9% human, 2.5% bovine and 2.5% canine ($p<0.01$). The coagulotypes were; 77.5% rabbit, 10% rabbit-bovine and 12.5% rabbit-human ($p<0.01$). The pigment production was predominantly creamy yellow 87.5% and golden yellow 12.5% ($p<0.05$). The haemolyin types were 50% α, 25% β, 10% γ, 7.5% δ with common associations α-β, y α-β-γ ($p<0.01$). 87.5% isolations presented capsule ($p<0.01$). Antibiotic resistance was 94.7% penicillin, 97.3% ampicillin , 42% cephfalotin and 44.7% oxacyllin ($p<0.01$). β-lactamase production type I was present in 68.4% isolations ($p<0.01$).The relation between β-lactamase production and resistant strains was observed in penicillin, ampicilln, cephalotin and oxacillin ($p<0.01$).

Discussion

Virulence factors suggest a multifactorial participation in the infection to animal and humans. In the dairy population it is important the findings about multiresistant and resistant *S. aureus* strains (Bartlett and Miller ,1993). The significant variation between the virulence factors associated to *S.aureus* strains, is a risk factor for the presentation of mastitis in the herd (Leitner *et al.,*2003). The presentation of haemolysins types α, β, γ and δ was considered as an important virulence factor related to the resistance and capsule production as observed in other countries (Younis t **al.**,2000). It is possible to reduce the glandular infection by *S. aureus* as well as the somatic cell count by developing prevention, control and surveillance practices to diminish health risks.(Foster, 1986;Watts, et al., 1986; Watts, 1988; Daley, et al., 1991; Blood, 1986; Mc Donald, 1987; Velázquez, 1994). (Schukken et al., 1990; Nickerson, 1988, Ziv, 1995).

Conclusion

The economic loss in dairy cows by mastitis has motivated intensive research to increase the cow's resistance to intramammay infections and the evaluation of phagocytosis activity in mammary gland (Capuco *et al.,*1986; Dekker *et al.*,1994; Marcus *et al.*,1994; Schutz *et al*.,1994 Marcus and Shuster ,1994), with the epiemiological surveillance related to the phenotypical expression of virulence factors associated with the potenial pathogenicity in animal and human population.

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STUDY OF THE RELATIONSHIP BETWEEN ANTIMICROBIAL CONSUMPTION AND ANTIMICROBIAL RESISTANCE – APPLICATION IN POULTRY BROILER PRODUCTION

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Introduction

It is of worldwide concern that antimicrobials use in food-production animals might contribute to antimicrobial resistance increase. Observational (2) or experimental studies (4) sustained the hypothesis of a relation between antibiotic consumption and antibiotic resistance. Nevertheless, epidemiological approach rarely has been applied in veterinary medicine.

Objectives of the study were to analyse bacterial antibiotic resistance data according to the previous antibiotic exposition of animals sampled in order to identify relationships between antibiotic consumption and resistance.

Material et methods

As part of the French antimicrobial resistance monitoring program 300 caeca samples of broilers from three different production types are annually collected since 1999 by veterinary officers on the slaughterline. Randomly collected caeca are sent to the laboratory with historical background of antibiotic consumption of the broiler flock sampled. One *Escherichia coli* and *Enterococcus faecium* isolate per sample is subjected to minimal inhibitory concentration determination for several antibiotics, secondly converted in a dichotomous susceptibility variable using CASFM thresholds. Relation between antibiotic-susceptibility and characteristics of the broiler flock sampled and antibiotics consumed were explored through PLS and logistic regressions.

Results

649 *Escherichia coli* and 540 *Enterococcus faecium* strains were included in this study.

Statistically significant associations were found between antibiotic consumption and antibiotic resistance ($p<0.05$). Consumption of betalactamines signicifanctly increased risk of isolation of an ampicillin resistant *Escherichia coli* strain (OR=3.2, $CI_{95\%}$ =1.8-5.8). Same findings were found for *Enterococcus faecium*. Consumption of quinolones significantly increase the risk of isolating a nalidixic acid resistant *Escherichia coli* (OR=1.9, $CI_{95\%}$ =1.1-3.7). Broiler production type was also found to be a significant factor. The risk of isolating a nalidixic acid resistant strain increased when the age at slaughter decreased, associated with an increasing rearing density.

Discussion

This study emphasised the usefulness of large databases and pharmaco-epidemiological approach to study the relationship between antibiotic consumption and antibiotic resistance. Relations assessed between carriage of resistant *E. coli* and *E. faecium* at slaughter and previous antibiotic use are consistent with medical findings (3) and other veterinary studies (1).

Further studies are now needed to explore in a more detailed way relationships between antibiotic use and animal carriage of a resistant *E. coli* or *E. faecium* strain. Particularly, age at treatment, treatment dosages and duration are to be explored to identify putative 'at risk' treatment practices. In this study, broiler production type was also found to influence carriage of antibiotic-resistant bacteria for some antibiotics. The role of the rearing conditions (density, age at slaughter…) should be further explored too.

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Foodborne diseases - Zoonoses

Oral Communications

COORDINATED APPROACH TO CONTROLLING FOODBORNE ZOONOSES – ACHIEVEMENTS AND FUTURE PROSPECTS

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Food safety is a relative concept and not an inherent biological characteristic of a particular food. We can define safe food as one that does not exceed an acceptable level of risk in relation to microbiological, chemical and nutritional aspects of the product. Decisions on acceptability involve not only science-based ones but also perceptions, opinions and values. Thus where science-based approaches balance risk against benefit and cost, value-based approaches balance risk against dread and outrage perceived by stakeholders especially consumers. This partly explains why microbiological risks vary so much in their acceptable risk along the foodchain. For certain pathogens such as Verocytotoxigenic *Escherichia coli* O157 (VTEC O157) the acceptable level of risk may be as low as zero along most of the foodchain, whereas campyobacter's acceptance level may be higher. Acceptable risk levels for *Salmonella* strains vary depending on factors such as serotype and antimicrobial resistance. However, irrespective of the acceptable risk it is incumbent on all of us including the consumer to limit and reduce as far as is practical the contamination of our foodchain with potentially harmful microorganisms and intoxicants. Our food-supply chains whether international, national or local provide numerous opportunities from farm to fork for the microbiological contamination of food and water for human consumption.

Given the enormous number and variety of potential contamination sources along the food processing chain, it is unrealistic to imagine that all food can be kept free from contamination throughout the process. However, it is now recognised that one key way to enhance food safety is to identify the critical contamination points affecting the safety of the final product. It should then be possible to introduce the most effective measures to minimise or eliminate the possibility of contamination from food production and processing to distribution, preparation and consumption. Advances in the twentieth century such as pasteurisation, refrigeration and more recent improvements in hazard analysis and control along the foodchain have contributed to improvements to the microbiological safety of most foods. Nevertheless, foodborne disease remains a significant cause of morbidity and mortality in Europe and the rest of the developed world. The most recent national surveillance study in England and Wales revealed that one in five people developed infectious intestinal disease each year, and that *Campylobacter* and *Salmonella* were the most common bacterial pathogens isolated (9). In the United States it has been estimated that foodborne diseases may cause up to 76 million illnesses, 325,000 hospitalisations, and 5,000 deaths each year (7). In the same study *Campylobacter*, nontyphoidal *Salmonella* and VTEC accounted for the vast majority of bacterial foodborne disease requiring hospitalisation. *Toxoplasma gondii* and Norwalk-like viruses accounted for the great majority of severe cases of parasitic and viral infections respectively. These two recent studies bear out the generally high estimated national and international human incidence of foodborne pathogens, especially *Salmonella* and *Campylobacter* in most parts of the developed world (Table 1).

Table 1. Estimated 2002 incidence of bacterial foodborne zoonoses Incidence per 100,000 population

	Europe* [(a)]	USA[(b)]	Australia[(c)]	Japan[(d)]
Salmonellosis	**51**	**15.1**	**39.5**	**5**
Campylobacteriosis	**53**	**13.8**	**112**	**2.5**
VTEC infections	**0.7**	**1.6**	**0.3**	**1.5**
Listeriosis	**0.3**	**0.3**	**0.3**	**ND**
Yersiniosis	**4.0**	**1.0**	**1.5**	**ND**

* Austria, Belgium, Denmark, France, Germany, Ireland, Spain, Sweden, United Kingdom
a) European Commission, 2004 (6), b) Anon, 2002 (1), c) Yohannes et al. (10), d) Anon, 2003 (2)

Taken together this data confirms the continuing importance of food as a source of human illness and, in particular, of foodborne zoonotic diseases arising from the infection of farmed livestock throughout Europe with these microbial pathogens.

Estimates for economic loss covering health costs, lost production and family-related expenses are imprecise due to the paucity and non-standardisation of data and approaches to provide the estimates. However, in England and Wales *Salmonella* cases alone could cost in excess of £100 million each year and in the US in 1996 it has been estimated that *Campylobacter* spp., *Salmonella*, VTEC O157 and *T. gondii* cost the public purse 0.8-5.7; 0.9-3.6; 0.16-0.3 and $3.3 billion respectively (4).

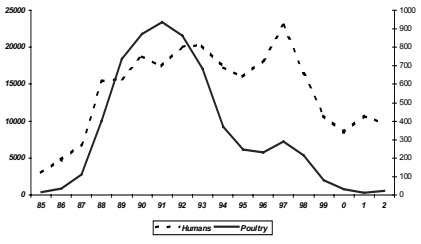
Integrated surveillance systems and molecular epidemiological tools are being used with increasing success to identify the contaminated food vehicles and sources associated with foodborne zoonotic agents and human outbreaks.. For example, *S. enteritidis* in poultry and in particular egg products, *Campylobacter* in poultry meat products, and *E. coli* O157 in ground and sliced beef , contaminated dairy produce and contaminated water sources. Many of these outbreaks and probably a number of sporadic infections can be avoided through correct hygiene procedures in the processing and handling of foods. However, only a co-ordinated farm to fork approach is likely to achieve permanent and significant reductions in foodborne infections in the future. Countries in the European Union operate within the new framework of the 2003 Zoonoses Directive 2003/99/EC (5) part of which is to monitor and control *Salmonella* and other specified foodborne zoonotic agents. The strategy focuses on the poultry breeding and layer sector and has, to a greater or lesser extent, demonstrated that an integrated and co-ordinated approach to controlling *Salmonella* in domestic livestock is feasible and leads to a sustained reduction in human incidences. This is best

exemplified by examining the relative effectiveness of the Danish control programmes via the published estimated food sources of human salmonellosis in Denmark in 2002 (Fig. 1)(3).

Fig.1. Estimated important food sources of human salmonellosis in Denmark, 1988-2002

Although admitted to be a rather imprecise assessment it clearly shows the dynamics in the changing sources of human salmonellosis over a 14-year cycle. Denmark experienced three waves of human salmonellosis, where the majority of cases were attributed to three different food sources. In the late 1980s broilers were the major food source, whereas in the mid 1990s pork products increased in significance and from the mid 1990s to the end of the century eggs and egg products predominated.
At each of the peaks of human salmonellosis new control programmes focusing on primary production were implemented and resulted in a reduction of human cases attributable to that particular food source (1988 – broilers, 1993 – pork, 1997 – table eggs).
In the UK there has been an association between the decline of laboratory reports of S. Enteritidis in humans and in the reduction of incidents in poultry in the last decade. Undoubtedly, the introduction of vaccination in poultry breeder and layer flocks in 1997 and 1998 has contributed to the sustained reduction of S. Enteritidis in UK poultry. flocks and probably impacted significantly on human cases (Fig. 2)

Figure 2. Salmonella Enteritidis in humans and poultry in UK 1985-2002



This provides clear evidence that intensive and co-ordinated *Salmonella* control programmes in animals can effect a reduction in human salmonellosis from that food source. It also provides sound evidence that controlling salmonella in animals is a very important control point in the entire foodchain and, at least in Denmark, is economically viable for the producers. It is too early to say how many other European countries can apply this approach since type and integration of poultry production varies considerably between countries. Recent reports indicate that a few countries have managed to reduce the prevalence of campylobacter in their broiler flocks but only time will tell us if this impacts on human cases and how economically viable these intensive biosecurity and monitoring programmes prove to be.

Some significant successes in reducing *Salmonella* infections in primary production with real impacts on human cases in many countries, should not detract from other real challenges for the future. *Campylobacter* infections are now the commonest cause of bacterial foodborne infections and in the most recent study in the UK, ranked second in the list of organisms isolated from cases of infective diarrhoeal infections. Human VTEC O157 cases have continued, implicating a variety of contaminated food sources and direct animal to human contact. *Cryptosporidium* either alone or as mixed infections with other foodborne pathogens continues to cause large outbreaks through contamination of water supplies. Increase and emergence of antibiotic resistance and multiply resistant bacterial strains, some of which have arisen from animals and their environment, highlights the immediate requirements for improved surveillance methods and alternative strategies for controlling infections. Potential emergence of new *Salmonella* epidemic strains e.g. multi-resisitant S. Newport in the US and S. Java in parts of continental Europe and continued evolution of VTECs highlights the need for robust early-warning systems and greater understanding of the mechanisms of genetic mutation and adaptation (8).

The farm to fork concept has encouraged closer co-operation between all sectors of the food industry and as stated above achieved some considerable successes in reducing *Salmonella* in livestock. However, it is likely that the most cost effective way within limited resources in targeting longer term strategies to control foodborne zoonotic infections in animals, is to focus on emerging trends in human infections caused by the major foodborne zoonotic pathogens, since most of these organisms are asymptomatic in animals. Thus integrated research and surveillance of animal and human foodborne zoonotic infections is crucial to future strategies. For example, rapid dissemination of changing trends between veterinary and medical sectors to improve response to emerging pathogens, changes in antimicrobial resistance patterns, co-ordination of surveillance systems that can accurately identify results of intervention methods implemented along the food chain and co-ordination and integration of research objectives. Thus the original farm to fork approach often ignored the key component along the food chain which should help to formulate future

strategies i.e. seamless and co-ordinated foodchain strategy. It is also now recognised that many of the most effective solutions will not be pathogen specific but focus on factors common to many different organisms in the foodchain. There are a number of recent initiatives that seek to address coordination at the national, European and global arenas and some examples are described below.

National

Close co-operation between veterinary and public health sectors is vital for the early identification of new and emerging zoonoses and to monitor and act upon changing trends in the epidemiology of foodborne pathogens. For example, in GB the Health Protection Agency and the Veterinary Laboratories Agency have initiated a programme for the harmonisation of laboratory methods for the detection and identification of a range of pathogens such as salmonella, campylobacter, VTECs including O157 and O26 and measurement of antimicrobial resistance. This will facilitate improved comparison of surveillance and incident data along the foodchain and will be used to compare temporal trends. Likewise standard approaches to the molecular typing of these organisms have already improved the management of outbreak investigations. For example, a recent local outbreak of S. Java in cattle in the England was enhanced by the rapid determination of the clonal type that was found to be unrelated to the European epidemic strain. In parallel, increased joint reporting of pathogens and antimicrobial resistance patterns from veterinary, food and medical sources increases the awareness of these organisms in terms of public health importance – initiatives that have been long established in some other countries e.g. Denmark and Sweden.

European

Following a series of food scares in the 1990s (eg BSE, dioxins…) which undermined consumer confidence in the safety of the food chain, the European Union concluded that it needed to establish a new scientific body charged with providing independent and objective advice on food safety issues associated with the food chain. The result was the European Food Safety Authority (EFSA). Established in 2002 and soon to be moving to a permanent site in Italy, EFSA provides independent scientific advice on all matters linked to food and feed safety - including animal health and welfare and plant protection - and provides scientific advice on nutrition in relation to Community legislation. The Authority communicates to the public in an open and transparent way on all matters within its remit. EFSA's risk assessments provide risk managers in Europe and elsewhere with a sound scientific basis for defining policy driven legislative or regulatory measures required to ensure a high level of consumer protection with regards to food safety. EFSA is now dealing principally with requests for risk assessments from the European Commission and plans to take on a wider brief from other European institutions in the near future.

EC COST Action funding is a very flexible and efficient means of linking research and surveillance activities across Europe. COST 920 – *Foodborne zoonoses : a co-ordinated foodchain approach* is an ongoing action that seeks to co-ordinate information along the foodchain to facilitate control of foodborne pathogens in 22 participating European countries. In particular, it draws together colleagues working along the foodchain to consider and present their most recent research and surveillance information on :

- comparability of surveillance data
- proactive approaches to new and emerging zoonoses
- integration of risk assessment into foodchain activities
- in-depth understanding of the foodchain hazards

The programme comprises four integrated working groups :

1. isolation, identification and typing methods
2. new and emerging foodborne pathogens
3. quantitative foodchain risk assessment
4. survivial of zoonotic pathogens along the foodchain

Outputs and proceedings of the various activities and meetings can be located at www.Cost920.com .

Complementary to the co-operative activities in COST 920 is Med-Vet-Net (MVN) a new European network of excellence for the integration of veterinary, medical and food sciences in the the field of food safety (15 partners in 10 countries). The objective of MVN is to improve research on the prevention and control of zoonoses while taking into account the public health concerns of consumers and other stakeholders throughout the foodchain and is due to begin in the latter half of 2004. This initiative has the scope to fund not only targeted research activities but the dissemination of results at meetings and will work closely with COST 920. Further information can be found at www.medvetnet.org/

Global

The World Health Organization (WHO) recognises the importance of foodborne diseases worldwide and has estimated that in less developed countries approximately 1.8 million people die, most of whom are children, from food and waterborne diarrhoeal diseases. A significant proportion of these are caused by zoonotic organisms originating from animals or their environment in the primary production sector of the foodchain. In 2002, the WHO published their Global Strategy for Food Safety with the goal to reduce the health and social burden of foodborne disease. Key approaches include the improvement of current surveillance of foodborne diseases through an interdisciplinary approach including all sectors dealing with foodborne diseases and food safety in both the health and agriculture sectors. Effective integrated surveillance is vital for the formulation of national and global strategies to reduce food-related risks. Improved microbiological risk assessment and risk communication are also very important by a) providing a tool to set priorities for future interventions that will improve public health through the reduction of microbiological hazards along the entire foodchain and b) developing methods to effectively communicate the risks

to stakeholders, including consumers, in a clear and understandable manner.

There are some good examples of how this strategy is being applied globally in a practical way to control foodborne diseases. Global Salm-Surv (GSS) is a global network of laboratories and individuals involved in surveillance, isolation, identification and antimicrobial resistance testing of *Salmonella*. It is targeted to microbiologists and epidemiologists who work in public health, veterinary services, food-related services and environmental health. Its aim is to strengthen the capacities and expertise of WHO member states in the surveillance and control of *Salmonella* infections and to contribute to the effort of reducing antimicrobial resistance in foodborne pathogens. It is being extended to other major pathogens including *Campylobacter*. Information can be found at www.who.int/salmsurv/en/ .Enter-net is a European funded global surveillance network for the enteric infections Salmonella and VTEC O157 and concentrates on the harmonisation of methods and maintenance of a timely international database. Considerable achievements have been made in the early identification of global outbreaks particularly *Salmonella* that have lead to effective public health measures being applied to limit the impact of the outbreak by, for example, identifying the contaminated source and removing it from the foodchain. This has proved to be a highly effective network by targeting and identifying potential human international outbreaks. It is hoped that it can be enhanced further by including animal data that will contribute to the identification of animal sources involved in future outbreaks and hence support the sustained control of foodborne pathogens by targeting primary production as well as contaminated food and food products. Details can be found at www.hpa.org.uk/hpa/inter/enter-net .PulseNet USA and PulseNet Canada are national networks of public health and federal food regulatory agency laboratories in North America who routinely perform standardized molecular subtyping of foodborne disease-causing bacteria and then share DNA 'fingerprints' electronically in real-time via Internet. The aim is to :

- detect foodborne disease clusters
- facilitate early indentification of common source outbreaks
- assist epidemiologists in investigating outbreaks
- assist in rapidly identifying the source of outbreaks

The following examples provide clear evidence of the contribution of PulseNet in recent foodborne outbreaks :

- 1997 : 16 VTEC O157 infections linked by Pulse Field Gel Electrophoresis (PFGE) in two states, 25 million pounds of ground beef recalled.
- 1998 : 486 *Shigella* infections in 3 states in Canada traced to parsley imported from Mexico.
- 1999 : Salmonella infections in 22 states traced to mangoes imported from Brazil.
- 2000 : Salmonella infections in 8 states, linked to orange juice produced using new, whole fruit pasteurization process.
- 2001 : Multistate *Listeria* outbreak linked to deli turkey meat.

The success of PulseNet in North America has provided the impetus for a similar PulseNet Europe network to be considered that proposes to harmonize with PulseNet in North America but to enhance the sharing of molecular data between the public health, food and agriculture sectors. This is an important initiative that attempts not only to enhance the current surveillance of human foodborne pathogens but to extend the network to the important primary production sector. If successful it will not only impact on the control of foodborne outbreaks in Europe but provide valuable new information on the sources and spread of potentially epidemic foodborne zoonotic pathogens in farmed livestock that will enhance active veterinary surveillance and help to sustain reductions in foodborne pathogens along the entire foodchain.

Summary

By the beginning of the twenty first century foodborne zoonoses have become the major cause of infectious intestinal disease in humans in many developed countries, replacing infections classically associated with poorly developed sanitary and housing conditions such as cholera, typhoid and dysentery.

The integration and globalisation of food production along the foodchain has facilitated the rapid spread of infections such as *Salmonella* and *Campylobacter*. The most notable example being the *S.* Enteritidis pandemic of the 1980s and 1990s. However, other factors including the emergence of new foodborne pathogens have also had a considerable impact. In particular, the emergence of VTEC O157 and spread of multi-resisitant *Salmonella* strains through the likely acquisition of relevant genetic material by an otherwise commensal or antimicrobial sensitive bacterium. These are powerful examples of the potential of new and emerging pathogens spreading rapidly through animal and human populations.

Paradoxically, the integration and globalisation of food production, also offers improved opportunities for the control of many of these pathogens.

Considerable progress has been made in the coordination of activities along the foodchain, and this paper has cited excellent examples of national, international and global sharing of data and intelligence that are having a practical impact on controlling foodborne pathogens in animals, foods and humans. The ability to rapidly share surveillance data is crucial in the battle to improve the quality of our food. The development of horizontal (geographical) and vertical (foodchain) data networks is very much in its infancy and is becoming a significant challenge, but much greater integration is vital if we are to contunue to build on recent successes. Furthermore, food safety risk analysis will need to combine robust mathematical models with secure quantitative microbiological data to reduce the level of uncertainty in many of the current risk assessments. This will facilitate accurate identification of those points along the foodchain that contribute most cost effectively to the control and spread of the organisms. As a result, improved cost effective control programmes should be developed, thus offering new opportunities to countries in which hitherto control methods have been considered too expensive. The lesson of the last thirty years has also clearly

indicated the importance of surveillance in any control programme, not only to identify emerging problems, but to demonstrate effectiveness of the programmes themselves.

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SOURCES OF *CAMPYLOBACTER SP.* CONTAMINATION OF PIGLETS IN FARROWING UNITS OF FARROW-TO-FINISH FARMS: FIRST RESULTS

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Introduction

Campylobacter sp. is one of the most frequent cause of human enteritis with *Campylobacter jejuni* more commonly implicated than *C. coli*. *Campylobacter* sp. has been isolated from raw beef, pork, limb, chicken and cooked meats. Campylobacters are often found in digestive tract of pigs [2, 8, 12, 14, 10, 3, 15, 4, 6]. *C. coli* is the large predominant species [12, 14, 4, 6] but *C. jejuni* was also isolated in association with *C. coli* [3, 15, 9]. *Campylobacter* colonization of the pigs seems to occur at an early age [3, 13, 15]. In France, little information about intestinal carriage of *Campylobacter* sp. in pigs is available. The purpose of the present investigation was to improve our knowledge of the epidemiology of *Campylobacter* in pigs.

Materials and methods

Sampling (table 1): The samples were collected from 9 pigs farms, randomly selected, situated in the western part of France. The farms were confined farrow-to-finish operations of intensive type and managed using the batch procedure and an all-in/all-out hygiene policy for farrowing, post-weaning and fattening sections. Three batches per farm were tested over a year. For each batch, 10 nursing dams randomly selected and 4 piglets from litters were tested for Campylobacter. Rectal fecal samples were collected once from piglets and the nursing sows. In addition, in 6 farms at each visit, water and piglets feed samples were taken.

Bacteriological analysis : All samples were transported to the laboratory at < 10°C. For water and "food" samples, the presence or absence of *Campylobacter* in each sample was tested by selective enrichment in Preston broth. For all the samples, some drop of each suspension were plated in duplicate on each following media : Butzler agar, Karmali agar. Agar plates were incubated at 42 °C for 5 days in microaerophilic conditions (5% O_2, 10% CO_2, 85 % N_2), and examined daily for presumptive *Campylobacter* colonies. Suspect colonies were confirmed as a member of the genus by examining motility, Gram staining and morphology. Three *Campylobacter* colonies per sample (when present) were randomly selected for genetic typing and stored at -80°C until further use.

Molecular analysis : Each isolate was identified as *Campylobacter jejuni*, *C. coli* or *Campylobacter sp.* by PCR. Multiplex PCR was performed using primers and conditions previously described by van de Giessen *et al* [11] . The PCR-RFLP typing method was used in order to obtain additional information about infection cycles of *Campylobacter* on the farm. For this analysis, all the isolates of one farm (farm A) were typed. The *fla*A gene encoding flagellin A was amplified with a pair of primers previously described by Nachamkin *et al.* [5] and Chuma *et al.* [1]. The 1700 bp product was digested overnight at 37 °C with the restriction enzyme DdeI according to the manufacturer's instructions. The digest products mixed with loading buffer, and PCR 100 bp ladder, were then electrophoresed in TAE buffer for 45 min at 100 Volt, 400 mA, through a 2 p.100 agarose MS-8 type gel with 1µg/mL ethidium bromide (Euromedex). The analysis of the gel electrophoresis image was done with Bio 1D++ software (Vilbert Lourmat).

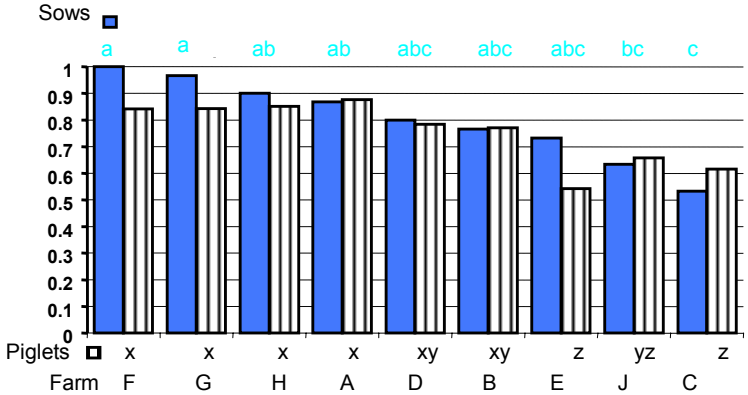
Statistical analysis : Data were analysed with SAS sofware (SAS Institute) using SAS analysis of variance (PROC GLM). A pig tested positive if it gave at least one isolate of *Campylobacter* using the isolation procedure described above. Data analysis were performed utilizing Chi square and Student-Newman-Keuls' test (SNK).

Table 1 : Characteristics of the 9 farrow-to-finish farms and of the sampling during the three visits in each farm.

	9 farms				
	F	D, I, B, H	C, E	G, A	
Number of sows	70	100 to 150	220 to 230	450	
Fattening pigs produced/year	1600	1700 to 2800	4000	9500	*Total samples analysed*
Sows tested/farm	3 X 10 (except farm F only 7 sows/batch)				**261**
Piglets tested/farm	3 X 4 X 10 (7) 24,9 (σ = 1,1) days				**1036**
Water tested/farm	3 X 2 (farms A, B, C, D, E, F)				**6 L/farm 6 farms**
3 dry feed samples/farm	3 x 3 x 25g (pellets, meal) (farms A, B, C, D, E, F)				**75 g/type of food/farm 6 farms**

Results

Campylobacter was not detected from water samples and feed samples. On all the 9 farms, pigs were heavily contaminated by *Campylobacter*. *Campylobacter* was recovered from 75 % of the faecal samples collected from the 1036 piglets and 79 % from the 261 sows. Nevertheless some differences between the 9 farms in the number of pigs tested positive for *Campylobacter* were statistically significant (p < 0.005 SNK test) (fig.1).



Values with different letters are significantly different (P<0.005)-SNK test

Fig. 1. Prevalence of *Campylobacter coli* in piglets and their nursing sows from the 9 farms.

The contaminated sows had more contaminated piglets than negative sows (fig. 2). A total of 1.100 isolates were obtained. On the basis of identification with multiplex-PCR, *C. coli* was the only species recovered from the faecal samples. First results of PCR-RFLP subtyping showed a large diversity of *Campylobacter* subtypes isolated from the pigs (farm A). Nevertheless piglets and their nursing sows in a same batch often harboured *Campylobacter* isolates with identical genetic subtyping profiles (fig. 3).

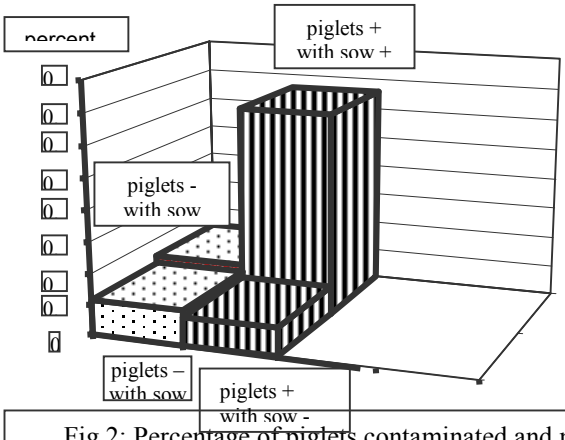


Fig 2: Percentage of piglets contaminated and not contaminated according to the status of their nursing

Discussion

The high prevalence rates reported in this study agree with other results indicating prevalence of *Campylobacter* of 85 % amongst piglets [14, 15] and at 75 to 100 % amongst sows [14, 15]. Young *et al.* [15] have described a predominant infection of pigs by *C. jejuni*. In our study and in agreement with Nesbakken *et al.* [6], *C. jejuni* had never been isolated from the faecal samples. These findings suggest that the prevalence of the respective species might differ considerably between breedings companies and countries. An other explanation may be the use of different identification procedures. This study confirms that piglets are already intestinal carriers of *C. coli* at the age of 25 days on the piggeries. The direct transmission of *C. coli* from the infected sows to piglets is attested by PCR-RFLP results. The similarities between genetic subtyping profiles of strains isolated from families of pigs (nursing sow and her piglets) and from subsequent groups of pigs housed in a same batch suggest that *C. coli* strains isolated are more dependent on the origin of contamination (sows) than on the farm [14]. Nevertheless, some genetic subtyping profiles are different between sow and her piglets in a same family of a batch [4, 14], and, some piglets were tested positive although their nursing sow was tested negative. This suggests that other sources of piglets contamination by *C. coli* than the nursing sows exist. Despite negative results in our water and feed samples analysed, the piglets environmental source (other sows of the batch and of the farm, hygienic practices of the farmer …) of contamination by *Campylobacter* sp. can not be exclude [7]. But adoption practices appear to be a major risk factor in the dissemination of *C. coli* into the farm [3, 13]. Contrary to other studies, focused on only 1 or 2 farms [14, 3], our survey reveals that there is a significant distinction between the level of contamination with *Campylobacter* of the pigs on these farms.

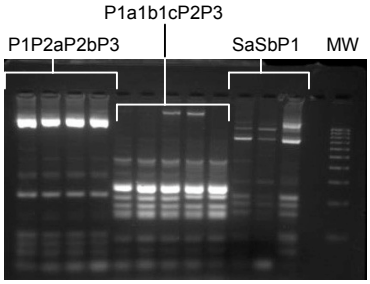


Fig. 3. Examples of PCR-RFLP *Campylobacter* subtypes isolated from piglets (P) and sows (S) of a same batch (legends: MW=molecular weight ; a to c= three isolates/animal)

Conclusion

Further studies are needed to identify risk factors in the dissemination of *Campylobacter sp*. in farms and to evaluate the impact of this infection of pigs on the meat and process contamination.

Acknowledgements

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AN INVESTIGATION OF THE EFFECT TRANSPORT AND LAIRAGE OF FEEDLOT CATTLE ON THE CAMPYLOBACTER PREVALENCE IN FAECES AND ON DRESSED CARCASSES

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Introduction

Campylobacter is an emerging zoonotic pathogen worldwide. In Ireland, there were 2,085 and 1,613 laboratory confirmed cases of illness due to *Campylobacter* spp. in 1999 and 2000, respectively (Whyte and Igeo, 2000; Foley and McKeown, 2001). The possibility of low infectious does combined with high incidence of infection and the serious potential sequelae for man makes campylobacteriosis an important public health disease.

Most patients with campylobacteriosis present as sporadic cases and person-to-person transmission is uncommon (Pebody *et al.*, 1997; Alterkruse *et al.*, 1998). Poultry has been identified as a significant risk factor for sporadic Campylobacteriosis (Kapperud *et al.*, 1992; Lior, 1996). However, cattle produce and direct contact have also been associated with Campylobatceriosis (Butzler and Oosterom, 1991; Skirrow, 1991; Pearson and Healing, 1992; Troutt and Osburn, 1997; Fitzgerald *et al.*, 2001; Neimann *et al.*, 2003).

Campylobacter is an organism often found in the gastrointestinal tract of cattle with reported prevalences ranging from 0-80% (Munroe *et al.*, 1983; Rosef *et al.*, 1983; Giacoboni *et al.*, 1993). The aim of this study was to investigate what effect, if any transport and lairage of cattle before slaughter had on the qualitative and quantitative shedding of *Campylobacter* in their faeces.

Material and Methods

A cohort of 109 heifers aged 27 to 30 months from a single Irish feedlot were investigated. The cattle were housed in four adjacent pens and remained in their assigned pen until slaughter. The cattle were then transported during March and April in three groups of 44, 44, and 21 respectively on three separate days for slaughter. The duration of the transport was six hours. The animals were held in lairage over night (12 hours) with *ad libitum* access to water. The abattoir used in this study was an approved Irish export abattoir, processing approximately 80,000 cattle per year with a throughput of 60 animals per hour during sampling for the study. The decontamination procedures used in this abattoir consisted of trimming and a hot water (62°C) carcass wash.

Individual rectal faecal samples were taken from the cattle at the feedlot on the day of transport approximately 4 hours prior to departure, then immediately upon arrival at the lairage, and again following lairage immediately prior to slaughter. At the abattoir all carcasses were swabbed in the chillroom within thirty minutes of entry, as previously described (Minihan *et al.*, 2003). The level of *pre-mortem* hide faecal contamination of the cattle was assessed immediately prior to slaughter by utilising a subjective tag scoring system (0-9).

Campylobacter was isolated using an enrichment (Preston broth) and selective agar (modified charcoal cefoperazone deoxycholate agar). Colonies with the characteristic gross morphology and curved or spiral Gram-negative rods were presumptively identified as *Campylobacter*. The limits of detection of *Campylobacter* was < 1 log CFU.gram^{-1} of faeces and 2 log CFU.4000cm^{-2} of carcass surface. Campylobacters were enumerated for 30 animals at each of the sampling points onto mCCDA. Colonies with the characteristic gross morhopology were counted as *Campylobacter* colonies. Each *Campylobacter* was identified to the species level by the use of a biochemical test (Camp ID^{TM}, Mast Diagnostics, Merseyside, England)or polymerase chain reaction (PCR) amplification of the 23S rRNA target.

Bacterial counts obtained were transformed to log $_{10}$ CFU/gram of faeces. The effect of transport and both transport and lairage combined on the quantitative shedding of *Campylobacter* per gram of faeces was compared using a paired Student t-test with significance defined at the 95% level ($P \leq 0.05$). The *Campylobacter* shed per gram of faeces for each sampling point was compared between each visit using a unpaired t-test with significance defined at the 95% level ($P \leq 0.05$). The prevalence of faecal *Campylobacter* shedding at each of the sampling points was compared statistically using a Chi-square test, with significance defined at the 95% level ($P \leq 0.05$). The "StatView 5" programme (SAS Institute Inc., USA) was used for the statistical analysis.

Results

Campylobacter spp were isolated from 191 (58%) of the of 327 faecal samples from all three sampling points. 82% (90) of the 109 animals in this study shed *Campylobacter* on at least one occasion during this study. At each of the three sampling points *Campylobacters* were isolated from 62 (57%), 60 (55%) and 69 (63%) of the farm, post-transport, and post-lairage samples respectively. There was no significant difference in the levels of *Campylobacter* faecal shedding at each of these sampling points.

Of the 191 *Campylobacter* isolates 179 were identified to species level, consisting of 70% (126) *C. jejuni* and 30% (53) *C. coli*. The proportion of *C.jejuni* to *C. coli* shed in the cattle faeces changed significantly between the farm and post-lairage sampling points ($P < 0.05$).

A trend of decreasing numbers of *Campylobacter* spp. shed in cattle faeces from farm to post-transport and post-lairage was observed during all three visits. The observed reduction in *Campylobacters* shed per gram of faeces between sampling at farm and post-lairage was statistically significant for visit one and for the combined data from the three visits ($P < 0.05$).

Campylobacter was not recovered from the swabs of the 109 dressed carcasses at the abattoir. Visual inspection of all the dressed carcasses in the present study revealed no visible faecal contamination. The mean hide tag score of the 109 animals immediately before slaughter was 4.8, ranging from 2 to 9.

Discussion

The prevalence of *Campylobacter* spp. shed in faeces in our study was high at 58% for feedlot cattle, but consistent with previous reports (Garcia *et al.*, 1985; Giacoboni *et al.*, 1993). However, others reported a contrasting lower prevalence of cattle *Campylobacter* spp. faecal shedding, ranging from 0.8 to 2.5% (Prescott and Bruin-Mosch, 1981; Rosef *et al.*, 1983; Warner *et al.*, 1986).

The shedding prevalence between farm and post-lairage only differed by 6% (57% to 63%). Transport and lairage neither increased nor decreased significantly the prevalence of faecal *Campylobacter* shedding ($P > 0.05$). These results are consistent with a previous study (Beach *et al.*, 2002). These insignificant changes in faecal shedding prevalence of *Campylobacter* spp. in response to the transportation and holding of cattle in lairage, contrast with those observed for other enteropathogens including *Salmonella* spp. in cattle and pigs, which have been shown to increase from farm to abattoir (Berends *et al.*, 1996; Barham *et al.*, 2002; Beach *et al.*, 2002). We postulated that differences in the faecal shedding prevalence of *Campylobacter* and *Salmonella* in might be accounted for by differences in the colonisation mechanisms of these bacteria.

Transport and lairage resulted in a significant reduction (1 $\log_{10}$ CFU.gram^{-1}) in the number of *Campylobacter* spp. shed per gram of faeces. This finding contrasts with a study which reported a ten-fold increase in faecal *Campylobacter* counts shed by broilers after transport (Whyte *et al.*, 2001). The authors postulated that the difference in the mean counts after transport and lairage could be due to differences in the mechanisms of colonisation and the responses of the hosts to the stresses of transportation and lairage.

In the present study, no *Campylobacter* spp. were detected on dressed carcasses, demonstrating that known positive cohorts of cattle, with a wide range of tag hide scores may be slaughtered and processed to produce clean carcasses by following good hygienic practices.

Conclusion

Transport and lairage did not result in an increase in the number of animals shedding *Campylobacter* spp. in faeces. In addition, transport and lairage result in a 1 $\log_{10}$ decrease of *Campylobacters* shed per gram of cattle faeces. The study demonstrated that an observed low level of this pathogen could be achieved on dressed carcasses when appropriate standards of hygiene are attained and maintained in abattoirs.

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SEROLOGICAL AND EPIDEMIOLOGICAL INVESTIGATIONS FOR ANALYSING RISK FACTORS OF SALMONELLA INFECTIONS IN PIG HERDS

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Introduction and Objectives

Salmonella causes important zoonotic diseases. Nearly 20 % of human salmonellosis in Germany are due to pork products (Steinbach et al., 1999). Salmonella contamination of food can occur in the primary production, at the slaughter and in the food production chain. In some countries Salmonella surveillance - and - control programs were implemented few years ago. In Denmark, for example, the pig herds are classified into three different categories, depending on the seroprevalence of meat-juice samples collected at slaughter. The true herd prevalence of Salmonella spp. in pigs and pork has markedly declined when comparing the status before starting the program and the situation 4 years later (Andersen et al., 2001). In Germany, a Salmonella control program is now running on a voluntary basis. For the implementation of effective control measures further investigations are needed to get more information about the infection dynamics of Salmonella in pig herds.

Material and Methods

The study was performed in 52 breeding herds. For the serologic monitoring, blood samples (10 per herd) were collected in 4-week intervals. The sampling was restricted to pigs at an age of 5 month.
The monitoring period was 3 years and in total about 13500 samples could be included. Serology was determined by use of an ELISA (Salmotype®, LDL, 04109 Leipzig).
For the risk factor analysis all herds were visited once. The data collection is based on a questionnaire and an examination of the pig herds and and their environments. The seroprevalences are evaluated via statistical description and by means of the (generalized) linear model.

Results

The statistical analysis of the optical density values of the ELISA test shows markedly seasonal variations. In the summer quarters 2 and 3 the average optical density values about all herds are much lower than during the winter quarters 1 and 4. The factor “time of the year” has a statistical significant ($p < 0.05$) stronger influence on the optical density values than the factor “herd”. Nevertheless the statistical analysis shows that the factors “herd” and “time of the year” do only explain 30% of the OD's variation.

Discussion

The investigations show a higher prevalence in pigs with antibodies against Salmonella during winter (quarter 1 and 4) than during summer. This could possibly explained with a higher rodent burden during the winter months which could be responsible for a higher Salmonella-infection-rate in this period.
The higher prevalence of other diseases and the strong climatic variations in winter can also lead to a higher susceptibility of the pigs for Salmonella- infections.
Our results show that high prevalence of Salmonella antibodies in summer has to be judged more critical than high prevalence in winter.
However, earlier investigations in Iowa achieved different results, the highest prevalence of Salmonella antibodies could be observed in quarter 3 (Baum et al., 1998).
Overall, this shows that additional risk factors have to be taken into account, and that factors on the farm level have to be investigated.

Conclusion

For the characterization of others factors influencing the OD- values further statistical analysis of the results of the questionnaire and the herd examinations are the objective of the ongoing data analysis. The factors that are focused at are such as: herd size, animal movement, biosecurity, rodent control, cleaning and disinfection, bird control, feed storage and many others more.

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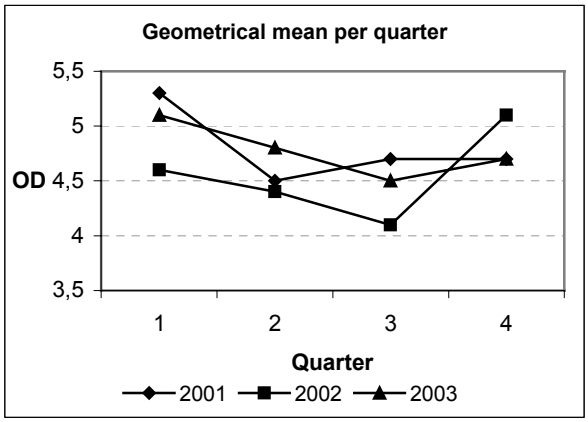
Figure 1: Geometrical mean of the OD- values per quarter



INVESTIGATION OF THE ORIGIN AND ATTEMPTED CONTROL OF *SALMONELLA* ENTERITIDIS PT6 INFECTION IN TABLE EGG PRODUCTION

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Introduction

Ovarian or vertical transfer of infection from breeding hens to progeny has been an important aspect of the epidemiology of *Salmonella enterica* serovar Enteritidis (*S.*Enteritidis) infection within the poultry industry. Although both poultry meat production and egg production have been involved in *S.*Enteritidis infection in humans, contaminated eggs are currently thought to be the biggest risk in the UK (ACMSF, 2001). There is little published work relating to the layer breeder sector. This paper describes studies on the distribution and attempted control of *S.*Enteritidis PT6 at various stages in layer breeding and commercial egg production.

Material and Methods

S.Enteritidis PT6 in the hatchery, breeder rearing, parent and commercial rearing farms was identified from notifications to the Zoonoses Order Database. *Salmonella* on the commercial laying farm was identified by follow up but not by routine monitoring. 200-400 samples were taken at each farm. The samples were taken directly into 225 ml of buffered peptone water (BPW) using gauze surgical swabs and consisted of approximately 25 g faecal material or 10 to 15 g dust or other dry environmental samples or surface swabs.

The BPW was returned to the laboratory under ambient conditions on the day of collection and incubated at 37^{o}C for 18 hours. Subsequent culture was in Diasalm (41.5^{o}C, 24/48 h) and Rambach Agar (37^{o}C, 24 h). Isolates were confirmed biochemically and serologically. Genetic typing of *Salmonella* was carried out using a combination of plasmid profile analysis, pulsed field gel electrophoresis and PsH.Sph1 Ribotyping as previously described (Leibana et al, 2001).

Results

The layer breeder hatchery was visited on three occasions after hatching the last eggs from the breeding flock which was infected with *S.*Enteritidis PT6 (PT6) had been terminated. At the first visit significant contamination was found in samples from hatcher incubators (4/96 [7.1%]), chick handling areas (9/58 [15.5%]), tray wash machine and surroundings (6/32 [18.7%]) and waste handling areas (8/9 [88.9%]). This contamination resulted from overdilution of disinfectants by inaccurate metering devices in the power washer and tray wash machine. Once these faults were corrected repeat sampling four weeks later identified only three *Salmonella* isolates from 340 samples and these were in waste disposal areas only. A third visit found only one isolate, in the footwell of a chick delivery vehicle, and this was *S.*Typhimurium DT208, which is normally associated with pigs.

Samples were taken before and after cleaning and disinfection in the infected breeding farm which previously supplied eggs to the hatchery and in two rearing farms. A low level of contamination was found prior to cleaning but after disinfection and fogging with formaldehyde based disinfectants little PT6 was found and there was no reoccurrence of infection in subsequently housed flocks.

PT6 was identified in chicks orginating from the contaminated hatchery in four of six commercial layer rearing houses. After two rounds of fluoroquinolone/competitive exclusion (FQ/CE) treatment no *Salmonella* was found in 60 faecal and environmental samples from each of five of the houses taken when birds were 16 weeks but in the sixth house no *Salmonella* was found in 48 bird level samples but eight of twelve dust samples contained PT6. Sampling carried out after cleaning and disinfection in this house showed significant residual contamination (2/53 floor surfaces, 4/34 ventilation ducts, 3/14 service area floor, 10/40 house surroundings). The house was redisinfected and no *Salmonella* was found in any of the subsequently housed flocks at 16 weeks of age.

Results of sampling four flocks after placement of laying birds from the pullet house where dust was found positive in a large cage laying house are shown in the table. Initially *S.*Enteritidis PT4 predominated in the cage house after further FQ/CE treatment but later in the life of the flock PT6 had increased. Both PTs survived cleaning and disinfection, which was carried out poorly, using a peroxygen disinfectant, and despite vaccination with killed (second flock) and live *S.*Enteritidis vaccines (third and fourth flocks) infection persisted. The administration of competitive exclusion to the third and fourth flocks after placement was also unsuccessful.

Samples taken from the five other houses on site showed similar, but higher, levels of infection with PT6 and similar lack of efficiency of cleaning and disinfection, vaccination and competitive exclusion. During this study all laying houses were sampled several times and *S.*Enteritidis PT6 had spread to these and persisted in each of the houses despite similar interventions.

Molecular fingerprinting of PT6 and other strains associated with the breeding company showed that two genotypes of PT6 were originally present in an independent breeding farm which has been contracted to the breeding company and this was thought to be the original source of the PT6 for the breeding company. One of these genotypes was not found elsewhere in the company, but one type was also found in the hatchery, rearing and breeding sites. Another related genotype of PT6 was also found in the rearing sites, breeding site, hatchery and commercial pullet rearing and cage layer farms. A further type was only present in the rearing site. PT3, 34 and 25 strains from the hatchery were all the same genotype, which was closely related to the PT6s, whereas one PT4 strain found in the hatchery was quite

distinct. The PT7 strain was closely related to the PT6 found on the rearing site.

Discussion

The work carried out in this study demonstrated the potential for contamination and cross-contamination in hatcheries and also the mistakes which can be made when too much reliance is placed on disinfectant metering devices. Correction of this problem rapidly curtailed the contamination issue but in other hatcheries contamination of incubators has been a much greater problem. This was not the case here because of routine use of formaldehyde evaporation during hatching and the reduced ability of *S.*Enteritidis to permanently colonise equipment, hatcheries and feedmills. Disinfection with formaldehyde based disinfectants was successful on breeding, and breeder rearing farms but peroxygen products were less successful on the pullet rearing farm and, in particular, on the cage farms. This correlates with previous experiences in which peroxygen disinfectants appear to be readily inactivated by residual organic matter. This can be partially overcome if they are used at high concentrations of 2-5% but then they become excessively corrosive for metallic equipment.

The persistence of *S.*Enteritidis in the cage layer houses is consistent with other problem farms operated as multistage sites. In these cases the combination of inadequate cleaning and disinfection, poor control of farm pests and presence of other infected flocks on site facilitates early infection of incoming pullets despite vaccination with killed bacterin or live *S.*Enteritidis or *S.*Gallinarum vaccines. The use of competitive exclusion in this case was not helpful but because the large houses were repopulated in stages administration to some birds was delayed for several days after exposure to infection.

Use of molecular typing for individual phage types of *S.*Enteritidis is difficult as these are highly clonal but the combined approach used in the study provided sufficient discrimination to demonstrate distribution of the organism via the breeding company and also diversification of genotypes, which also occurred with phage types in the hatchery, during the course of the outbreak.

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Distribution of Salmonella in cage layer house containing previously infected pullets

	Ends of Egg Belts	Dust	Floor beneath Cages	Bulked faeces/ Droppings belts	Egg Belts	Mouse Droppings	Rat Droppings	Flies
4th week post move*	3/42 (7.1)[a]	3/28 (10.7)[a]	1/14 (7.1)[b]	1/98 (1.0)[b]	0/14	0/1	0/4	0/1
Last month of lay	3/28 (10.7)[a1,c2]	7/28 (25.0)[a3,c2,d2]	8/42 (19.0)[a4,c3,d1]	4/84 (4.8)[a1,c3]	-	-	1/1[c]	1/2 (50.0)[c]
Post C&D	1/28 (3.6)[c]	cages 1/56 (1.8)[c]	12/84 (14.3)[a2,c10]	17/42 (40.5)[a1,c16]	0/28	0/5	0/1	0/4
Second Flock:								
Last month of lay	25/28(89.3)[ace]	48/56(85.7)[acf]	49/56(87.5)[acef]	23/28(82.1)[ace]	4/11(36.4)[ae]	1/1[a]	-	-
Post C&D	-	cages 1/33(3.0)[a]	16/35(45.7)[ac]	5/12(41.7)[ac]	1/23(4.3)[c]	0/1	1/1[a]	-
Third Flock:								
Last month of lay	11/15 (73.3)[acg]	10/15 (66.7) [acg]	10/10 (100.0) [acg]	4/20 (20.0)[c]	-	-	-	-
Post C&D	-	cages 0/5	2/15 (13.3)[c]	1/5 (20.0)[c]	0/5	-	-	-
Fourth Flock:								
8 weeks after placement†	2/9 (22.2)[ac]	2/10 (20.0)[c]	0/5	3/15 (20.0)[ac]	-	-	0/1	-

Key: * After fluoroquinolone/competitive exclusion treatment; † After chlortetracycline treatment; [a] *S.* Enteritidis PT4; [b] *S.* Typhimurium DT193; [c] *S.* Enteritidis PT6; [d] *S.* Agona; [e] *S.* Indiana; [f] *S.* Mbandaka; [g] *S.*Enteritidis PT7; - not sampled; number after superscript = no. of isolates of each serovar/phage type

Cryptosporidium spp. PREVALENCE IN LAMBS AND EWES FROM THE NORTHERN REGION IN THE STATE OF MEXICO

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Introduction

The genus *Cryptosporidium* has been recognized as a significant enteropathogen of humans and livestock. Cryptosporidial infection of livestock may have an important economic impact to farmers because of high morbidity an sometimes mortality rates among farm animals (Casemore *et al.,* 1997). *Cryptosporidium* oocysts excreted with faeces from infected farm animals can be a source of human infection having great influence on public health (Lee *et al.,* 2001). There is few information regarding the occurrence of cryptosporidiosis in sheep (Casemore *et al.,* 1997), mainly in small flocks. The infection in these animals is common and in some cases the infection often causes death in diarrhoeic lambs (Tzipori *et al.,* 1981; Kaminjolo *et al.,* 1993; Olson *et al.,* 1997).

The State of Mexico is an important non-technified (rustic) sheep production zone in which the existence of gastrointestinal and respiratory syndromes are known as well as high morbidity and mortality regarding these problems.

The aim of the study was to determine the prevalence of *Cryptosporidium* in sheep (lambs and ewes) in the Northern region in the State of Mexico.

Material and Methods.

20 flocks from the Northern region in the State of Mexico (Jiquipilco and San Felipe del Progreso) were chosen at random. 502 faeces samples were taken directly from the anus by using a plastic bag to identify *Cryptosporidium spp*. They were properly identified and were taken in a refrigerated box (4ºC) to the laboratory until processed. Smears were stained by using the modified Zeihl-Neelsen technique. A positive control was run in each smear to compare the samples (Henriksen and Pohlenz, 1981). Smears were observed under the light microscope by using immersion oil objective (100X). All the processes were performed under proper biosafety conditions at the laboratory facilities. Faeces were taken

The comparison of the groups by production stage and size flock was perfomed by using the independent group proportion hypothesis test ($p<0.05$). The punctual prevalence was compared with a hypothetical proportion according to published reports (Fátima *et al.,* 1995). The statistical software Stata version 5.0 (1999) was used.

Results.

Table 1 shows the sample distribution according to the number of exposed population and production stage.

TABLE 1.- SAMPLE DISTRIBUTION PER PRODUCTION STAGE.

EXPOSED POPULATION		NUMBER OF SAMPLES PER PRODUCTION STAGE (%)	
LAMBS	EWES	LAMBS	EWES
522	927	214 (40.1)	288 (31.1)

In table 2, prevalence distribution of the sampled population is shown, according to the flock size and age group.

TABLE 2- *Cryptosporidium spp.* PREVALENCE BY FLOCK SIZE, GROUP AND PRODUCTION STAGE.

			PREVALENCE		
Flock size (heads)	SAMPLED ANIMALS	Positive Samples	GROUP	LAMBS	EWES
	502	129	25.7^{x}	20.09^{A}	29.86^{B}
≤ 100	307	91	29.64^{a}	29.85^{A}	29.58^{A}
> 101	195	38	19.48^{b}	15.64^{A}	31.25^{B}

We found a higher prevalence in lambs than in ewes ($p>0.05$) in flocks with less than 100 animals. In the ones in which there were more than 101 animals, ewes had a higher prevalence than lambs ($p<0.05$).

Discussion

Our results demonstrate that there is a high prevalence of *Cryptosporidium spp.* in the region in comparison to other studies. Majewska *et al.* (2000) in Poland found a 10.1% prevalence in sheep; Valenzuela *et al* (1991) in Chile found a 7.7% prevalence in lambs; Gorman *et al.* (1990) in central Chile found a 6.4% prevalence in sheep; Santos da Silva *et al.* (1990) found in Brasil 10% prevalence in lambs; Villacorta *et al.* (1991) found in Spain (Galicia) a 1.45% prevalence in lambs;Olson *et al.* (1997) found in Canada a 23% prevalence in sheep; Ozer *et al.* (1990) found in Turkey a 12% prevalence in diarrhoeic lambs with less than one month of age; Minas *et al.* (1993) in Greece (Larissa) found a 4.6% prevalence in diarrhoeic lambs; Kaminjolo *et al.* (1993) found in Trinidad and Tobago a 20% prevalence in diarrhoeic and non-diarrhoeic lambs; Abou Eisha (1994) in Egypt (Ismailia Governorate) found a 24% prevalence in lambs and a 2.4% prevalence in ewes; Nagy (1995) found in Hungary a 22.6% prevalence in diarrhoeic lambs aging 1 to 5 weeks old; Kambarage *et al.* (1996) found in Tanzania (Morogoro region) no animals infected with the parasite; Nouri and Karami (1991) found in Iran a 17.2% prevalence in sheep. In contrast, Muñoz *et al.* (1996) in Spain found a 45% prevalence in lambs, Causapé *et al* (2002). found 59% prevalence in Spain too and Fatimah *et al.* (1995) found in Malaysia a 36% prevalence in diarrhoeic and non-diarroheic lambs. According to Ortega Mora (1999) ewes can represent a risk factor for lambs because of an increase in the secretion of oocysts during

the perinatal period. In our study, ewes presented a higher prevalence than lambs, which may be related to the phenomena described by Ortega Mora (1999).

Conclusion

We conclude that there is a high prevalence in sheep mainly in flocks with a high number of animals.

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DOSE-RESPONSE FROM FOODBORNE DISEASE OUTBREAK DATA

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Introduction

In France, outbreak diseases are to be notified (1). In this context, a network has been organized at a national level : for each of the 99 French districts, the veterinary services (DDSV – Direction Départementale des Services Vétérinaires), the public health services (DDASS – Direction Départementale des Affaires Sanitaires et Sociales) and the district public laboratory are associated in order to investigate the outbreaks. Investigations are centralised by the national institute for public health surveillance (InVs - Institut de Veille Sanitaire) and the food directorate of the Ministry of Agriculture (DGAl - Direction Générale de l'Alimentation) in relation with the national reference centres concerned.

This network generates an important volume of data which take into account exposed people, foods and pathogens incriminated making it possible the evaluation of the dose-response relationship. Unfortunately, these data may not be sufficient or not be formatted in the right way to derive dose-response relationship. For these reasons we investigated which data currently collected are interesting and which additional data need to be collected. We also wanted to have a direct contact with the field in order to determine the pertinence, the quality and the limits of the data we would collect. Our main objective was to set a methodology making possible the long term collection of data adapted to the evaluation of the dose-response relationship.

Material and Methods

During the usual investigation of an outbreak, the QMA team (Qualité Microbiologique des Aliments) is in charge of performing analysis of the samples collected by the veterinary services of Paris and 2 other districts closed to Paris (Hauts-de-Seine and Seine-Saint-Denis).

From November 2003 to June 2004, an eight month pre-study has been conducted in Paris. Numerous contacts have been established with veterinary and public health services. The pre-study was initially based on *Salmonella* because, in France, this pathogen is the most frequently isolated from foods implicated in outbreaks (2). Moreover *Salmonella* represents, for many countries, one of the pathogens with a great impact on public health (number of cases, hospitalisations and deaths) (3). However, this methodology could also be applied to other pathogens such as *Campylobacter,* Shiga-toxins producing *E. coli*, *S. aureus*, S*higella*, etc…

We presented at first the project to the veterinary inspectors and medical staff in charge of the investigations. To the medical services, we proposed an additional questionnaire in order to get data concerning risk factors and then to better characterize exposed people (severity of the symptoms, hospitalisation, former exposition to the pathogen, nutritional state, quantity of incriminated food ingested, general state, medical treatment, sensibility of the person, etc…). With the veterinary services, we defined a procedure allowing us being in the field during the investigation. In this context, as soon as the veterinary services are aware of a case, they contact the laboratory (on a voluntary basis). Immediately, we reach them everywhere in Paris in less then 1 hour.

During the usual investigation of the veterinary services, we had time to collect additional data which can be used for the evaluation of the dose-response relationship (temperature parameters, better understanding of the conditions responsible for the outbreak, etc…). Then, after inspection, the collected samples (set-aside meal, remainings of the foods, etc…) are sent to the laboratory and the QMA team searches the incriminated pathogen. Depending on the quantity of sample available, an enumeration is performed on one or more samples. The isolated strain of *Salmonella* is then serotyped by the laboratory.

At the end of this pre-study, a debriefing was organized with the veterinary services. To have a better knowledge in the field, we decided to participate to all investigations for which veterinary services contact us even if the symptomatology was not representative of a *Salmonella* infection.

Results

The pre-study allowed us to participate to the investigation of 9 outbreaks (33 % of the outbreaks notified in Paris for this period).

On these 9 investigations, some of them allowed us to get additional data (history of time-temperature, quantity of food consumed, characterization of exposed people,…) but not enough to assess the dose-response relationship. For most of them the pathogen was not identified or not enough sample was available to enumerate the pathogen incriminated (no data concerning the dose).

For Paris, one *Salmonella* outbreak investigation allowed us to get enough data to appreciate the dose ingested and the effect for the exposed people. It was a family outbreak where 3 children and their father were ill (all of them consumed the cheese St-Nectaire). At first, detection and enumeration were performed on the remaining of the cheese. Secondly, the veterinary services recalled 2 entire cheeses of St-Nectaire of approximately 1,5 kg each and detection / enumeration were performed on many samples (the cheese consumed by the family was from the same lot and the same retail shop than the entire cheese). We also had data on the quantity of cheese ingested by each exposed people, the history of time-temperature (retail, home and storage

after consumption by the family), and the characterization of exposed persons. This outbreak was interesting since it was due to *S. Dublin* and, even if all the exposed people were ill, we estimated a low ingested dose showing the importance of taking into account the virulence of the strain when studying the dose-response (4, 5). Moreover, 7 agars have been tested (XLD, other selective or chromogenic agars) and only 2 of them allowed us to obtain characteristic colonies. This showed us the great importance of the methodology used for detection or enumeration of the pathogen for a dose-response study.

At the same time, we also developed some contacts with other veterinary services (especially with District 93). We had recently the opportunity to investigate an other interesting *Salmonella* outbreak in a other family. *S. enteritidis* was implicated (approximately 15 ill persons). We had precise data on exposed people (severity of the symptoms, hospitalisation, former exposition to *Salmonella*, nutritional state, quantity of incriminated food ingested, general state, medical treatment, sensibility of the exposed persons, etc…). We can determine the dose of *Salmonella* ingested (high) taking into account a very good homogeneity of the level of contamination in the cake. This outbreak is still under investigation.

Then, in Paris, we had a third interesting outbreak due to *S. aureus* enterotoxin (type E) in Salers cheese. Again we had precise data on the characterization of exposed people and quantity of cheese ingested. This outbreak is also interesting due to the fact that the quantity of E toxin ingested seemed to be very low (under the limit of quantification during the analysis). Strains will be tested, in the future, for their capacity to produce this E toxin.

Discussion - Conclusion

This pre-study shows us that, in the context of the French surveillance of outbreaks, it is possible to collect pertinent and good quality data in order to assess the dose-response relationship. This work requires the laboratory to be extremely available to collect the additional data. It is important to be present each time the veterinary or public health services contact the laboratory even if it is known that all the data will not be available to evaluate the dose ingested and the response of exposed people.

Being the laboratory associated to the veterinary and public health services is important because it allows to establish consistent contacts with actors of the investigation. Moreover, in terms of methodology it is important to minimize the stress before enumeration (impact of the temperature and storage on the number of cells enumerated). Being in the field is also very important if it is sought to appreciate the quality and the pertinence of the data collected.

Only some of the outbreaks are interesting to evaluate the dose-response relationship. Thus, we think that family outbreaks are more interesting than others. Actually, we frequently have remainings of the meal, data on history of time-temperature, additional data on exposed persons and the analysed samples represent really what have been eaten. Concerning investigations in food catering such as schools (legislation with set aside meals) (6), the analysed samples and the ingested food can have different history (set aside meal are not always stored just before the meal, stress due to the cold storage can be of great importance for the determination of the ingested dose).

In the context of this pre-study, the tested methodology is adapted to *Salmonella* but it must be clear that this methodology is also applicable to other pathogens for which dose-response should be developed.

At this time, the project will be continued taking into account more outbreaks and trying to better characterize strains, food samples and exposed people. Contacts with veterinary and sanitary services from other districts will be developed (presentation of the project, expertise, training, …).

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EXAMINATION ON THE OCCURRENCE OF SELECTED ZOONOTIC PATHOGENS REGARDING THE CURRENT SITUATION OF THE FINNISH REINDEER HUSBANDRY

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Introduction

Reindeer husbandry represents an important economic factor and a valuable cultural heritage. About 185 000 semidomesticated reindeer (*Rangifer tarandus tarandus*) live in the northern regions of Finland. Transmission of infectious agents to man may occur through direct contacts to free-ranging animals including cervids, by contamination of the environment through faecal shedding or by consumption of venison. In contrast, to domestic animals, however, the epidemiological situation in free-ranging animals and in their habitat is difficult to assess. There is a lack of information regarding the human health risk due to faecal shedding of pathogens by reindeer. Bacteria, such as *Campylobacter* spp, *Enterococcus* spp, *Escherichia coli* (*E. coli*), *Salmonella* spp, *Yersinia* (*Y.*) spp and the parasites *Cryptosporidium* spp are among the most important agents in causing zoonosis like enteric and other diseases and were isolated before from healthy and dieseased domestic ruminants (De Rycke *et al*., 1986; Munoz *et al*., 1996; Busato *et al*., 1998 and 1999). The objectives of this study, that was performed as part of the EU-project RENMAN, were to figure out the occurrence and prevalence of important zoonotic pathogens in reindeer.

Material and Methods

In this study, 2 243 faeces samples from healthy reindeer, adults and calves, of both genders were examined for the occurrence of *Campylobacter* spp, *Enterococcus* spp, *E. coli*, *Salmonella* spp, *Yersinia* spp, and in addition, for the occurrence of the parasites *Cryptosporidium* spp. The samples were taken in the course of one year (June 2001 - April 2002) from Finnish and Norwegian free-ranging and corralled reindeer herds, considering parameters such as degree of intensification of husbandry, location and season. Samples were taken off the ground or per rectum from slaughter animals, sent to Kiel, Germany, directly after collection and conserved frozen until further processed.

The examination for *Campylobacter* spp was done by incubating faecal material in Preston broth for 24 h in a microaerophilic atmosphere at 37 °C. A loopful of the enriched suspension was plated on Preston agar and incubated for 48 h under the above described conditions. *Campylobacter*-like colonies were analysed by Gram-staining, catalase and oxidase tests, and further biochemical reactions.

To detect *Enterococcus* spp, faecal material was diluted in glucose-azide broth and incubated for 48 h at 37 °C. A loopful broth was then spread both on kanamycin-aesculin-azide agar and Slanetz and Bartley agar. After 48 h at 37 °C suspicious colonies were Gram-stained and their biochemical reactions were analysed further by catalase and oxidase tests.

Escherichia coli was isolated by adding faeces to Gram-negative broth. After 24 h of incubation at 37 °C a loopful of broth was plated onto Endo-c agar and incubated under the above mentioned conditions for 24 h. Typical metallic shiny colonies were subcultured on blood agar, incubated for 24 h at 37 °C and tested for their biochemical reactions. PCR was used to detect the occurrence of shigatoxin1 and 2 genes (*stx1*, *stx2*), the intimin gene (*eae*) and EHEC-hemolysin gene (hly_{EHEC}). *EHEC EDL 933* (*stx1,2* positive) was used as a positive control and *E. coli ATCC 11 229* (*stx1,2* negative) was included as negative control. The amplified products were analysed by electrophoresis and were visualised following ethidium bromide staining (100 µl/100 ml gel) at UV-light.

For the selective enrichment of *Salmonella* spp faeces was inoculated into tetrathionate broth and incubated for 24 h at 37 °C. Two enrichment steps were repeated the following two days. On the fourth day one loopful of the cultured medium was plated both on *Salmonella-Shigella* agar and Leifson agar. After 24 h of incubation at 37 °C presumptive *Salmonella* spp colonies were Gram-stained and tested biochemically.

Cultural examination of *Yersinia* spp was performed by adding faeces to Gram-negative broth and incubating for 48 h at 21 °C. One loopful of broth was then plated on *Yersinia*-selective agar and incubated for another 48 h at 21 °C. Colonies with the typical bull's-eye-appearance were subcultured on blood agar and Gram-stained and biochemical tests were subsequently carried out. To detect the *Yersinia*-genes encoding *16SrRNA*, *yadA* and *v-antigen* PCR was performed.

For the detection of *Cryptosporidium* oocysts, immunomagnetic separation was applied using Dynabeads anti-*Cryptosporidium*. Twenty µl of the immunoconcentrate were used for a direct immunofluorescence test (*Cryptosporidium*-Antigen-IFT). *Cryptosporidium parvum* oocysts from a calf served as the positive control. Using a fluorescence microscope at x400–x1000 magnification *Cryptosporidium* oocysts appear as 6-10 µm in size, round or oval in shape with bright green fluorescence.

Results

In 2 224 (99.2%) out of the total number of 2 243 faecal samples one or more of the examined bacteria species were isolated. *Campylobacter* sp, identified as *C. hyointestinalis*, was detected in one sample only (0.04%). *Enterococcus* spp were isolated in 2 084 (92.9%) samples. *Escherichia coli* were isolated in 2 123 (94.7%) samples. Only few of the isolated *E. coli*-strains possess genes encoding *stx1* (0.14%), *stx2* (0%), *eae* (0.61%) and hly_{EHEC} (1.08%), There was no evidence of the occurrence of *Salmonella* spp nor *Cryptosporidium* spp. These results are shown in **Table 1**. One hundred and eight (4,8%) strains of *Yersinia* spp were isolated, consisting of *Y. enterocolitica* Biogroup 1A (n=29), *Y. intermedia* (n=2), *Y. kristensenii* (n=72), *Y. mollaretii* (n=3) and *Y. rhodei* (n=2).

Regarding the degree of intensification of reindeer husbandry, the season or the geographic origin, no significant differences were found for *Enterococcus* spp and *E. coli*, whereas the prevalences of *Yersinia* spp differed significantly: prevalences for *Yersinia* spp in free-ranging reindeer in summer and autumn were significantly higher than in fenced reindeer during winter.

Discussion

Faecal samples of reindeer were examined for the occurrence of important enteric pathogens in order to get information about the human and animal health risk. All bacteria investigated in this study may be found in Northern Europe in the environment in aquatic, terrestrial and animal reservoirs (Kapperud, 1981) and were isolated from the intestinal tract of healthy or diseased ruminants worldwide (Adesiyun *et al*., 1998; Busato *et al*., 1998). In reindeer, *Enterococcus* spp and *E. coli* occurred in very high prevalences, showing the affiliation of these two species to the normal intestinal flora of healthy reindeer. Concerning *E. coli*, there are only few reports on diseases caused by shigatoxin-producing bacteria in ruminants (Sherwood, 1985; Mainil, 1999), however these bacteria are of extreme importance in causing severe diseases in humans (Griffin & Tauxe, 1991). As the genes encoding *stx1*, *eae* and hly_{EHEC} were detected only in very low numbers of the isolated *E. coli*-strains, the human health risk due to *E. coli* excreted by reindeer can be considered very low at the moment. These results comply with another study detecting no *E. coli* O157:H7 in 1 387 faecal and 421 meat samples from reindeer (Lahti *et al*., 2001). *Yersinia* spp was isolated in 108 samples. The identified species *Y. intermedia*, *Y. kristensenii*, *Y. mollaretii* and *Y. rhodei* have been isolated before from various environmental samples (fresh water, soil, *etc*.), food, healthy animals and healthy and diseased humans (Baier & Puppel, 1981; Sulakvelidze, 2000). Even though these species are widely distributed in nature, their actual impact on human health is a matter of controversy. *Campylobacter hyointestinalis* was isolated from one sample only. As the cultivation of *Campylobacter* spp. is exceedingly difficult, the real prevalence might be higher. Hitherto *Campylobacter hyointestinalis* has been associated only sporadically with human gastrointestinal disorders (Edmonds *et al.*, 1987, Gorciewicz *et al.*, 2002). Even though the prevalence for *Campylobacter* spp in this study was very low, it shows that reindeer can be carriers of *Campylobacter hyointestinalis*. This is approved by another study detecting *Campylobacter hyointestinalis* in a prevalence of 6% in Finnish reindeer faeces (Hänninen *et al*., 2002).

Conclusion

Summarizing it can be stated, that the examined enteropathogens were either not detected at all (*Salmonella* spp and *Cryptosporidium* spp), in very small numbers (*Campylobacter* spp) or if detected, their virulence and pathogenicity was very low (*E. coli* and *Yersinia* spp). In the present situation in northern Europe the potential human and animal health risk by reindeer, excreting various important enteropathogenic bacteria and *Cryptosporidium* spp, has to be estimated as very low. These results are very important especially regarding the status of reindeer meat as a natural product for the consumer, as for the production no antibiotic treatment is required so far.

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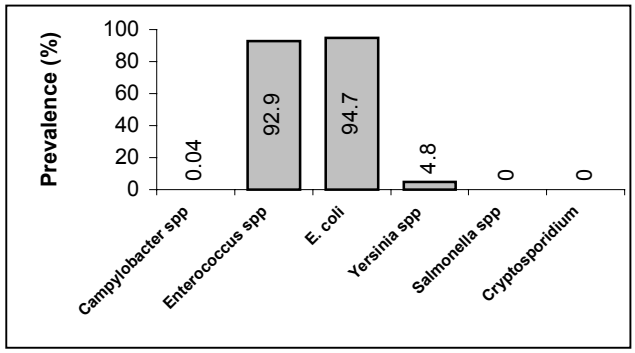
Figure 1. Prevalences of analysed pathogens in faeces of reindeer (n= 2 243)



Foodborne diseases - Zoonoses

Posters

MOLECULAR CHARACTERIZATION OF *STREPTOCOCCUS SUIS* STRAINS BY PCR-RIBOTYPING ASSOCIATED WITH RFLP ANALYSIS

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Introduction

Streptococcus suis is an important pathogen of swine, causing meningitis, arthritis, pericarditis, polyserositis, septicemia and sudden death of weaning piglets as well as growing pigs. In addition, *S. suis* is a zoonotic agent (1). To date, 35 serotypes have been described (1, 2, 1/2, 3 to 34), and among them, serotype 2 has always been considered as the most virulent and prevalent type isolated from diseased pigs (2, 3, 4).

In the present study, we report the development of a molecular typing method based on PCR amplification of a larger fragment of rRNA genes, including a part of 16S and 23S genes and the 16S-23S rDNA intergenic spacer region (PCR-Ribotyping), followed by RFLP analysis with *Rsa*I endonuclease. The genetic relationships between 138 *S. suis* strains belonging to various serotypes, isolated from swine or human cases, were also performed.

Material and Methods

One hundred and thirty eight strains of *S. suis*, epidemiologically unrelated, isolated from diseased pigs suffering from meningitis, septicemia, or arthritis (84 strains), from nasal cavities or tonsils of clinically healthy pigs (22 strains) and from human meningitis cases (29 strains) were studied. Among these strains, capsular typing using the coagglutination test has revealed serotype 1 (1 strain), serotype 2 (98 strains), serotype 1/2 (7 strains), serotype 3 (6 strains), serotype 4 (1 strain), serotype 5 (1 strain), serotype 7 (12 strains) and serotype 9 (10 strains). Two strains were autoagglutinable.

The template DNA of each strain was amplified with the forward primer 16S-489(f) complementary to the 3' end of the 16S rRNA gene and the reverse primer 23S-206(r) complementary to the 5' end of the 23S rRNA gene (5). The PCR-Ribotyping products were digested with *Rsa*I endonuclease (5).

The patterns were digitized and analyzed by using the Biogene package (confidence interval of 8%). The numerical index of discrimination was calculated. The relationships between patterns of strains isolated from pathological cases or from clinically healthy pigs, including serotype, and origin of the strains (pig or human) were analysed by using the Fisher exact test ($n \leq 5$) or the Chi-square test ($n > 5$). Differences were estimated significant when $P < 0.05$.

Results

In our conditions, the *in vitro* stability of patterns and the reproducibility of the method were 100 % because a similar pattern was shown for each strain after the two independent DNA extractions. The index of discrimination was superior to 0.95, the threshold value for interpreting typing results with confidence.

The patterns were composed of 6 to 21 fragments of 134 to 925 bp. Fourty two patterns were identified among the 138 *S. suis* strains, analyzed. These strains diverged by up to 33 % (67% homology). At a level of 72 % homology, five groups A to E were identified. At 75 % homology, the group A was divided in two subgroups a and b.

Among the 113 strains isolated from diseased pigs and humans, 38 patterns were identified. The majority of strains (78 of 113) were in group A. No significant association between the strain origins and the groups was observed ($P > 0.05$). However, the pattern R17 were significantly associated with strains isolated from clinically health pigs ($P = 0.011$).

Among the 98 strains of serotype 2, 30 patterns were identified. 54 percent (53 / 98) and 24 percent (24 / 98) of *S. suis* serotype 2 strains were included in subgroup a ($P = 0.021$) and group C ($P = 0.002$) respectively. The patterns R6 was significantly associated with *S. suis* serotype 2 strains ($P = 0.019$). The *S. suis* capsular types 1, 1/2, 3, 4, 5, 7 and 9 were clustered in the subgroup b ($P < 0.001$). Significant associations were observed between these strains and the patterns R26 ($P < 0.001$) and R29 ($P = 0.002$).

Nine patterns were identified among the 29 strains isolated from humans, whereas 38 patterns were obtained from 109 strains isolated from pigs. Twelve of the 29 (41%) strains, isolated from humans, clustered in group C, whereas 96 of 109 (88%) strains were distributed among the other groups ($P < 0.001$). The patterns R6, R36, R11 and R38 were significantly associated with strains isolated from humans ($P < 0.05$), because 22 (76%) strains had these types.

Discussion-Conclusion

The PCR-Ribotyping followed by RFLP analysis with *Rsa*I endonuclease, because of its reproducibility and discriminatory power, can be used to characterize *S. suis* strains. Pulsed-field gel electrophoresis (PFGE) was previously described (6). However, PFGE is laborious and time-consuming technique whereas the PCR-Ribotyping offer the advantages of simplicity and rapidity conferred by the PCR procedure. The typing of 138 *S. suis* strains confirmed that the strains isolated from humans was less genetically diverse than strains isolated from pigs. For the first time, one molecular pattern was significantly associated with *S. suis* serotype 2 strains (R6) and one pattern was associated with strains isolated from clinically health pigs (R17).

In conclusion, this genetic tool could be a valuable help in distinguish individual isolates of *S. suis* during further epidemiological investigations.

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SALMONELLA CONTROL STRATEGIES IN PIGS

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Introduction

As part of the follow-up to the EU´s "White Paper on Food Safey", European member states must develop a programme for salmonella reduction throughout the pork production chain. S. enterica *typhimurium* is the most common serovar in pigs and attention is therefore focused on reducing the incidence of S.typhimurium on the farm. Factors influencing gut health have a significant effect on the incidence of *S. typhimurium*. For example, feeding coarsely-ground, instead of finely-ground diets, can positively influence gut health and thereby reduce the incidence of salmonella. Addition of potassium diformate (KDF) to the diet has also been shown to reduce the incidence of salmonella by positively influencing gut health. KDF delivers formic acid to the small intestine, where it acts against gram negative bacteria including *Salmonellae*. A series of controlled studies and field trials were carried out to determine the effects of KDF on salmonella status in piglets and in growing pigs.

Materials and Methods

In 3 controlled trials (T1- T3), 20 weaned, Salmonella-negative piglets were divided into a control group (CG) and a treatment(TG) and fed *ad libitum* with the following diets:

T1& T3 CG: finely ground, pelleted diet
TG: coarsely ground, pelleted diet + 1.2 %KDF
T2 CG: coarsely ground, pelleted diet
TG: coarsely ground, pelleted diet + 1.2 %KDF

Treatment piglets were orally infected with a bouillon containing 10^9 CFU/ml S. Derby. At day 0 of T1 + T2 each of the piglets was orally infected, In T3, 2 piglets per group were experimentally infected and returned to the other uninfected group members 4 days post-infection (DPI). Salmonella excretion was observed via rectal swab for a period of -3 – 32 DPI. In T1 and T2 piglets were sacrificed every 4 days beginning at day 16. In T3 the piglets were sacrificed at days 24, 25 and 26, and analyses of chyme carried out.

In a field trial carried out in Denmark, (T4; Olesen, 1999) 15 grower-finisher herds with recurrent salmonella problems were categorised according to the Danish salmonella monitoring programme from level 3 (poor) to level 1 (good). Farms categorised in levels 2 and 3 must actively work towards improving salmonella status. In T3, a level of 0.6% KDF was introduced to the diets at time 0 and salmonella monitoring was carried out for 3 months before, to 5 months after, KDF addition.

Results & Discussion

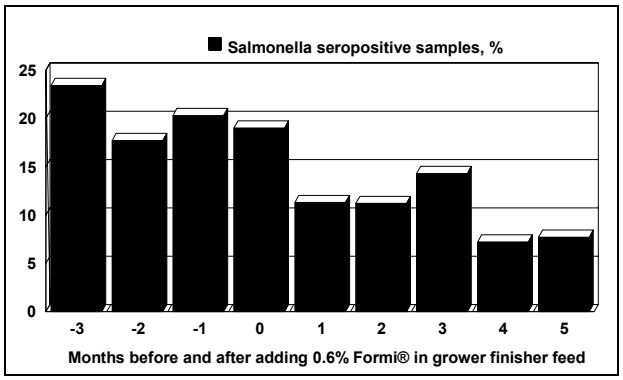
In trials T1 and T2 each of the experimentally infected piglets excreted Salmonella 2 to 3 days post-infection via the faeces. Results showed that both coarse grinding of ingredients and the addition of KDF reduced the faecal excretion ratio and shedding duration (Table 1). Reduced excretion of salmonella in the experimentally infected piglets resulted in a significantly lower infection rate of the other piglets of the treatment group in T3. Addition of KDF resulted in reduced counts of E. coli and increased counts of Lactobacilli and gram positive bacteria in the chyme of some parts of the digestive tract. Feed conversion ratio of the pigs fed the coursely ground, KDF- diet was better than that of pigs fed the control diet.

Table 1: Effect of coarse grinding and potassium diformate on frequency and duration of salmonella shedding in piglets.

		Control	Treatment
T1	Ratio (in %) (positive/tested piglets)	77.0±14.2	39.2±19.5
	Duration of Salmonella excretion (in d)	19.2±7.4	12.6±7.1
T2	Ratio (in %) (positive/tested piglets)	49.4±20.0	34.4±15.7
	Duration of Salmonella excretion (in d)	12.3±8.1	9.3±5.4

In T4, Incidence of salmonella was markedly reduced within 1 month of KDF addition, and over a longer period was reduced even further. Farms that had been categorised in level 3 and 2 were re-categorised to reflect the improved salmonella status (figure 1).

Figure 1 :Effect of 0.6% KDF on incidence of salmonella in Danish grower-finisher herds.



Conclusions

Addition of potassium diformate at 0.6-1.2% is an effective strategy to prevent and reduce salmonella infection in piglets and grower-finisher pigs.

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RIFT VALLEY FEVER IN SENEGAL: 10 YEARS OF SURVEILLANCE

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Introduction:
RVF is a viral disease of veterinary and medical importance. Periodic severe epizootics are accompanied by epidemic human disease in Africa (Egypt 1997, 1993, Mauritania 1987, 1993, 1998, Eastern Africa 1997-98) with recent extensions in the Arabian Peninsula (Saudi Arabia and Yemen in 2000). In Senegal, following the first RVF outbreak in 1987 in the Senegal River Delta, a program of surveillance of the disease in domestic ruminants was conducted from 15 years (from 1988 to 2003). The objectives were to establish an early detection of the disease based on sentinel herds sero-monitoring and disease reporting through the country..

Material and Methods
A network of sentinel herds (small ruminants) located in potential high risk areas for RVF was visited during the raining season (from June to November) with clinical examination and cattle herds randomly selected during the Pan African Rinderpest Campaign were sampled.
Collected samples (sera) were analysed by VN and Elisa for IgM and IgG antibodies in order to reveal recent and past viral circulation.
Virus isolation from organs and tissues was performed on Vero cells and suckling mouse.

Results

Animal RVF antibody prevalence.
The serosurveys conducted in sheep and goats showed the following variations:
- the RVF seroprevalence reached a peak of 70% after 1987 epizootic, dropped to 30% in 1988 and then decreased continuously until 1993. This fall in RVF prevalence in animal population corresponded to period of low rainfall (Thonnon et al, 1999).
- during periods of heavy rainfall, such as 1994, 1999, 2002 and 2003, RVF activity re-emerged as epizootics amongst herds in the Senegal River basin and some adjacent areas such as Ferlo.
In the Ferlo area, an enzootic cycle of RVF virus was shown involving species of mosquitoes like *A vexans* and *A ochraceus* during the raining season (Thiongane et al, 1994).
Throughout the country, many suspicions of clinical RVF were confirmed by laboratory analysis but others transboundary animal diseases were detected.

Public information.
Communication and training materials (5 periodic bulletins, 400 booklets, 200 video and 2000 posters) (Figure 4) were produced to raise local awareness of RVF consequences on livestock and human health. A computerized regional database with more than 20 000 informations (sero-surveillance surveys, suspicions and outbreaks notifications) collected in Senegal and neighbouring countries from 1988 to 2003

Discussion.
RVF surveillance can be accomplished by a variety of approaches but we choose the serological survey system according to local conditions, specially herd ownes agreement, cost and effectiveness. Moreover, specific diagnostic tools with ELISA assay was chosen and permits separation of IgG and IgM, and IgM are a valuable indicator of recent infections. The presence of virus circulation increased the risk of an epizootic, and hence an epidemic, in the rainy seasons in relation to vector activity. The etiological diagnosis must be associated with sustained awareness of RVF in order to prevent major RVF epizootics.
Data obtained from satellite imagery will help to predict and prevent future RVF epizootics and epidemics and must be used in association with animal surveys which are sensitive and inexpensive tools (Davis et al, 1985).

Conclusion
RVF virus transmission appeared to be endemic in the northern Senegal, mainly in the Senegal river basin, with fluctuations according to rainfall. According to the risk for livestock and human populations, a serosurvey based on small domestic ruminants appeared for us a sensitive tool for the detection of RVF circulation.

Acknowledgements.
We are grateful to veterinary agents and we appreciated the collaboration with herd owners who permit us repeated blood samplings during many years. This work was supported by the FAO-EMPRES Programme.

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Free communications

Oral Communications

THE EFFECTS OF A NON STARCH POLYSACCHARIDASE ENZYME PREPARATION FROM THERMOMYCES LANUGINOSUS ON THE RUMINAL VOLATILE FATTY ACID PRODUCTION, ENERGY AND PROTEIN METABOLISM AND MILK YIELD OF DAIRY CATTLE

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Introduction

External Non Starch Polysaccharidase (NSP) enzymes are used in two ways. Firstly: enzyme pre-treatment of roughages and forages is regarded very promising. Nakashima and Orskov (1989), Beauchemin et al. (1995), Stokes (1992), Chamberlain and Robertson (1992), Kung et al. (2000), Stokes (1992).

Secondly: direct feeding of enzymes in the daily ration is expected to aid the digestive processes in the stomach(s) and intestines, presuming the enzymes preserve their polysaccharidase activity in the gut. Hristov et al. (1998) attested the stability of these enzymes in the ruminal environment and proved their quick inactivation at the low pH of the abomasum.

Direct feeding of enzymes to cows increased the milk production by about 5 to 10% at the beginning of the lactation (Kung et al., 1997; Nussio et al., 1997) and in other experiments (Lewis et al., 1999; Schingoethe et al., 1999.) in the middle of the lactation. Schingoethe et al. (1999) reported an increment in the butterfat and milk protein production of the treatment cows, however, authors failed to explain the background of the improvement. Other experiments (Lewis et al., 1996; Howes et al., 1998; Yang et al., 1999) have shown that saccharidases increased the ruminal concentration of volatile fatty acids (VFAs). Direct feeding of these enzymes increased the microbial protein synthesis and improved ruminal digestibility of the fibre fraction (Yang et al., 1999; Beauchemin et al., 2000). In cows with positive energy balance enzymatic treatment proved inefficient (Beauchemin et al., 2000).

The positive and in some respect controversial data of the relevant literature have prompted us to study the effects of an enzyme preparation (Rumino-Zyme) high in xylanase activity on the ruminal volatile fatty acid production, on fat and carbohydrate metabolism, and energy balance and on milk production of dairy cows.

Materials and methods

The enzyme preparation: In our laboratory Thermomyces lanuginosus, known to produce cellulase free extracts high in xylanase and low in β-xylosidase, β-glucosidase and α-arabinosidase activity (Purkarthofer and Steiner, 1995; Bennett et al., 1998.) was used to produce an enzyme preparation as reported elsewhere (Kutasi et al., 2001). The product (Rumino-Zyme) is a light-brown granulate (particle size: 400-500 μm) of 90% dry matter (DM) content, which contains thermal resistant endoxylanase from the fungus Themomyces lanuginosus. Enzyme activity of the product is 2 000 IU*/g. The preparation hydrolyses xylans and arabino-xylans into mono-, bi-, tri- and oligo-saccharides.

Place and time of the study: The experiment was carried out at a loose housing dairy cattle farm of 2 000 Holstein Friesian cows between October 1999 and February 2000.

Animals and diets: By pairing on basis of equal production and parity, two hundred ear tagged Holstein Friesian cows of 2nd and 3rd lactation was assembled into an experimental and a control group of equal size. Housing and feeding regime of the experimental and control cows was identical with the exception that daily ration of the experimental cows contained 34 g enzyme preparation (Rumino-Zyme) in 1 kg concentrate from about 3 weeks prior to expected calving till the 110th day of lactation.

Data recording and samplings: Of the 100 experimental and control cows, 10 cows in each group were designated for taking ruminal fluid, blood and urine samples by about two weeks intervals from the beginning of the experiment (9±4.6 days before the expected parturition) till its end (107±4.6 days after parturition).

VFA concentration of the ruminal fluid samples was measured gas chromatographically.

Fat- and carbohydrate-metabolism and -balance were monitored by determination of the glucose, acetic acid and NEFA concentrations in the blood samples. Data of the acid-base balance was also considered. Subclinical fat mobilisation syndrome was studied on basis of the NEFA concentrations and AST activity of blood samples. Occurrence of hyperketonaemia was judged on basis of the aceto-acetic acid concentration of the blood samples.

Milk yield of the experimental and control cows were recorded by milking. Butter-fat concentration was measured once a month. Feed intake of the cows was measured per feedings.

Body condition of the cows was scored at the time of taking ruminal fluid samples (Mulvany, 1977).

Results and discussion

VFA concentration of the ruminal fluid: Ruminal acetic-acid concentrations of the groups were almost identical prior to and right after calving and no statistically significant difference was found between the groups in the later phase of the experiment till the 6th and 7th samplings at day 75 and 107 post partum, respectively, where the experimental cows produced significantly more acetic acid than the controls.

The average propionic-acid concentration of the experimental cows proved inferior to the controls at the 1st sampling and in the first month of lactation there was no major difference between control and experimental cows. From the 5th samplings onward, however, the propionic-

IU: one unit of xylanase activity expressed as μmol of reducing /xylose equivalent/ sugar released in one min

acid concentration showed steady increase in the experimental animals over the controls.
The concentration of n-butyric-acid in the ruminal fluid samples of the experimental and control cows was almost identical prior to calving (1st sampling), then in the next two samplings this parameter of the experimental animals lagged behind those of the controls. In the 2nd part of the experiment (from the 4th samplings onward) the experimental cows produced more n-butyric-acid in the rumen in comparison with the controls.
The concentrations of the total VFAs followed the pattern of the three organic acids discussed above. There was a higher VFA concentration in the experimental cows in the 2nd half of the experiment.
Energy and protein metabolism: Glucose concentrations of the blood samples of the control and experimental cows ranged between 2.6-3.3 and 2.6-3.5 mmol/l, respectively throughout the examination with no statistically significant differences between control and treatment cows.
The serum aceto-acetic acid concentrations of non-treated cows were higher than those in the experimental cows throughout the experiment. The difference between averages of the groups proved significant on days 22, 75 and 107 post partum. It is accepted that aceto-acetic concentration higher than 0.1 mmol/l indicates the presence of hyperketonaemia (subclinical ketosis) (Brydl, 1999, Brydl et al., 2000, Radostits et al., 2000). The group average of the control cows on day 22 post partum was higher than 0.1 mmol/l and the difference between the experimental and control cows was statistically significant ($P<0.01$). Further to this, analysis of the data revealed 11.1, 16.7, 14.3 and 12.5% incidence rate of subclinical ketosis in the control group on day 10, 22, 32, and 58, respectively. The incidence of hyperketonaemia in the experimental group was considerably less with 8.3 and 11.1% on day 10 and 22 after calving. There was not hyperketonaemia on day 32 and 58 in this group.
The NEFA concentration in the plasma of the control cows on day 10 after calving elevated over the physiological level (0.2 mmol/l, Gönye, 1987, Gaál, 1999). The 0.183 mmol/l difference between the pre-calving NEFA concentration (Day -9) and that measured on Day 10 after calving proved statistically significant ($P<0.01$) in this group.
AST activity of the control and experimental cows ranged between 43.6-103.7 and 67.8-95.4 (U/l), respectively with no significant between group difference. The within group proportion of cows that had AST activity higher than 100 U/l was bigger in the control than in the experimental group (e.g. 55.5% vs. 33.3% on day 10, or 37.5% vs. 22.2% on day 107).
Prior to parturition the average urea concentration of the blood in the experimental cows was significantly higher than that of the control cows. After parturition the blood concentration of the urea in both groups have elevated slightly over the accepted physiological limit value (3.3-5 mmol/l, Brydl, 1993). The difference between the control and experimental cows proved statistically significant on day 22 after calving. Urea concentration of the urinal samples varied in both groups within the physiological range (130-300 mmol/l, Vrzgula, 1985, Brydl, 1993).
Laboratory findings concerning the acid-base metabolism of the experimental and control cows indicated the presence of balance throughout the experiment. The average Net Acid-Base Excretion (NABE) was higher in both groups than the physiological limit value (>100 mmol/l, Kutas, 1965), while the urinary pH values remained within the range of pH 7.8-9.2.
Milk production: The experimental cows produced more milk from the very beginning of the lactation. The difference between the groups varied between 0.49 and 3.43 l/day.cow in favour for the experimental groups with an overall surplus of 2.14 l/day.cow. Experimental cows produced somewhat more (0.09%) butter-fat in the average of the experimental period. No difference was found with respect to the milk protein content.
Feed intake: The experimental cows ate more Total Mix Ration (TMR) in the first half of experiment (4 decades). The feed consumption of the experimental cows in this period proved significantly higher ($p<0.001$) than that of the controls. The TMR intake was numerically more in the 6th and 7th decades in the experimental cows, and in the 8th decade the difference between the experimental and control cows became statistically significant ($p<0.001$). At the end of the experiment (9th, 10th, 11th decades) the control cows consumed more TMR ($p<0,001$). At the end of the experiment the experimental cows consumed less TMR for production of 1 litre of milk.
Body condition of the cows: At the beginning of the experiment there was only 0.2 score difference between the groups in favour of the controls, which then became less and from about the third samplings the condition of the experimental cows increased over the controls, but the 0.2-0.4 score difference was statistically not significant. Body condition of the cows reached minimum at about 32±4.6 days after calving and proved inferior throughout the experiment to the score taken prior to calving. The about 30% difference between the body condition scores of the dry (experimental and control) cows and those measured at about 32 days after calving proved statistically significant ($P<0.001$). The condition scores of the experimental cows never went below 2.6 (to the contrast of the controls), and the decline of body condition in the post-parturient period was about 20% less ($P<0.001$) in the experimental group.

Conclusions

The lignolytic enzyme preparation applied in the present experiment and fed to dairy cows at 34 g/day dosage increased the appetite of experimental cows and VFA concentration in the rumen from about 32 days after calving and onward. Increased appetite and VFA production has been followed by about 5 to 10% increase in the milk production and almost 0.1% increase in the butterfat production. Due to more balanced energy metabolism post-parturient weight loss of the treatment cows is reduced, which decrease the risk of clinical manifestation of fat mobilisation syndrome.
Data of the present experiment supply further proof for the beneficial effects of lignolytic enzyme preparations in dairy cows.

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MINIMAL PIGLET LOSSES WITH THE TENDERFOOT° „VARIO STEP“SYSTEM AFTER WEANING A CASE REPORT

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Introduction

In Germany the most breeding farms have high losses (> 15 %) of piglets in the first weeks after birth.The reasons can be leg lesions,arthropathia and overlying.

Material and Methods

For a Dalland° breeding herd with 400 sows a new farrowing house with the Tenderfoot° „Vario Step Floor System“ was build.During the last years production dates like farrowing rate,leg lesions,arthropathia,overlying and other parameters were registrated by a computer program.In a comparison with the old and the new stable floor different parameters for losses during the last and this year were evaluated.

Tenderfoot° Vario Step System with a suckling sow

automatic lift for the piglet floor

lift down

lift up

Results

During the year 2003 in the first three weeks after birth there were losses of 11.97 % in the old farrowing house.In the year 2004 we found losses of 8.7 % in the new stable with the Tenderfoot° vario step system.In 2003 losses by leg lesions under the piglets were 0.45 % and in 2004 they were 0.13 %.In 2003 the losses with arhropathia were 0.83 % and in 2004 they were 0.05 %.In 2003 the losses of overlying were 4.98 % and in 2004 they were 2.14 % (Table).

Table
Losses of piglets during the first three weeks after birth with out and with using the Tenderfoot°vario step system

time	total	leg		
period	losses (%)	lesions (%)	arhropathia (%)	overlying (%)
12 months				
2003	11.97	0.45	0.83	4.98
6 months				
2004	8.70	0.13	0.05	2.14
% of reduction during the two periods	27.3	71.1	94.0	57.0

Discussion

In this breeding farm,which used the Tenderfoot° vario step system in the farrowing house since 2004 the farmer could reduce the total losses by 27.3 % and the losses of overlying by 57 % in the first 6 month of this year with the new floor.The losses of lesions,as arhritis and clows,were reduced by 71.1 % and of arthropathia by 94 % with the new floor system.After the birth of the piglets the mechanic and pneumatic lift for the piglet bed was automaticly letted down,when the sow stood up.When the sows came down,the piglet bed got up and the piglets could suckle.Because of this automatic lift the losses were reduced,as we could show in the new farrowing house with the Tenderfoot° vario step floor system. The Tenderfoot° grill grate is protected with Plastisol°,warm,step - and slide - save,with reduced

arthritis and clow lesions.In a comparison study with other floor systems it could be shown,that the Tenderfoot° floor have the lowest lesions at all body parts of sows and piglets (G.Jacobs,2002).In the Dalland° breeding farm the number of weaned pigs could be increased from 23.5 piglets per sow and year to 24.7 in the first two quartals of this year.The benefit per sow and year increased by more than 14 Euro till now with the Tenderfoot° vario step system in the new farrowing house.

Conclusion

In a Dalland° breeding farm with high health and normal losses during the fattening period,the Tenderfoot° vario step system was installed in a new build farrowing house.Already in the first six months the losses during the suckling periode were reduced by 27.3 % and the benefit increased by more than 14 Euro for each sow.

THE DEVELOPMENT OF DENSELY POPULATED LIVESTOCK AREAS IN THE EUROPEAN UNION A CASE STUDY IN THREE DIFFERENT EUROPEAN PIG PRODUCTION AREAS

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Introduction

The paper analyses the development of pig production over the last 150 years in three European regions. These regions are characterised by high pig densities per square kilometre. Study areas are:

- the provinces Parma, Reggio Emilia and Modena (called Emilia) in the north Italian region Emilia-Romagna
- the provinces Noord-Brabant and Limburg (called Zuid) in the southern part of The Netherlands
- the counties Emsland, Cloppenburg, Vechta, Grafschaft Bentheim and Osnabrück (called OBE-region) in Lower Saxony, northern Germany.

These regions represent main national pig production areas.

The main goal of this paper is to create a model of the development of pig production areas with high pig densities in Europe. Today the system of intensive pig and pork production and the environmental impacts from intensive agriculture get high attention from the public. Very often, however, agricultural history and the high-capacity production which was built up over a long time period do not get the right attention.

Material and Methods

In a first step the three different developments and historical structures of pig production were compared from a historical point of view. Individual aspects during the agricultural development, such as property structures (owner, tenants), different target markets (sausage production, export market, domestic market), sector organisation (co-operatives, private enterprises), political reactions on environmental problems etc. were included. For each research area an individual development structure was made up. The results of the case studies were compared and it was distinguished between individual and typical structure elements and driving forces.

Results

The three study areas show periods of stable production patterns in pig farms and under the influence of different driving forces the transformation into another stable system. For Emilia as well as for Zuid three developmental phases are distinguished, for the OBE-Region only two (see figure 1). The individual histories are different: while in Emilia the transformations occurred around 1910 and 1960, Zuid changed its pig production structure the first time after the Second World War and again in the 1990th. The OBE-region shows only one transformation: in the post-war period of the Second World War. As one can easily see (see table 1) there are considerable differences in the individual structures. Whereas in Emilia pig production started in cheese factories, in Zuid and the OBE-Region small and medium sized family farms were the owners of the pigs. Another important distinction is the market orientation. The Italian region was and still is focussed on the production of traditional sausages and hams (like Parma ham). The Dutch region supplied foreign markets already, whereas the German region always concentrated on the domestic market. Nevertheless there are typical structures in each study area.

For the model two stable phases could be developed: the first one reached over a period of 100 years from the middle of the 19th century to the middle of the 20th century. It was based on self-sufficient family farms who more and more integrated pig breeding into their households. They used whey as one part of the basic feed and replenished it with imported feed. Co-operatives and private enterprises, involved in slaughtering and meat processing, were established in the regions and promoted pig production. The main driving forces in this period were: the disposal of whey out of milk processing, good traffic connections to the sea ports for imported feed and to the developing markets for meat products, agricultural innovations and their dissemination, skills of working family members and the introduction of co-operatives. This phase is called "phase of steady growth". The second phase started after the Second World War. It was based on the fast growing family farms, which were highly specialized. Vertical integrated production structures were not yet wide spread, production control systems were established. Depending on the overall growth of pig numbers, regions with high pig densities increased and caused environmental problems. To get rid of the nitrogen loads, different strategies were developed: use of feed with reduced nutrients (e.g. protein), transformation of the manure in their components, shipping the manure into other regions and, as a final possibility, reducing the pig numbers. Besides, various outbreaks of Classical Swine Fewer lead to problems in consumer acceptance. The main driving forces were growing meat demand, political regulations to protect the environment, consumer demand for controlled and save food. "Phase of instability" could be the headline of this period.

Figure 1: Comparison of the development phases in the three case study areas

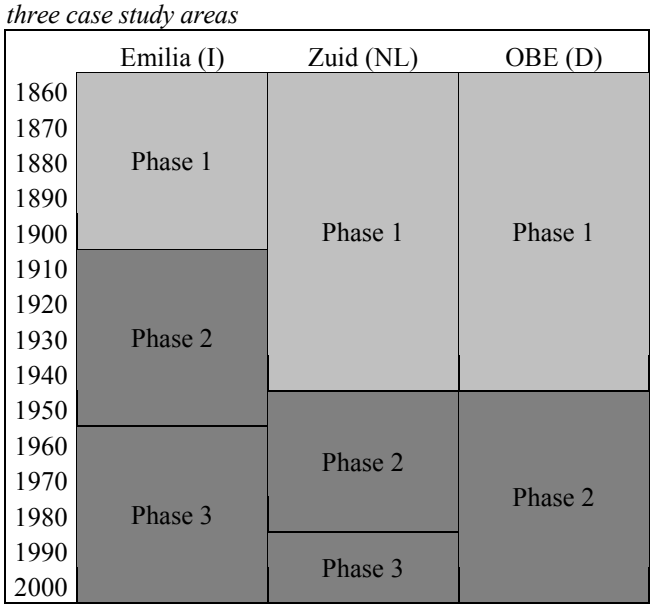

Table 1: Individual and typical structure elements in the different phases of the three study areas

Phase		individual structure	typical structure
Emilia/Italy			
1	1860 to the beginning of 20th century	- regional tenant system (*Mezzadria*) and large estates - mixed culture (*Cultura Mista*) and milk production as a basic element - pig production only of owners of cheese factories - production of preserved sausages, this means fattening of 160 kg pigs	- mainly small subsistence farms - cheese production leads to whey feeding - higher disposal of feedings through imports
2	1910 to the 1950th	- regional tenant system (*Mezzadria*) - emergence of co-operatives with implementation of pig production - production of preserved sausages, this means fattening of 160 kg pigs	- family farms - co-operatives strengthen agriculture - cheese production leads to whey feeding
3	1961 till now	- decline of the regional tenant system - increase divide between cheese factories and pig production - high presence of hired labour - production of preserved sausages, this means fattening of 160 kg pigs - reduction of the pigpopulationr	- high growth of pig numbers in family farms - processing of the manure - introduction of control system (specific to the traditional product)
Zuid/The Netherlands			
1	mid 19th to mid 20th century.	- small/medium sized family farms - return of whey to the delivering farm - mainly focussed on export markets leads to different weights and qualities	- mainly small subsistence farms - cheese production leads to whey feeding - imported feed as basis of production - co-operatives strengthen agriculture
2	from 1950th to the end of 1980the	- setting up of contracts between chain members - mainly focussed on export markets - delivery of manure to manure banks	- high growth of pig numbers in family farm - amounts of manure leads to environmental problems - introduction and extension of control systems
3	1990th	- delivery of manure to manure banks - reduction of the pig number with growing concentration in specialized farms and production areas	- high growth of pig numbers in family farm - distribution and processing of manure
OBE/Germany			
1	mid 19th to mid 20th century.	- regional tenant system (*Heuerlinge*) and inherit farms (*Erbhöfe*) - dealers dominate, transport the pigs to slaughterhouses out of the region - home market orientation leads to standard production	- mainly small and medium sized subsistence farms - cheese production leads to whey feeding - imported feed as basis of production
2	from 1950th till now	- decline of the regional tenant system - poultry farmers step into pig production - setting up of slaughter houses and processing industries in the region - marketing mainly by dealers - introduction of feed with reduces nutrients - delivery of manure to manure exchange and distribution agencies	- high growth of pig numbers in family farm - distribution and processing of manure - (hesitant) introduction of quality assurance and traceability systems

Discussion

For the near future another organisational pattern can be expected: the increasing demands for safe food and for traceability systems will force the pig and pork producers to invest in supply chains. So far of the three study areas only the Dutch have installed such systems. The Emilian pig production, based on traditional sausages, will also establish such systems in the near future and take advantage of the growing export market. The German study area, focussed on the domestic market, will lose market shares to international competitors if supply chains will not be established.

Conclusion

The results have to be compared with the historical developments in other European pig production areas. A global perspective would be helpful to estimate the market chances and risks which the leading pig production regions in the world have to face in a growing global market.

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BIOLOGICAL CONTROL OF NEMATODES IN GOATS BY THE NEMATOPHAGOUS FUNGUS *DUDDINGTONIA FLAGRANS*

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Introduction

The high prevalence of nematodes resistant to anthelmintics in French dairy goat flocks and the reduction in the number of anthelmintic treatments allowed per year in the organic production system force to develop new schemes for the control of nematode infections.

Different options are currently studied. Amongst them, the biological control method is based on the use of nematophagous fungi that form traps and consume parasitic nematode larvae in faeces. Administered as chlamydospores to sheep or cattle, the predacious fungus *Duddingtonia flagrans* has demonstrated its ability to highly reduce (over 90%) the developing larvae of a large range of nematodes in faeces (Larsen, 2000).

This method has been tested in goats with different aims :- Efficacy of spore administration in goat faeces in laboratory conditions
- Determination of the optimal dose
- Efficacy in field conditions.

Material and Methods

Animals

For all the laboratory trials, culled dairy goats were purchased in local farms. For the farm trial, a flock of young kids naturally parasite infected was used.

Fungus

Spores of the Danish Troll A isolate of *D. flagrans* were produced on millet seeds and packaged in aluminium foil bags by Chr. Hansen Biosystems A/S, Denmark.

Experimental schemes and parasitological procedures

For all the trials, half of the animals received the spores at the daily dose rate of 5×10^5 spores/kg BW, except for the dose titration trial, while the other half acted as control. After 6 days of administration, faeces were harvested individually. The egg excretions were determined using a modified McMaster technique. Faeces were then cultured for 12 to 14 days at 23 °C in a climatic chamber. At the end of this time, infective larvae were harvested by Baermann technique.

Specific parts of each trial are detailed afterwards.

- Efficacy in laboratory conditions

The efficacy of *D. flagrans* was determined against the 2 main prevalent nematodes in goats: 2 gastro-intestinal species: *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (experimental infections).

- Dose titration study

A dose effect relation was investigated using both experimental (*T. colubriformis*) and natural mixed infections (*T. colubriformis*, *T. circumcincta* and *Haemonchus contortus*). The tested doses were 1.25×10^5, 2.5×10^5 and 5×10^5 spores/kg BW. 2 ways of administration of spores were used: forced administration and administration at trough.

- Efficacy in farm conditions

Spores were administered daily at trough to half of the goat kids during the grazing season. Different parameters (weight, egg excretion, pasture infectivity and serum pepsinogen) were monitored during the whole grazing season. Kids were weighed at the beginning, middle and end of the grazing season. The egg outputs were measured every 3 weeks. Blood samplings were performed at the beginning, middle and end of the grazing season in order to measure the serum pepsinogen concentration reflecting the infection by abomasal nematodes. Pasture infectivity was evaluated every 3 weeks by washing the sampled grass and extracting the washed larvae by centrifugations using a sucrose solution.

Statistical analysis

The data were expressed as mean egg output and larval output per group. The percentage of development of larvae from the faecal samples of the different groups was calculated as the ratio between the total number of larvae and the total number of eggs in the faeces×100. The percentage of reduction of larval development was calculated as (1-(percentage of development in the fungus group/percentage of development in the control group))×100.

The comparison of means was made with the non-parametric Mann and Whitney-test using Systat 9.1 for Windows, 1998, SPSS Inc. (Chicago, USA).

Results

Efficacy in laboratory conditions

Table 1: mean number of eggs per gram (epg) and larvae per gram (lpg) obtained per group, in faeces of goats infected by T. colubriformis or T. circumcincta and receiving or not the spores

	epg	lpg	development
T. colubriformis			
Fungus treated goats	405 [a]	6,28 [1]	1,5
Control goats	384 [a]	62,30 [2]	16,2
T. circumcincta			
Fungus treated goats	167 [a]	3 [1]	1,8
Control goats	143 [a]	26 [2]	18,2

The reduction of larval development in goat faeces containing *D. flagrans* spores when compared with the larval development in control faeces was 91 % and 90 %, respectively for *T. colubriformis* and for *T. circumcincta* (table 1).

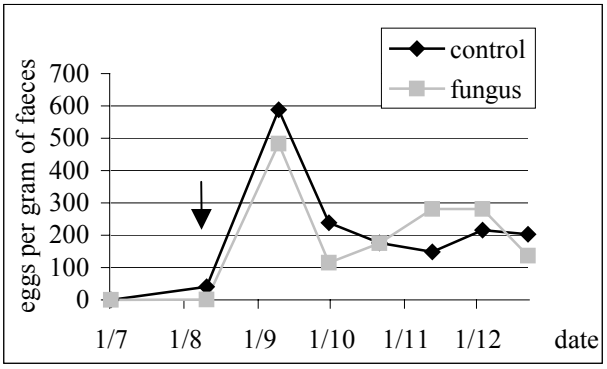
Dose titration study

In laboratory conditions, whatever the dose rate, the larval development reduction was higher than 75 % when compared with a control group and there was no difference between the doses.

On the contrary, with trough administration, a significant increase in the larval development reduction was observed from the 2.5×10^5 spores/kg BW/day (83.9 %) to the 5×10^5 spores/kg BW/day (97.7 %) when compared to the control. and

Efficacy in farm conditions

Figure 1 : evolution of egg outputs in the fungus treated group and in the control group during the grazing season



beginning of administration of spores to kids of the fungus group

None of the parasitological parameters (figure 1 for the egg outputs) showed any statistical difference between the fungus treated and the control groups.

The growth of kids of the 2 groups was the same (mean growth in the 2 groups : 13 kgs).

Discussion

These trials demonstrated the high ability of *Duddingtonia flagrans* to trap the infective larvae of gastro-intestinal nematodes in goat faeces. When compared with control faeces, the larval development reduction was 90 % for the 2 species tested, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* with a daily dose of spores of 5×10^5 spores/kg BW. This is in agreement with previous results: with the same dose, Fontenot et al (2003) reported reduction of the larval development of *Haemonchus contortus* higher than 80 % in sheep faeces.

All the tested doses, 1.25×10^5 to 5×10^5 spores/kg BW, gave the same reductions in conditions of controlled administration. In goats, Terrill et al (2004) showed a non significant effect with an apparent dose response, the highest dose, 5×10^5 spores/kg BW, leading to the highest reduction of the larval development of *H. contortus*, *T. colubriformis* and *Cooperia* spp.. On the contrary, in farm conditions, a dose response was shown in our study.

The minimal dose to recommend appears to be 5×10^5 spores/kg BW on a daily basis. A particular attention should be paid to the way of administration in order to limit variations in ingestion: Knox et al. (2001) demonstrated that variations in voluntary consumption may highly influence the efficacy of *D. flagrans* until total failure.

These variations in voluntary consumption may partly explain our results with young kids. When spores were administered at trough during the whole grazing season, we were not able to see any positive effect whatever the parameters. These results agree with other studies in goats : Wright et al., 2003 gave spores to goats at the daily dose rate of $7.5*10^5$ /kg BW during 26 days. Tracer kids were then introduced during 14 days. The faecal egg counts of the groups receiving or not the spores showed no significant difference nor the pasture larval counts. However, these authors demonstrated an effect of the administration of spores on the latter contamination of the pasture as the worm burdens of the tracer kids were significantly reduced in the group grazing the fungus treated pasture.

Conclusion

If the efficacy of *D. flagrans* against nematode larvae in controlled conditions of administration and culture has been demonstrated, its efficacy under field conditions should be further evaluated. Cost efficiency and environmental impact of daily feeding of fungal spores should be also evaluated before marketing *D. flagrans* spores as a method of nematode control.

Acknowledgements

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PIG PRODUCTION IN POLAND AND CENTRAL EUROPE

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Introduction

Poland is a country located in Central Europe, with a population of about 40.000000 people. The total number of farmers exceed 2 millions. From them more than 700.000 produce pigs. Poland was the first country in this part of Europe to liberalize in 1989 agriculture sector. Due to this, most of the large government and collective farms have been privatized. The new private farms are smaller compared to their former socialist organizations. Following the political changes the farming business has gone through a very hectic period of transformation. Instead of producing for a fixed price set by the government, pig producers have to operate on a liberalized market. The market changes were not limited to the internal market but also had an effect on international trade. More products from abroad could enter the markets and compete with local products.

Pig production is the most important farming and creates Poland as the largest producer of pigs in Central Europe (Table 1). Total number of pigs produced in Poland is similar to that in all other – nine new members of UE.

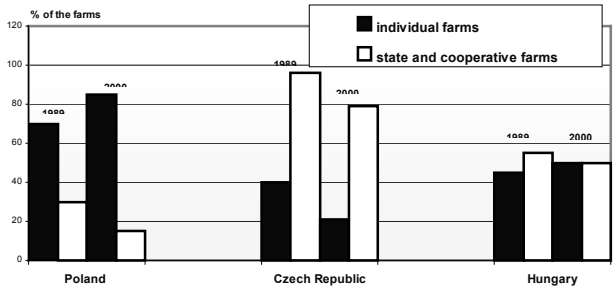
Country	Number of pigs
Bulgaria	1 850 000
Czech Republic	3 286 200
Estonia	340 000
Hungary	4 500 000
Lithuania	1 052 950
Poland	*19 605 300*
Slovakia	1 553 380
Slovenia	606 300
Romania	5 101 258

Tab.1. Production of pigs in Central Europe in 2003

The main breeds used in Poland are: Landrace, Large white, Duroc, Pietrain and Hampshire.

The structure of pig farming was different in various CEC. For example in Poland just before 1989 the percentage of private farms reached approximately 70%, wheren in Czech Republic was only 4% such farms and in Hungary 45%. In 2000 percentage of private farms increased in all mentioned countries and reached the level of: 85%; 21% and 59% respectively in Poland, Czech and Hungary. Similar trend were observed in all other CEC.

Fig. 1. Pig producers in selected CEC according to the production sector in 1989 and 2000



Source: ITP

Annual pig meat production reached about 2,5 million tons.

Year	Number of swine slaughtered	Average weight at slaughter	Pork production in 1000 kg
1991	22.339	115	2578
1992	23.576	112	2652
1993	22.827	111	2532
1994	19.870	112	2226
1995	22.694	113	2576
1996	23.571	113	2657
1997	20.990	112	2430
1998	20.800	111	2601
1999	24.427	109	2675
2000	22.658	110	2500
2001	22.000	108	2415
2002	23.600	107	2600
2003	25.500	106	2820

Tab.2. Swine population and pork production in Poland during 1991-2003

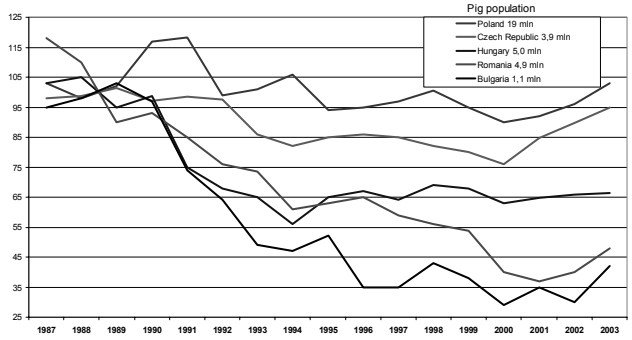
Number of pigs produced annually by one farm is very low (approx. 16) in comparison of fatteners produced by average farm in EU (94 pigs)

Country	Number of pigs in herds			
	1-9	10-49	50-199	> 200
Denmark	7.1	18.8	24.0	50.1
Finland	7.1	13.7	42.8	36.4
France	71.5	6.0	5.8	16.7
Spain	84.5	7.0	4.1	4.4
Holand	3.1	5.7	25.5	65.7
Germany	50.1	20.6	15.5	13.8
Portugal	85.2	10.7	2.9	1.2
Sweden	20.1	25.6	25.4	28.9
POLAND	**67.4**	**27.9**	**4.4**	**0.3**

Tab.3. Herd structure in Poland and in certain UE countries in 2000 (%)

The number of sow in Poland circulated around 1.6 million. Annual production of fatteners reached in 2003 - 25 millions.
It should be underlined that many (in numbers not in percentage) farms produce more than 5000 fatteners/per year.
It might be stated that in general pig production in Poland is extensive. Production of piglets amounted in average 14.0/per sow/per year. Weaning period is about 35 days and number of days till slaughter reached between 160-220 days. In majority of farms feed conversion exceeded 3.1 kg/kg body weight. It should be underlined that agriculture production in Poland is not subsidized. Taking into account presented information it is clear that in some cases the cost of production is higher that of the imported product. As a result of this in 2001 many pig producers stopped or reduced their production capacity.

Fig. 2. Dynamic of pig production in selected Central European Countries 1987 - 2003 (Production of pigs in 1998=100)



Source: ITP

Unfortunately, structure of pig farming in Poland is old fashion more than 80% of farms are selling less than 50 fatteners per year. Even more traditional (old fashion) pig production is in Romania, where 5,4 milions pigs is produced by approx 2 mln farms. The size of the pig farm is connected with area of the land related to the farm. Average size of the land in Poland is 7,6 ha and is significantly lower than in others EU countries. The structure of agriculture is related also to the number of people working in this sector. In Poland 27% of emploes work in agriculture. It is typical that during period of very low prices small farms survive difficult periods whereas many of medium and large farms are going to bankrupt.
During last 10 years due to new economical situation and cooperation of farmers with foreign companies and investors major steps forward have been made by some, most active producers. In particular due to the use of new genetics, imported feed additives and new technologies of production thousands of pig producers meet international standards in terms of cost of production and quality. Many (in numbers - not in percentage) farmers produce more than 22 fatteners/per sow/per year. Significant influence on progress in efficiency standards plays foreign pig producers which established pig farms in Poland. Among them are farmers from Denmark and USA. It should be stated that currently about 20% of UE pork production takes place in new member states. Among them Poland produce 11,6% of UE pork.
Control of health status of pigs is done by approximately 2000 veterinarians – owners of private animal clinics.
System of postgraduate studies in the field of pig specialists exist in Poland since 1996. During this time about 150 veterinarians received title of The Veterinary Pig Specialist. Such groups of veterinarians offer very professional wide range veterinary service. They offered consultancy not only in the field typical for veterinarians but also in areas connected with management, feeding, organization of production, insemination, etc.
Discussing health status in pig farms in Poland it should be stated that health problems in pig industry are similar to those observed in other European pig producing countries.
Poland is free from classical swine fever (CSF), FMD, SVD and ASF. Classical swine fever causes significant problems in some CEC. For example in 2003 more than 150 outbreaks of CSF were noted in Romania and more than 10 in Bulgaria. Like in other countries the main economical problem are caused by respiratory diseases. Most of the pulmonary problems are created by widespread of PRRS virus. Approximately, 60-70% of medium and large farms are infected with etiological factor of this disease. Application of vaccination program is based on detailed evaluation of type of PRRSV existing in the farm and serological profile of the herd. In general proper introduction of vaccine gives reasonably good effects. The second most popular respiratory disease is Mycoplasmal pneumoniae of swine (MPS). Control of this disease is performed by means of immuno- and chemioprophylaxy.
Since 3 years the number of cases of new disease is rapidly growing. There are Streptococcosis caused by Streptococcus suis type 2 and in some cases by Streptococcus suis type 1. The second such disease is Glässer diseases caused by Haemophilus parasuis.
Still, some enteric diseases are difficult to control. To these diseases swine dysentery and adenomatosis should be included. The growing problem among suckling piglets is connected with isosporosis.
The main reason of low production of piglets by sows (only 14.0 weaned piglets/per sow/) are “old fashion” approach of majority of farmers to reproduction. Also average veterinarian is not interested in organization of production and reproduction. It might be stated that such methods like: synchronization of oestrus, diagnosis of pregnancy, synchronization of farrowings are applied very seldom.
In summarizing, despite of very positive changes achieved by part of pig producers, more emphasis should be done to improve efficiency of pig production and health control in swine sector in CEC. In Poland important step – connected with international trade of live pigs-is introduction of program of Aujeszky Disease eradication. To do this, as soon as possible, philosophy of producers and approach of large part of veterinarians must be changed. Such changes are necessary for being competitive in common EU market.
It should be noted that during 2 month after 1 of May 2004 (day of accession to EU) prices for pigs in Poland rised about 15-20%.

References

FAO, EUROSTAT, ITP

CONCENTRATION OF DIFFERENT MYCOTOXINS IN FEED AND STRAW ON 6 IRISH RACEHORSE FARMS

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Introduction

Mycotoxin contamination of feed can affect equine health and performance. This can result in reduced animal well-being and in major economic losses for the equine industry. In addition to reduce growth rates, reproductive performance and physical performance, mycotoxins also act to weaken the immune system, leading to greater susceptibility to disease and secondary infections, and consequent increases in veterinary costs [1] [2] [3] [4] [5] [6]. Historically, the principle focus for investigation into mycotoxin contamination has been on grain, yet evidence is now accumulating that roughage and even straw used as bedding can as bedding or 'environmental enrichment' can play a role in mycotoxicosis.

Mycotoxins are naturally occurring, toxic chemical compounds produced by filamentous fungi (molds). These molds can produce a variety of mycotoxins, such as aflatoxin, fumonisin, deoxynivalenol (DON), ochratoxin A, T-2 toxin and zearalenone.

Accurate diagnosis of a mycotoxicosis is difficult because affected animals may exhibit few or many of a variety of symptoms. The fact that most of the symptoms are rather unspecific and can be caused by many other factors makes it often difficult to properly diagnose mycotoxin problems.

The aim of the present trial was to investigate the concentration of different mycotoxins in feed and straw of 6 Irish racehorse farms in order to get a measure for actual challenge levels.

Materials and Methods

A total of 175 feed and straw samples were collected during the time period October 02 - March 03 in 6 Irish racehorse farms. All samples were analyzed by ELISA for the following six mycotoxins: aflatoxin, ochratoxin, fumonisin, deoxynivalenol, T-2 toxin and zearalenone. The samples were grouped as straw, hay/haylage, grain and mixed feed and the data were analyzed by descriptive statistics.

Results and discussions

Zearalenone and DON were the 2 main mycotoxins found in the samples (Table 1 and 2). The maximal concentration measured for aflatoxin was 17.7 ppb, for fumonisin 627.0 ppb, for ochratoxin 22,1 ppb and for T-2 toxin 208.1 ppb.

Mycotoxin concentrations varied from farm to farm with the mean farm concentrations for zearalenone ranging from 20 to 117 ppb and the mean farm concentrations for deoxynivalenol ranging from 50 to 270 ppb. Many of the samples were contaminated with multiple Fusarium mycotoxins. This is of concern, as mycotoxins can act synergistically.

Overall, the survey demonstrates that mycotoxins are present in considerable concentrations in both the bedding and the feed. Bedding and feed qualities have to be managed properly and mycotoxins should be taken into consideration when performance, health or reproductive problems do occur on horse farms.

Table 1: DON concentrations of horse feed and bedding

	DON		
	Mean, ppb	Maximum, ppb	%Positive
Hay/haylage	167.0	1424.0	22.7
Straw	200.0	1300.0	24.4
Feed	160.7	1087	33.8
Grain	132.0	500.0	31.8

Table 2: Zearalenone concentrations of horse feed and bedding

	Zearalenone		
	Mean, ppb	Maximum,pbb	%Positive
Hay/haylage	38.0	291.2	18.2
Straw	62.2	1334.0	22.0
Feed	77.3	298.0	61.8
Grain	3.0	65.8	4.5

Conclusions

Bedding and feed qualities have to be managed properly and mycotoxins should be taken into consideration when performance, health or reproductive problems do occur on horse farms.

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MODELLING THE ANIMAL – ENVIRONMENT INTERACTION AND ITS IMPACT ON THE WELFARE AND THE ECONOMY OF FARM ANIMAL PRODUCTION

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Abstract
Welfare, the level of animal comfort can make a great difference in economical results between herds with a similar general production system. The goal of our effort in the field of animal welfare is to create the virtual system of evaluation of animal welfare from the stand point of physical conditions maintained in the stables, the system and condition of feeding, the maintenance of care including veterinary care. The mutual interference between the genotype, feeding and environment is used for modelling this process as it is expressed in the form of the body mass growth and the product formation by the animals. The described modelling approach is based on the self-regulating model of body mass growth accomplished directly from the data commonly used by farmers with the best animal care.

Introduction
When investigating the Animal – environment interactions we have to handle two distinct systems. The environment, the background of which are the local climatic conditions. They can change in a circadian and seasonal rhythm according to a great variability of radiant temperature, air temperature, wind velocity, humidity and barometric pressure, rain and snow falls. The homoeothermic animal is characterized by the constant value of its core temperature. This feature maintains his vital functions at a given appropriate level. However for this privilege, the homoeothermic animal must pay by producing the thermostatic heat in the same amount as he simultaneously leaves to the environment. The only external source of energy needed for the biological processes accomplished in the organism is the feed. However the feed energy consumed for the thermostatic heat production is lacking for the synthesis of proteins lipids and sugars needed to the body mass growth or milk or egg production. That means, the production of the farm animals depends not only on their genetics and feeding, but also on their relation to the environment. This implies that the growth process has to be interpreted not only by the empirical descriptive models based on the use of the logistic or exponential growth functions (Emmans 1997, Moughan, P.J., Verstagen, M.,W.,and A., Visser-Reineveld (editors):1995, and many others). Neither only in the other way based on biochemical interpretations, (see McNamara, J. P., France, and J., Beever,: D.2000). The self regulating growth model created by Ludvik Novák (1996) produces the growth curve as a process of conversion of the feed energy into the thermostatic heat and the mass of synthesized proteins, lipids and carbohydrates. The calculation is carried on independently of the growth of the real organism (ie the considered farm animal) from the data about the feed, environment and the biological characteristic of the simulated organism. The self regulating model was validated in experiments with the regulated feeding in Wistar rats (Novák, Pipalová 1996), on the growth of broilers and pigs (Novák, Zeman, 1997, Novák, P., Novák,L. et al. 1998, Novak. L. 2003, Novák, P et al. 2004, Novák, L. et al 2004.).

Material and Methods
Our effort in the field of animal welfare is focused on the development of the virtual model for the evaluation of animal welfare based on physical conditions maintained in the stables, the system and conditions of feeding, maintenance care and the veterinary care. The final objective of the elaborated methodology is to include the influence of the local climate and various stressing factors linked with the technology and the maintenance care on the economic profitability of the farms. This is a very important fact because it enables to solve problems about the economical impacts of the EU legislation concerning the welfare of farm animals.

Results and discussion
The mutual interference between the genotype, feeding and environment is one of the areas suitable for modelling the growth of the animals. Our modelling approach is based on the body mass growth from the data commonly used by farmers with the best care devoted to the animals
In comparison with classical approaches used in the solution of welfare problems (Broom. 1986), the virtual system elaborated by our team is characterised by the fact that the core of the system generates automatically the body mass growth from the basic elementary biologic data about the organism. The relation of the organism to the ambient surroundings is described by the physical data (e.i. core temperature, thermal insulation of the core regarding the environment, radiant and air temperature, relative humidity, air movement, barometric pressure). The relation of this data to the organism's welfare is interpreted according to the biophysical rules; they do govern the energy balance between the organisms and their surroundings (Novak, L., Novak, P., Schauberger, G.:2000).
All the processes are carried out **inside the stables.** That means the construction of stable building modifies the influence of the surrounding local climate and the **concentration of animals** inside the stable invokes the need for the **adequate ventilation, heating or cooling**. The high concentration of wastes and urine, sources of ammonia, sulphuric compounds raises up new problems leading to unpleasant odours.
The interaction between the energy intake through the feed, the cooling power of the environment and the actual state of the body mass maturity on the welfare of the growing organism are presented in **Scheme 1**. The intake of metabolizable energy and its partition between the total heat production (THP) and the formation of the net energy for production (NEp) is influenced by the cooling power of the environment (CPE). If in this model the prices of the feed and the slaughter prices of the animals

are supplied, it is possible to figure out the economy of raising the organism. Such a possibility under the actually defined conditions is presented in the demo-model at: http://fvhe.vfu.cz/2502/2210/files/EKONWE_dEM2002cz5.xls. The most important thing on this methodology is its independence on any coefficients. They should be measured in real previous experiments, because the input data are values that describe the energy input through feed intake in [MJ/day], the actual body mass of the modelled organism [kg], the value of genetic body mass [kg] and the cooling power of the environment [MJ/day], calculated for the actual core temperature [^{o}C], the thermal insulation [m^2K/W] of the core against the temperature [^{o}C], relative humidity [%] and movement [m/s] of the air in the livestock building. The relation of the microclimatic values within the livestock building to the local climatic conditions depends on the construction of the building expressed in the thermal insulation of the walls, windows and doors [m^2K/W], further on the volume of the air-flow exchanged by the ventilation system V [m^3/hour], and the local meteorological values. (Schauberger 1988a and 1989)

Conclusions

In comparison to the classical approaches used in the resolution of welfare problems, the virtual system elaborated by our team is characterised by the fact that the core of the system generates the body mass growth automatically from the basic elementary biological data about the organism and its feed. The relation between the animals and the ambient surroundings is described by the physical data (i.e. core temperature, thermal insulation of the core to the environment, radiant and air temperature, relative humidity, air movement, barometric pressure,). The relation between these data and animal welfare is interpreted according to the biophysical rules; they do govern the equilibrium of energy exchange between the animals and their surroundings

Aknowledgements

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Scheme 1.

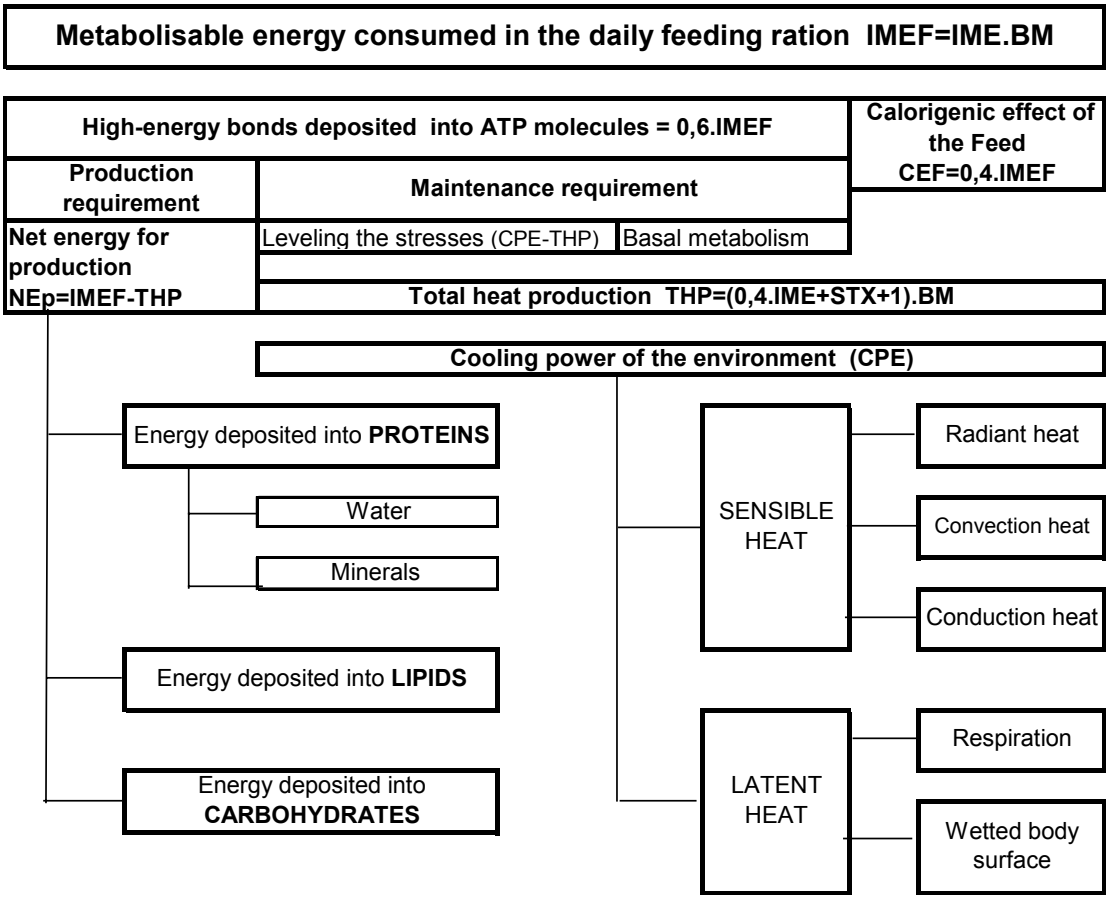
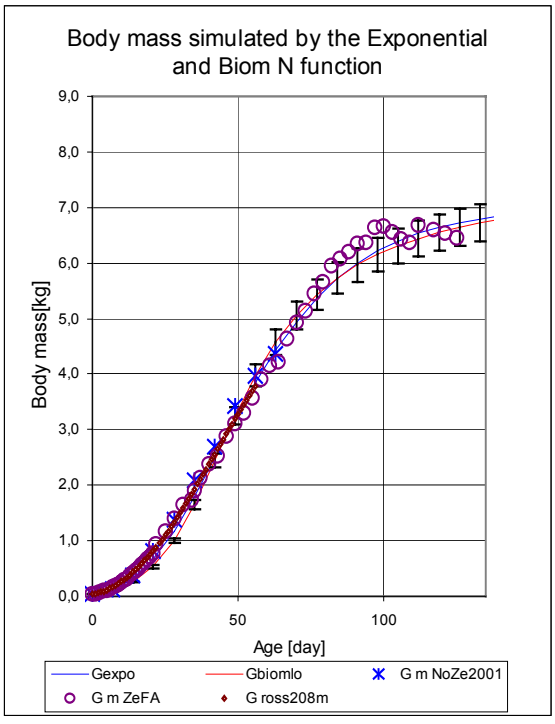
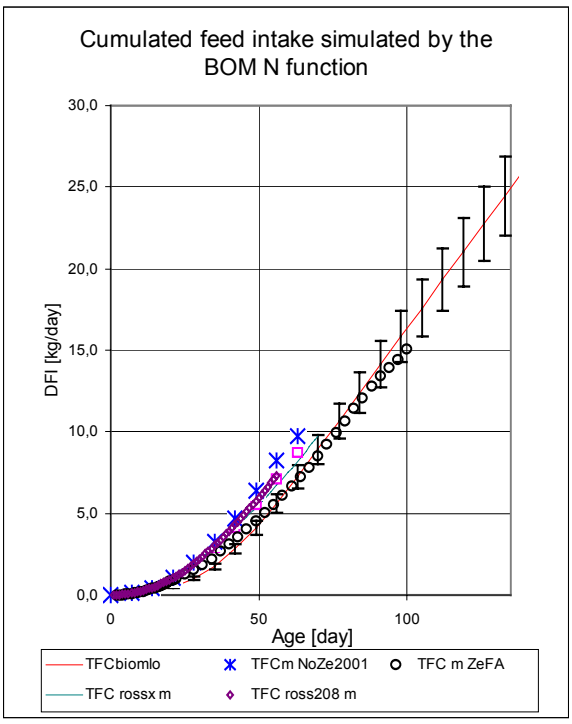


Fig. 1., Body mass growth and feed consumption in ROSS 208 cockerels ; calculated and measured values of body mass growth and feed consumption.Input data Zelenka, Fajmonova et. al 2001, Novak P. Zeman et al 2004

Interval	G_0/Gli	dGm	dGm biolo	DFI	IME	STX	SMEF	cOP	cPB	IMEF	THP
0	0,035	0,100	0,115	0,0095	4,720	0,15	12,06	0,5	0,16	0,114	0,075
7	7			0,240	2,242	0,15	12,06	0,55	0,16	2,990	2,810





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A SURVEY ON SOME BACTERIOLOGICAL AND PATHOLOGICAL ASPECTS OF SHEEP LIVER ABSCESSES

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Introduction

Hepatic abscesses can be seen in any species but are more prevalent in ruminants especially Cattle. The liver is particularly susceptible to abscesses because it receives blood from several sources, including the hepatic artery, the portal system, and the umbilical vein in fetus and neonate. Local suppurative infections of the liver don't cause clinical sign's of hepatic dysfunction unless they are particularly massive or extensively metastatic. Hepatic abscesses in goats have been described, too, but the information about them in sheep is very little. This study was carried out to find the occurrence of liver abscesses in sheep slaughtered in Garmsar.

Material and Methods

Livers from 493 randomly selected sheep were examined. Specimens were obtained at the local slaughterhouse on 21 days spread over two seasons. The animals were selected from both sexes and divided to three age groups (group A: less than 1 year, group B: 1-2 years and group C: more then 2 years). Liver was examined, and the number, location and size of abscesses were record and then sampling for bacterial culture was done. Statistical analysis is performed using Z-test and $\chi 2$ methods.

Results

Of the 493 sheep examined, 39(7.9%) had abscesses. The rate of liver abscesses in male and female was 5.3% and 73.8%, respectively. According to presence of abscesses there was significant difference between the two sexes ($p<0.05$). The rate of liver abscesses in different age groups A, B and C were 2.4% , 8.7% and 21.6%, respectively and there was a significant difference between them ($P<0.05$). Most of the abscesses (82.1%) were found in right lobes of livers and there was a high significant difference between different liver lobes ($P<0.01$). Also, most of the abscesses (92.3%) were found in diaphragmatic surface of the liver. All of the abscesses were less than 1 cm in diameter. The following bacteria were isolated (Table 1): Staph.aureus (22 cases), C.pseudotuberculosis (18 cases), Strp.agalatia (6 cases) and F. necroforum (1 case).

Tab1: Frequency of bacteria isolated from liver abscesses

Bacteria	Frequency
C.Pseudotuberculosis	5(12.8%)
A.pyogen	4(10.3%)
Staph.aureus	6(15.3%)
F.necroforum+Staph.aureus	1(2.6%)
A.pyogen+staph.aureus	10(25.6%)
A.pyogen+staph.aureus+strep.agalactiae	3(7.7%)
staph.aureus+strep.agalactiae+ C.Pseudotuberculosis	1(2.6%)
staph.aureus+strep.agalactiae	1(2.6%)
A.pyogen+ strep.agalactiae	1(2.6%)
No growth	7(17.9%)
Total	39(100%)

Discussion

The abscesses usually found in the liver at the time of slaughter or necropsy are often well encapsulated with thick fibrotic walls and therefore hematological analysis and liver function tests are not useful indicators of liver abscesses. Economic loss may still be significant, however, because the rate of gain may be reduced by 3% to 8% due to decrease in feed intake, and feed efficiency may also be reduced. Also, hepatic abscess leads to the rejection of the affected livers at the abattoir. Liver abscesses occur in any species, but the abscesses of significant economic impact occur in feedlot cattle. However, it can occur in other ruminants, like sheep. Results of this study showed that 39 sheep (7.9%) had liver abscesses. We couldn't find the rate of hepatic abscess in slaughter sheep as most of the studies on liver abscess have been done in cattle. Therefore, a comparison of the results of this study with other studies was impossible. But this figure is less than the usual range for cattle, 12% to 32%. In this study female sheep had liver abscess more than male. It is expected that the rate of abscess is higher in older sheep, and in a herd, female sheep are older than males. It should be noted that although many factors are involved in occurrence of liver abscess, but diet is the most important one. The question may rise, as male sheep is breed as feedlot animal rather than females, and receive more carbohydrate food than females; therefore they must be more vulnerable to liver abscess. One can imagine that the female sheep are fed with the risky diet before slaughtering, and due to their higher age, they are more exposed to liver abscess.There was a significant difference between different age groups with respect to liver abscess affection, such that the abscess occurrence is higher for older sheep. Presence of isolated bacteria indicated abscesses forming following ruminitis and reaching bacterial flora from rumen to liver. Observation of more abscesses on diaphragmatic surface and right lobes can be due to being more exposture of these parts to portal vein blood stream.

Conclusion

According of results of this study hepatic abscess isn't an important disease in sheep in Garmsar.

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RESEARCH ON IMPACTING FACTORS ON LOW PROLIFICACY IN SOWS IN ROMANIA

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Introduction

Undoubtedly the level of prolificacy is influenced by many types of factor among which: genetical, managerial, diseases and environmental factors are the most involved. The purpose of this article is to summarize and rank the influence of a number of factors on decreasing level of prolificacy and to show the impact of their combination.

Material and Methods

The research was carried out on Landrace and Large White sows. The sample consisted of 21,509 farrowings of sows that were born in six years and raised in breeding farms in Romania, a country with temperate climate. The prolificacy variable (total number of piglets born) was divided into three classes; the criterion for dividing was the mean and one unit of standard deviation (X±S) of each breed. The same criterion was used for all continuous variables of study.

Using MINITAB a 2-step analysis was performed, with the odds ratio as the relationship estimator between studied variables and the level of prolificacy: 1) A univariate analysis was performed to screen the relationship between potential risk factors with influence on the classes of prolificacy. 2) The multivariate analysis of the factors that passed the previous screening test was performed using multiple logistic regressions. Regression models were run for small prolificacy (P_S) vs. normal prolificacy (P_M) and high prolificacy (P_H) vs. normal prolificacy (P_M), with normal cases (P_M) as comparative basis. In the present study logistic regression models and results in odds ratio (OR) in terms of only medium prolificacy vs. low prolificacy (LP) comparison level are shown. For each impacting factor the comparison was made between middle class of variables vs. both boundary classes. At other times comparison was made between classes that were most appropriate by average of prolificacy (9.93 piglets) vs. rest of classes. Post statistical analysis factors were ranked by OR.

Results

When the univariate study had been completed, 29 variables were found associated with the prolificacy level. Multivariate analysis suggested that 21 studied variables (or classes of variable) were associated with prolificacy. Practically, the multivariate study emphasized that the sows with previous failure lactation were 13.29 (with Confidence Interval 95% between 11.34÷15.57) more times risk to LP than sows with normal lactation length. The sows with previous short service period experience were 5.58 folds (CI 95% between 4.10÷7.60) more risky to LP than sows with medium service period. The conceptions after mating between boars 3 and 2 years younger than sows were 3.89 and 2.80 times respectively, times more at risk to LP than conceptions between partners of the same age. Short and long interval between farrowings were 3.82 and 1.48 times respectively more risky to LP than medium interval. Primiparous were 1.95 times more risky to LP than second parity sows. Prolonged and short gestation vs. normal duration of gestation were 1,69, and 1,33 times respectively, more risky to LP than NP.

Comparing medium levels of environmental factors with high duration of sunshine, high temperature-humidity index, high relative humidity, and high level of air temperature we noticed a higher risk of LP. For the above factors: OR=1.24; 1.18; 1.16; and, 1.15, respectively. *(2)*

The combination of the first three risk factors, compared to the sample average, leads to the following observations:

- prolificacy of sows with farrowing coming after a previous lactation failure was 14.5% less;
- prolificacy of sows becoming pregnant after a previous lactation failure and a short service period was 22.9% less;
- prolificacy of sows with farrowing after failure lactation, short service period and high age difference (sow-boar) at mating was 54.7% less.

Discussion

The results of the study suggested essential implications of these factors on the level of prolificacy. Moreover, the factors where also involved in decreasing the number of piglets weaned per sow per year (NPWY). The combination of risk factors decreases the NPWY. The sample of sows with this low level of prolificacy can be considered as belonging to *reproduction herd-disease category. (1)*

At the end of the study we observed that intensification of Pig farming requires carefulness at each stage of the breeding process. Each step needs to be optimized

Conclusions

- Factors having an impact on prolificacy where drawn out: they can be considered as risk factors in Romania.
- Combinations of risk factors, decrease prolificacy to the point where the number of piglets weaned per sow per year is dramatically impacted.

Acknowledgements

Present research was possible owing to a CNCSIS grant from the Ministry of Education and Research from Romania. Special gratitude goes towards Prof. Horia Cernescu and Prof. Emil Sas for their kind professional guidance.

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SANITARY EFFECTS OF RAIN DEFICIENCY DURING SUMMER 2003 ON HEALTH IN SUCKLING COW HERDS IN SOUTH-BOURGOGNE (FRANCE)

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Introduction

During 2003, an early and unusual severe rain deficiency associated with high temperatures occurred in France especially in the central area of the country. This situation resulted in a severe grass deprivation for the bovine herds. As a corrective measure, the farmers fed their beef cattle with large quantities of straw in order to maintain lactation in the suckling cows at the pasture.

Material and Methods

Thanks to routine data collected by a local organisation in charge of traceability of cattle (EDE) [(1)], we carried out an epidemiological study aiming at accurately follow up the natural mortality rates over time. In fact, each animal dying on our county must be notified to EDE and the date of the death noted. Furthermore, some information is collected about the cause of death through veterinary inspections realised on request of the Sanitary Authorities (DDSV) [(2)]. In Saône-et-Loire there are nearly 6000 bovine herds regrouping about 250000 cows including 220000 suckling cows.

Results

From June to November 2003, the mortality rate increased within cattle population of 6 months to 1 year of age in comparison to 2002 (rate multiplied by about 1.5) (Figure 1). For the cattle of more than 2 years, the mortality rate clearly increased during the first quarter of 2004, reaching 0.32% in March instead of 0.20 to 0.24 % during the previous years (Figure 2).

Figure 1 : Monthly mortality rate of cattle of 6 months to 1 year of age, evolution since January 2002 to April 2004.

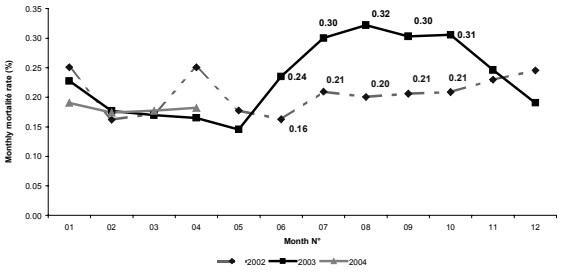
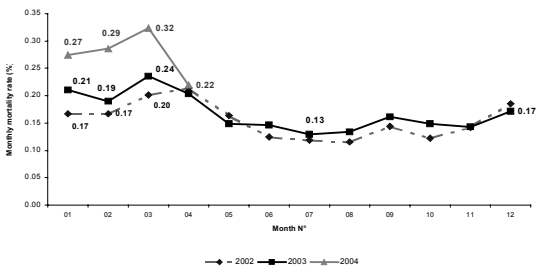


Figure 2 : Monthly mortality rate of cattle of 2 years of age and more, evolution since January 2002 to April 2004.



The relative part of the different syndromes reported by the veterinarians was not very different during the first quarter of 2004 in comparison to the same period of 2003. The relative prevalence of metabolic diseases slightly increased in 2004 (8% during the first quarter of 2004 instead of 5% at the same period of 2003).
In cattle of 2 years of age and more, the mean age at death was significantly higher during the first quarter of 2004 than that during the same period of 2003 (resp. 6.7 years and 6.1 years, p-value < 0.001).

Discussion

About sanitary effects in cattle following the drought period of 2003, some differences might be in part correlated with the age of the animals. The grass-fed calves showed earlier and greater mortality than adults, probably due to a less adaptability to the diet that included large quantities of straw.
During the first 3 months of 2004, the calving might have triggered the enhancement of the mortality in adults (stress). In addition, because of the drought, the cows have lost weight and the mineral and trace element deficiency has been more severe. The muscular depletion is often accompanied by kidney damages like nephritis. The oldest cows might have been particularly weakened and this could explain the increased average age of cows which were lost.
No special infectious aetiology can be put forward trying to explain the increased mortality of adults, suggesting that the grass deprivation has indifferently triggered many disorders, without giving greater place to a given biological disturbance. However, even if the feed tended to be better balanced with addition of molasses to straw, the ruminal flora has certainly been modified, probably causing long-term metabolic disorders.

Conclusion

The severe drought experienced during 2003 had serious sanitary effects on suckling cattle. The main clear expression was on increased mortality. We are now trying to investigate further the different pathological processes leading to death through a retrospective analytic epidemiological survey.

(1) Etablissement Départemental de l'Elevage
(2) Direction Départementale des Services Vétérinaires

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THE BACTERIOLOGIC STUDY OF HEPATIC ABSCESS IN SLAUGHTERED CATTLE IN SHAHREKORD ABATTOIR (IRAN)

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Introduction

Local suppurative infections of the liver (hepatic abscess) do not cause clinical signs of hepatic dysfunction, unless they are particularly massive or extensive metastatic. Many bacterial causes have been isolated from bovine liver abscesses Liver abscesses occur at all ages and in any species, but the abscesses of significant economic impact occur in feedlot cattle (1,2, 3, 7, 9).This study was carried out to find the occurrence and bacterial causes of hepatic absesses in sacrificed cattle in Shahrekord abattoir.

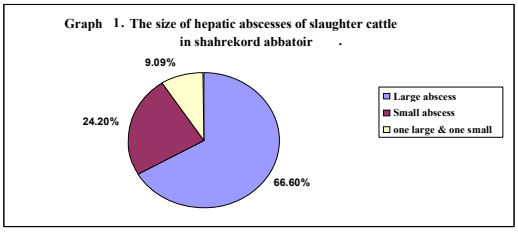
Material and Methods

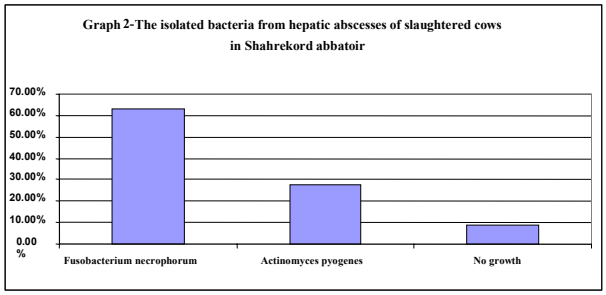
During June to September 2003 five hundred sacrificed cattle in shahrekord abattoir were inspected for hepatic abscess. In the case of hepatic abscess after recording of animals' sex, age, production and abscess characterizations (number, size, location), whole abscess with some normal liver tissues which adhered to it , was dissected from the liver and transferred to the bacteriology lab. Aerobic, anaerobic and microaerophilic bacterial cultures from hepatic abscesses were carried out with standard methods.

In this study 500 sacrificed cattle for hepatic abscess were examined and bacterial culture of 33 abscesses was done.

Results

Thirty three cattle (6.6%) from 500 inspected sacrificed cattle were involved with hepatic abscess, from which 18 abscesses were found in females (54.5%) and 15 were in male animals (45.5%). Twenty three livers from 33 infected livers had only one abscess (69.69%) and 10 livers had two abscess (30.30%). *Fusobacterium necrophorum* was isolated as unique bacterial cause of 21 abscess and *Actinomyces pyogenes* was isolated from only 9 abscesses and from 3 abscesses no bacteria grew.





Discussion

Different studies on the occurrence of bovine hepatic abscess showed different results (3, 6, 7, 8).

In the present study occurrence of bovine hepatic abscess was 6.6% which is in accordance with other studies.

It has been reported that *F. necrophorum* is the primary etiologic cause of 80 to 97% of bovine hepatic abscess (1, 2, 5,). Other bacterial agents such as *Actinomyces pyogenes, Streptococcus sp, Staphylococcus sp and Bacterioides* were also isolated from liver abscess (1, 2, 6, 7, 8).

In the present research *F. necrophorum* was isolated from 63.63% of hepatic abscesses of slaughtered cattle and *A. pyogenes* was isolated from 27.27%.

Conclusion

The results of the present study showed that *F. necrophorum* is the most important bacterial cause of hepatic abscesses in slaughtered cattle in Sharekord abattoir and A. pyogenes is the second important bacterial cause of hepatic abscesses.

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AFTER BSE – A FUTURE FOR THE EUROPEAN LIVESTOCK SECTOR

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Introduction

The present paper is the executive summary of a report commissioned by the council of the European Association for Animal Production (EAAP). A working group* was appointed to debate on the question. It included animal scientists, veterinarians, but also distinguished experts from other sectors like consumer concerns, economics and social sciences.

- -The BSE epidemic, which began in 1986, is now gradually coming to an end. Though knowledge is incomplete, enough is known about the disease to be reasonably confident that such an epidemic will not recur.
- -Three principal questions remain unresolved: the origin of the BSE epidemic; the future of vCJD; and what to do with the 16 million tonnes of animal by-products produced annually by the slaughter industry.
- -Loss of value and cost of disposal of MBM (Meat and Bone Meal) exceed 1.5 billion Euro per year. Though new EU legislation could permit over 80% of this material to be used again in livestock feeds, the best option is to continue the ban on its use.
- -The cost of the epidemic has been enormous, and is estimated here at about 10% of the annual output value of the European beef sector. The discounted present value of these costs is estimated at € 92 billion.
- -The progress of the epidemic was marked by many deficiencies and failures, of which two are particularly noted:
- The inadequacies of public information, particularly in the UK

Failure to prevent international spread through contaminated meat and bone meal.

- -Ongoing changes in the industry are documented: changing consumer requirements; concentration of processing and retailing power; declining producer prices, and reduction in numbers of full time producers. These changes represent both the causes and effects of a continuing shift in the terms of trade to the disadvantage of producers. To ensure fair trading, increased controls to prevent abuse of economic power may be necessary.
- -The ten countries which are destined to join the EU have 40% more farmers than in the EU 15. The challenge of accommodating them in a common EU policy, market and budget has major implications for the existing EU livestock sector.
- -European production costs for milk, red meats and cereals (the raw material for white meat production) are higher than in the traditional exporting countries for these commodities. This is partly due to relative scales of production units. With progressive trade liberalisation, continued pressure on producer prices is inevitable.
- -Steady increases in unit scale and intensification, particularly in pig, poultry and dairy enterprises, have generated problems of nutrient overload in some regions. The industry will need to acknowledge and address these problems.
- -In the present context it is ironic to note that the situation on animal disease in Europe has never been better. AlI major diseases are eradicated or under controI. For the future the emphasis will be on the control of enzootic diseases, largely through husbandry practices; reduction, and eventual elimination of routine use of antibiotics in feeds; and intensive research to core with emerging diseases.
- -Scientists have lost credibility as a result of the BSE crisis. While it is more critical than ever that public policy be informed by the best scientific advice, those involved in providing such advice must more carefully identify and distinguish the factual basis from the value judgements involved.
- -Scientific innovation has also lost favour with the public, particularly where it affects food and health. The livestock sector will need to weigh carefully the technical benefits against the risks and public acceptability of technologies such as GMOs, BST in milk production, growth promoters in meat production.
- -Given that over 95% of European livestock production is destined for European consumers, the production industry must concentrate on securing their loyalty by fulfilling their expectations on
  - food safety;
  - transparency and accountability;
  - quality and variety, including response to the demand for regional and organic products.
- -New ways need to be found to build the community of interest of producers, processors, and retailers in meeting these goals.

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* composition of the working group (alphabetic order) : J.J. Badiola (Sp), G. Brem (D), F. Crespo (OIE), P. Cunningham (Irl, chairman), JC. Flamant (Fr), J. Graham (UK), J. Hodges (A), K.K. Jensen (DK), S. Jutzi (FAO), F. Madec (Fr, secretary), B. Mepham (UK), A. Nagy (Hungary), A. Nardone (It), P. Sandoe (DK).

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HISTOLOGICAL STUDY ON THE UTERO-VAGINAL JUNCTION ON THE OVIDUCT OF THE LAYING HEN IN ROSS BREED

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Introduction

The histological structure of the oviduct of the domestic fowl has almost completely been investigated by many workers. They studied mainly the histological structure of The oviduct itself. Richardson (1935) observed in detail the function and histologic Structure of the gland of the oviduct.at the present the ovicuct is generally divided Into-5 portion:the Infundibulum, the Magnum,the Isthmus, the Uterus, and the Vagina.each portion has its particular structure and physiological function for egg production.the region connecting the uterus and the vagina was studied histologically. It was found that there were a special mucosal zone at the caudal end of the uterus and another at the beginning of the vagina.that was characterized by the following References Structure:the caudal end of the uterus formes a ring shaped zone, about 0.5-1 cm wide, immediately befor the vaginal orifice.it is covered with a graish-white mucous membrane and has low,some what longitudinally arranged mucosal folds. The histologically feature of this zone is the presence of many large simple or branch tubular glands in lamina properia.

Material and Methods

The oviduct collected from10 mature white Roos laying hens.after slaughtering them.and selected samples between terminal portion of the uterus and vaginal orifice that a narrow, ring-shaped zone, about 0.5-1 cm wide.to accomplish fixation ,small sampeles put in formalin %10 .then, via routin histological methods ,5-10 micron sections were prepared and stained with H&E and PAS .

Results

As thorown into regular longitudinal folds distinctly graish-white and somewhat low. Microscopically,a few tubular glands that located in lamina properia. Glandular epithelium was simple coloumnar with spherical nucleous and nucleolous,with PASdescribed above, a characteristic mucousal zone was recognized at the caudal end of the uterus. The structural features are summerized: Macroscopically, the mucous membrane is + granules .and 19-21 micron hight .

Discussion

The most important point in this study is presence and growth of special glands in utero-vaginal-junction. So this study is different from tht of Fujii (1963).kelany et al (1993) observed these glands and explain that they are coil tubular with PAS+ epithelium. But the glands were observed branch tubular in this study.

Conclusion

This study shows that the whole structure of oviduct consists of Exocrin glands that make different material for egg formation but in the utero-vaginal-junction are present especial glands that differ from other glands in respect to their shape and size. These glands were dist-tinguished as sperm host glands in this study.

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THE EFFECTS OF PRESTORAGE INCUBATION OF QUAIL BREEDER EGGS ON HATCHABILITY AND SUBSEQUENT GROWTH PERFORMANCE OF PROGENY

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Introduction

A number of methods have been investigated to improve the hatchability of eggs stored for more than seven days. Recently, it was reported that pre-heating of poultry eggs before storage resulted in more live chicks and a lower level of embryonic mortality compared to eggs that were not heated [2, 6, 7, 10]. However, no previously conducted research has tested pre-incubation storage (PRESI) as a method for improving the hatchability of quail eggs, and its interaction with breeder age and length of storage period. The specific goal of this study was to determine if PRESI would improve the hatchability of quail eggs. The second objective was to determine the interactive effect of different breeder age and length of storage period with PRESI treatment on hatchability and subsequent growth performance of quail.

Materials and Methods

Freshly laid quail eggs were collected from two flocks aged 20 and 37 wk-old. At the time of collection, 900 eggs selected at random from each flock were weighed and randomly distributed into two groups of 450 eggs recpectively exposed to an egg storage lasting 5 or 15 days. Eggs in each egg storage treatment groups were randomly allocated to two groups of 225 eggs exposed to the PRESI treatment for 8 hours or not (control, 0 h). Eggs for the 15-d storage group were collected 10 d prior to the eggs collected for the 5 d storage group, so that all the eggs from all groups could be set in the incubator at the same time. A total of 8 interactive groups constituted of this study (2 level of breeder age x 2 level of PRESI x 2 level of storage period). Forty-five eggs constituted a replicate in each treatment group.

Management

Eggs at 8 h PRESI were incubated at a standard dry-bulb incubation temperature of 37.5 ^{o}C. After the PRESI treatments were completed, all eggs including control and 8 h PRESI were stored at an average temperature of 15 ºC and relative humidity of 65% for 5 or 15 d and were turned twice a day. All eggs were weighed after storage, and eggs from each group were incubated in a commercial setter and hatcher for 17 d. The setter was operated at 37.5±0.5 ºC dry bulb temperature and 29.0±0.5 ºC wet bulb temperature. The hatcher was operated at 37.0 ±0.5 ºC dry bulb temperature and 31.0±0.5 ºC wet bulb temperature. Eggs in setter were turned 15 times per day. Trays representing all treatment groups were distributed in all positions in the setter and hatcher (45x8=360 eggs per tray and 5 trays in the incubator). Newly hatched chicks in all groups were reared under the same growing conditions in brooding cages (colony type) in an open-sided house with mechanical ventilation. Chicks belonging to the same group were randomized into five replicates at hatch. All chicks were brooded and reared at 28 ^{o}C for the 1st weeks, 27 ^{o}C for the 2nd week, 24 ^{o}C for the 3rd week, and 18-21 ^{o}C from the 28th day until 42 days of age. Standard production practices and standard quail feed produced in the centre were used during the treatment. All birds had *ad libitum* access to feed and water. Twenty-four h lighting were used throughout the growth period. Data for the growth performance were collected from the hatch to 42 d of age.

Data and Statistical Analysis

Three days after removing the chicks from the hatcher; all unhatched eggs were broken open to determine the fertility. If the eggs were fertile, period of embryonic development was determined according to Hermes [9]. Hatchability of fertile or total eggs was calculated as the number of chicks hatched per fertile or total eggs [1, 19]. The fertility results were reported as apparent fertility. Individual body weights of quail were measured at hatch and 42 d of age, and cumulative feed conversation (grams of feed intake per grams of body weight gain) was calculated for 42 d of age. Mortality was recorded on a per group basis as it occurred.

The hatchability and growth period data were analyzed by three way ANOVA with two levels of PRESI (0 and 8 h), two levels of breeder age (20 and 37 wk of age) and two levels of egg storage (5 and 15 d). The main treatments as well as the interactions were analyzed for significance at the 5% level. All data in percentages were transformed using arc sine square root transformations prior to analysis [16]. The statistical analysis for body weight apparent fertility, hatchability of total and fertile egg, feed conversion and mortality were calculated on the basis of the replicates. All tests were performed using SPSS® computer software 10.00 [17]. The PRESI treatment, breeder age, and length of storage were the main effects.

Results

The main effect of PRESI, breeder age, and egg storage on egg weight loss, apparent fertility, hatchability of total and fertile eggs, and embryonic mortality are presented in Table I. There was a significant difference for the egg weight losses during storage due to main effects of PRESI and egg storage treatments. There were significant PRESI x breeder age interactions for the hatch of total and fertile eggs. Breeder age x egg storage interaction was found significant for the hatch of total eggs. Hatch of total and fertile eggs were found significant due to main effect of PRESI treatment. The mortality rates of embryo belongs to two PRESI and two breeder age treatment groups were found to be not statistically different while the differences due to storage period were significant from 14 to 17 d of incubation.

The subsequent growth performances of progeny in main groups are presented in Table II. The body weight at hatch in the main groups was found similar. It was

found that no significant differences for the body weight at 42 d of age of quail hatched from young and old breeders or 8 h PRESI and control treatments while the differences due to storage period was found to be significantly different. There was significant PRESI x breeder age and PRESI x egg storage interaction for FCR of progeny, respectively. The same interactions were observed for mortality of progeny. The cumulative feed conversion ratio (FCR) of quail hatched from both breeder age and storage treatment groups were found to be significantly different. Main treatment of breeder age on mortality of progeny was found to be significantly different.

Discussion

In this study, egg weight losses in 8 h PRESI and 15 d storage were .49 and .24 g greater than control and 5 d storage, respectively. This result was expected, as exposure to PRESI and long time storage would increase the opportunity for water vapour to escape from the egg. Although long storage time did not affect true fertility, the present study and the work of Petek et al. [14] have demonstrated that the long period egg storage prior to incubation decreased numerically apparent fertility. The fact that the collection of the eggs for the 5 and 15 d storage groups was separated by 10 d might have accounted for the differences in fertility. In this study, 8 h PRESI of the eggs prior to storage significantly affected hatchability of total and fertile eggs. In the same time, 5 d storage was numerically improved hatch of total and fertile eggs. This result was expected on in accordance with the previous reports in quail and other species related to egg storage and PRESI [2, 5, 6, 10, 14, 21]. Some embryos of eggs stored for long periods could not start developing immediately after normal incubation temperatures are provided. Another possibility is that the development of embryos from long period stored eggs proceeds at a slower rate thorough the first period of incubation. As reported previous studies about broiler breeder [4, 18] the age of quail breeder significantly affected the hatchability of total eggs. The eggs obtained from young breeder produced more chicks. Embryonic mortality rates in each stage of incubation examined in this study were not significantly affected by the main and their interactive effects, except for the main effect of storage period on mortality from 14 to 17 d of incubation (Table I). However, embryonic mortality of eggs 8 h PRESI treatment in each period was numerically reduced compared to non heated group. Most probably, embryos in PRESI are being pushed the optimal stage of development to safely store eggs (4, 8). A comparison of the main effects between two PRESI treatments, two storage period and two breeder age showed that 8 h PRESI treatment had significant beneficial effect on hatchability and embryonic mortality; however, embryos of eggs stored 15 d resulted in noticeably lower hatchability and mortality from 14 to 17 d of incubation and the eggs obtained from young breeder significantly produced more chicks. The significant PRESI x breeder age interaction for hatchability of total and fertile eggs revealed that superior effect of the 8 h PRESI was the highest when combined with the young breeder's age. There was significant breeder age x egg storage interaction for the hatchability of total eggs. This led to the conclusion that the depressive effect of 15 d storage on hatchability was highest in old breeders. In this study, neither the hours of PRESI nor the breeder age between the main groups significantly influenced the subsequent body weight of progeny. The result for the body weight of progeny was not concurrent with the previous findings [15, 20, 22]. Egg storage for 15 d significantly depressed the body weight of quail due to probably, increased second grade of chicken in prolonged storage time. The feed conversion ratio of progeny hatched from 15 d stored eggs and old breeder group were significantly greater than progeny hatched from 5 d stored eggs and young breeder, respectively. Quail in groups of 5 d and young breeder consumed less feed for body weight gain. Findings about the body weight and FCR related to the storage period were not corroborate with previous observations in which body weight and FCR are not affected due to length of egg storage [14]. The mortality rate of progeny was not affected significantly due to main effects in the present study, except for main effect of breeder age. The survival rate of quail hatched from old breeders was found to be lower. Superior effect of PRESI for survival was greater in progeny obtained from young breeder and stored 5 days.

Conclusion

The results of the present study show that 8 h PRESI have a positive effect on the hatchability of eggs and subsequent growth performance of progeny. Further researches are necessary to determine the optimum length of PRESI time and storage durations for obtaining maximum hachability. Meanwhile, it should be kept in mind that the economic cost of PRESI must be evaluated in comparison with its beneficial effects.

References available on request.

Table I. The effects of PRESI, egg storage and breeder age on egg weight loss, embryonic mortality, fertility, hatchability of total and fertile eggs.

Main Treatment Effects	Fresh egg weight (g)	Egg weight loss during storage (%)	Apparent fertility %	Hatchability of Total eggs %	fertile eggs %	Embryonic mortality during incubation (%) 1-7 d	7-14 d	14-17[3] d	Total
PRESI (h)									
0	12.33	0.32	90.8	79.7	85.3	3.83	2.96	4.32	10.00
8	12.35	0.81	91.8	82.6	90.4	2.59	1.48	3.70	7.00
Breeder age									
Young	12.48	0.64	93.7	84.7	88.3	3.83	2.72	3.83	9.33
Old	12.23	0.74	88.9	77.6	87.4	2.59	1.73	4.20	7.67
Egg Storage (d)									
5	12.36	0.49	93.6	82.1	88.1	3.33	1.44	5.22	10.00
15	12.33	0.73	89.0	80.2	87.6	3.06	3.19	2.50	7.00
ANOVA									
PRESI	n.s	0.017	n.s	0.001	0.001	n.s	n.s	n.s	n.s
Breeder age	n.s	n.s	n.s	0.001	n.s	n.s	n.s	n.s	n.s
Egg Storage	n.s	0.001	n.s	n.s	n.s	n.s	n.s	0.01	0.01
PRESIxBreeder age	n.s	n.s	n.s	0.005	0.019	n.s	n.s	n.s	n.s
PRESIxEgg Storage	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Breeder agexEgg Storage	n.s	n.s	n.s	0.001	n.s	n.s	n.s	n.s	n.s
PRESIxBreeder agexEgg Storage	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
SEM	0.40	0.02	0.25	0.25	0.25	0.03	0.03	0.02	0.31

a-b within columns , values with different superscript differ significantly at * P<0.05

Table II. Main effects of PRESI, breeder age, and length of storage period on growth performance.

Main Treatment Effects	n[1]	Body Weight (g) at hatch	Body Weight (g) 42 d of age	FCR[2]	Mortality (%)
PRESI (h)					
0	717	8.6	185.3	4.05	8.04
8	743	8.4	180.5	3.80	7.00
Breeder Age					
Young	762	8.7	182.0	3.56	6.74
Old	698	8.3	183.9	4.28	8.29
Egg Storage (d)					
5	739	8.5	191.3	3.65	7.46
15	721	8.5	174.4	4.21	7.58
ANOVA					
PRESI		n.s	n.s	n.s	n.s
Breeder age		n.s	n.s	0.001	0.001
Egg Storage		n.s	0.002	0.001	n.s
PRESIxBreeder age		n.s	n.s	0.001	0.001
PRESIxEgg Storage		n.s	n.s	0.022	0.001
Breeder agexEgg Storage		n.s	n.s	n.s	n.s
PRESIxBreeder agexEgg Storage		n.s	n.s	n.s	n.s
SEM		0.023	2.492	0.025	0.025

[1]: Number of chicks at hatch, [2]: Feed conversion ratio

a-b within columns , values with different superscript differ significantly at * P<0.05

Quality in food chains - Pork

Oral Communications

CONSUMER PERCEPTIONS OF OUTDOOR PIG PRODUCTION, PORK QUALITY AND THEIR WIILINGNESS TO PAY FOR NEW QUALITY ATTRIBUTES

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Introduction

In pork production, attempts have been made over the past decade to find ways to redress consumer concerns over animal welfare and to reduce environmental pollution within an overall framework embracing the economic and social aspects of agricultural production. However the organic sector still occupies only a fraction of the market. Questions have been raised about the long-term financial benefits of 'organic farming' and whether or not organic products will ever occupy more than "niche" markets. What will decide the success and size of the organic pork market will be the image of consumers, the quality of the products and, above all, that consumer perceptions will generate a willingness to pay for the extra production costs.

Material and Methods

Consumer opinions: four focus groups were conducted in four countries with men and women from the town and the country. They discussed pig production and pork quality in general terms [3].

Consumer expectation: These were conducted in the form of a questionnaire asking about quality expectations, attitudes and buying intentions, both with regard to pork produced in conventional indoor systems and extensive outdoor systems.

Consumer choice: photographs of 16 pork chops [4, 5] were systematically modified at two levels of each of colour and fat cover, and were also printed with labels of system of production (indoor or outdoor) which were permutated with labels on origin (home produced or imported). The percentages of consumer choices were categorised as consistent (>5 out of 8 replicates) or inconsistent (<6) choices.

Willingness to pay: After choosing their preferred photograph, each consumer gave a figure for the price relative to a given price. The given prices were 5.01, 5.64, 13.42, 4.86 €/kg for the British, French, Danish and Swedish tests respectively.

Outdoor pork production: littermates, all free of the n and RN$^-$ alleles, were allocated to either a conventional indoor system with a totally slatted floor, at 0.65 m²/pig and controlled ambient temperature at 22°C or to an outdoor system with sawdust-shave bedding (1.3 m²/pig) with free access to an outdoor area. Pigs were slaughtered at the average live weight of 110 kg.

Meat quality characteristics: after thawing at ambient temperature, the chops were grilled with a double contact grill at 280°C for 6 minutes. A 10-member trained taste panel assessed each chop for odour, tenderness, juiciness and flavour and consumers rated the overall appreciation.

Results

Consumer opinions: Consumer focus groups in France, England, Sweden and Denmark were conducted to obtain insights into the decision-making involved in the choice of fresh pork and attitudes towards today's pig production systems. Many positive perceptions of pork meat were evoked. Negative images of the production systems in use today were expressed, but rationalised in terms of consumer demands, market competition and by comparisons to previous systems of production. Knowledge of production systems appeared of little consequence in terms of any pork market potential as several groups freely remarked that there was no link between the negative images of production methods and their purchase behaviour. The consumer groups were confused and mistrusted the information on pork quality and origin that was available at the point of purchase [3].

Statistical analysis of the interviews [1], concerning pig production and pork quality, gave 3 main contexts that contained 80% of the words from original discussion. These related to the broad contexts `political', `quality' and `animal production'. Rural men group contributed significantly more to the political context and rural women more to the quality context.

Table 1. Choice based on appearance and labels
Values are the percentages of consumers. Data taken from reference 2

		DK	FR	SW	UK
Colour	Pale	26	17	19	20
	Dark	29	49	35	33
	Inconsistent	45	34	46	47
Fat cover	Lean	33	50	49	37
	Fat	13	5	6	17
	Inconsistent	54	45	45	46
System	Outdoor	45	59	44	36
	Indoor	8	8	10	7
	Inconsistent	47	34	46	57
Origin	Home produced	63	77	71	47
	Imported	4	1	3	3
	Inconsistent	33	22	26	50

Consumer expectations A total of 2451 consumers from Denmark, Sweden, France and the UK completed a survey questionnaire. In all four countries, pork from outdoor production systems was expected to be better in terms of leanness, freshness, healthiness, tenderness, nutritional quality, juiciness. It was also expected to be produced locally without hormones and drugs and be better for animal welfare. However, these quality expectations were poor predictors of attitudes and intentions and varied substantially between countries.

Consumer choice: Looking at the inconsistent choices (Table 1), it is clear that for the 'origin' and 'system', they were more consistently chosen than 'colour' and 'fat cover' except for the UK consumers, who were the least concerned by the information given. The type of colour seemed not so important overall with only a small percentage more choosing the dark colour over the pale red colour. For fatness, all 4 countries and particularly France and Sweden, preferred the leaner option.

Labelling With photographs of fresh pork labelled with origin and system of production, in all 4 countries there was a strong preference for those chops marked 'home

produced' (labelled with the name of the country) and 'outdoor' (Table 1). French and Swedish consumers were particularly attentive to the origin labels with over three-quarters of them making a consistent choice.

Table 2 Information and willingness to pay
Values are in €/kg and percentages. Data from reference 2

	without information	with information	increase (%)
British	5.05	5.25	4.04
French	5.83	6.11	4.78
Danish	11.29	10.96	-2.94
Swedish	5.00	5.29	5.82
means	6.79	6.90	2.93

Willingness to pay: Willingness to pay was tested with and without information on photographs of pork chops. Overall, most consumers were willing to pay more for the information concerning origin and system of production, although Danish consumers were not willing to pay more. On average consumers suggested that they would pay 3% more for such information on the fresh chops (Table 2).
Meat quality: The objective quality of grilled pork from indoor and outdoor (an available outside yard area) was tested (Table 3). There were no significant differences in tenderness, juiciness and flavour judged by trained panellists nor in overall appreciation by consumers and were of medium to good quality.

Discussion
European consumers generally have positive attitudes to pork production although this may not lead to higher consumption. Pork is generally seen as being suitable for different dishes although not as a meat for special occasions and may be perceived as being relatively fatty and unhealthy compared to beef and poultry.
The meat chosen for this study was the pork loin chop as it was essential to select a cut which was recognised in the 4 countries and which exhibits variation in the chosen 4 appearance characteristics. The characteristics chosen were those 4 most frequently cited by consumers at the point of purchase. A new finding was that most often 2 characteristics are used in selection. Marbling and drip were of equally low importance in with equal numbers of consumers choosing the marbled and non-marbled but pork with visible drip was rarely chosen. Colour was important to consumers however overall, preferences for both the darker and the lighter coloured lean were found in the 4 countries. Larger differences were found among consumers from 26 countries [5].
Relationships between choices and socio-economic consumer profiles were weak and therefore it is difficult to targeting any particular segment of consumers for the appearance characteristics. However, the choice of leaner pork by young consumers needs to be taken into account for future successful marketing of pork.
In terms of production of pork, the fatness and colour will depend on the genotype, feed, age and on the post-slaughter conditions. In previous trails on organic pig production, few differences were found in appearance. These results, together with those showing little differences in objective eating qualities, suggest that the perceived quality of pork is a matter of expectations. Consequently, consumers choose in line with their expectations of a better quality.
With little or no advantage in appearance and eating quality, attitudes, expectations and price of organic pork will be crucial in determining the size of its market share. Information, with the product or in forming attitudes, will play an important role in this. Any information will have to be clear and meaningful to consumers as a lack of information or misinformation may seriously weaken its image. In the meat industry, labels or trade marks differ between countries. However, they are usually symbolic and give little guidance as to the essential elements expected by consumers. One piece of information which most Europeans stress is the 'own country' label as origin [3] which is easily understood and appears to reassure people about safety and quality.

Table 3 Eating quality of pork from outdoor and indoor production systems
Values are means (scale 0 to 10) for French panels

		Outdoor	Indoor
Trained panel	Tenderness	5.6	5.5
	Juiciness	3.8	3.6
	Flavour	5.8	5.8
Consumer	Appreciation	6.4	6.4

Consumers were strongly influenced by the information concerning the origin (preferring pork labelled with their own country) and system (preferring the outdoor production) when judging pork quality.
Consumers appeared to be prepared to pay, on average, only about 3% extra, even when all characteristics of appearance and labelling were available. After tasting, consumers were prepared to pay between 4 and 10% extra for the labelled pork. So, with a system of organic production costing 30 to 35% extra, this willingness to pay would equate to about a 15% substitution of the conventional pork market. However, pork labelled 'home country' is likely to be equally as attractive as pork labelled 'organic production' and it is unlikely that all the meat from the carcass could be commercialised as such.

Acknowledgements
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IMPROVING QUALITY AND SAFETY IN PORK CHAINS - ADDRESSING THE CHALLENGE OF CHAIN WIDE INFORMATION MANAGEMENT

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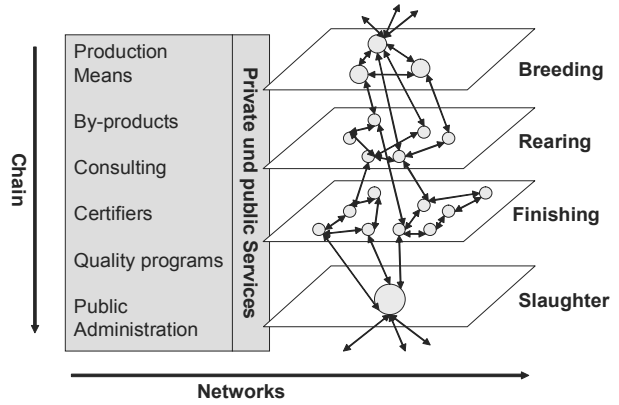
Introduction

To maintain and improve consumer trust in the quality and safety of food, legislative actors have induced new framework conditions on food production: The EU General Food Law (EU 178/2002) demands traceability, transparency and a "stable to table"-approach throughout agricultural production while governmental food safety inspection is currently under reorganisation towards a „control of control"-principle. EU has consolidated its Food hygiene legislation into a consistent set of four new regulations, often referred to as the Hygiene package (EU 852, 853, 854 and 882/2004). They include specific rules for the documentation of products and processes and information sharing between chain actors. This new legal environment provides a unique chance to boost innovation and to improve chain wide cooperation in pork production.

It has been widely agreed that information and communication systems linking different parts of food chains can aid to meet individual information needs of chain actors. The potential for pork chains mainly lies in the fields of animal breeding, animal health management, improved cooperation between abattoir and meat processing and risk based meat inspection. Effectively implemented and supported by innovative information technology it can assist to improve productivity, raise consumer confidence and finally result in higher profits (1, 4). Despite this broad consensus successful applications are missing in practice. Main reasons are a high demand for coordination and negotiation including the question of cost sharing as well as a lack of organisational support and technical tools.

Material and Methods

Figure 1: Generic model of a pork netchain (from production to slaughter)



The theoretical framework used in this paper combines the models of netchains and of processes from EN ISO 9000:2000. A netchain (Figure 1) has been defined as a contraction of chain and network to extend the concept of supply chain towards the reality of vertical and horizontal dependencies and interactions in chains (3). The process model describes product and optimal information flows between clients and suppliers for their individual decision making. Extended to the entire netchain, a multitude of such relationships exist. Their information exchange should be jointly coordinated. Then information can be exchanged along the chain and processed according to needs of their actors (6). Traceability is a means to couple yet unlinked information.

In this Dutch German R&D project of GIQS (German abbreviation for trans border quality assurance) the concepts of chain quality management are turned into the real world of pork production. As a sub-project a food chain information system is developed to support animal health management and a risk based meat inspection. Aim of this research is to find the critical success factors and to reduce motivational limits of its practical implementation. Three pilot chains in Germany and the Netherlands implement and validate the system.

This sub-project concentrates on two key aspects:

1. Organisational aspect to set up organisational structures as "Trusted third parties" that moderate communication and cooperation between chain actors.
2. Technical aspect, to develop chain wide IT systems that integrate existing solutions, standard systems and available data sources that reduce implementation time and cost. Here special focus lies on:
   a. Identification of the products,
   b. Capturing of relevant data - preferably from existing sources,
   c. Data processing - Tools and methods that maximise the use of food chain information for the various chain links.

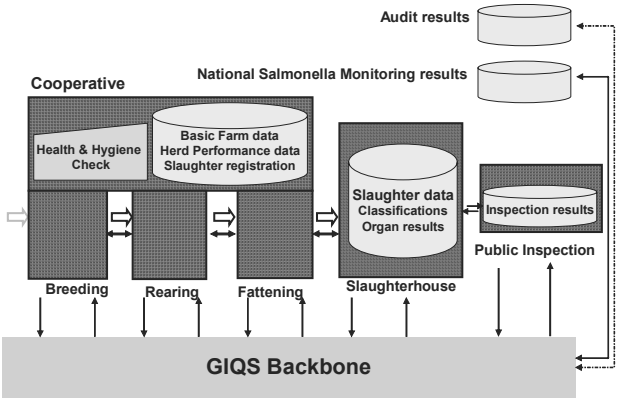
Results

The central food chain information system is built on standard systems and links existing data sources to enable cost effective and practical solutions. Therefore the concept of inter enterprise data warehousing has been adopted. Following DEVLIN's (2) definition, relevant quality information is selected and obtained from a variety of data sources along the whole production chain and structured according to the information needs of the different users. Through specific access rights and internet based interfaces information is made available to various end users, who can use it in their individual context (2). Similar to a backbone in the body, the GIQS Backbone performs a centralised data management for all chain actors.

The architecture of the GIQS Backbone supports a variety of analyses, including elaborate queries on large amounts of data. Information on chain oriented health management can be specifically processed:

- as static reports
- as interactive reports
- as ad hoc analysis through OLAP (Online Analytical Processing), an decision support application that allows the user to quickly analyse information that has been summarized into multidimensional views.

Figure 2: GIQS Backbone – integration of eight data sources in one pilot chain



To efficiently and consistently make available the information on products and processes over a longer period of time, information sets from different data sources are integrated. As an example, chain data from eight different sources are integrated in one of the pilot chains (Figure 2).

The coupling of information to the different herd groups allows a fast tracking and tracing throughout the chain. Depending on their access rights and specific information needs chain actors are provided with individually processed information:

- **Farmers:** Regular reports with analyses and benchmarks on animal groups and over time:
  - **General overview -** a quick one page overview on core figures on health, classification and performance results of recent groups
  - **Sorting –** report on economic losses from suboptimal feeding and sorting (male/female, slaughter date)
  - **Organ results and health check –** overview and comparison of health status during fattening and organ results at slaughter; comparison of animal groups and over time.
  - **Hitlist organ results** – an anonymous benchmark with similar farms of the cooperative.
- **Veterinarians, extension service, consultants:** use their clients' reports and benchmarks for advisory service; OLAP tool for a profound analysis of specific problem complexes on farm level and with farmers suppliers.
- **Chain Coordinator:** OLAP tool for chain management, supplier analyses and benchmarking;
- **Slaughterhouse:** OLAP tool for logistics and process optimisation, supplier benchmarking;
- **Public meat inspection services:** traffic light system decision model for the new risk based meat inspection (5).
- **Meat processing:** Reports on meat quality, muscle characteristics in combination with yield of the different processing steps.

Discussion

Information exchange in netchains is more and more established, but often paper based and point-to-point. A variety of databases contains valuable information. To set up a cost effective quality information systems that meet the above described demands, existing information sources should be linked or integrated into a comprehensive system. Data warehousing technology is a means to integrate this information and support effective information exchange, continuous improvement and provide an added value for all stakeholders of a netchain.

Powerful analytical tools are of high importance to make use of available information on the various aspects of pork production to chain actors. A key driver is the aim to reduce uncertainty through prior knowledge on emerging decision alternatives at control points and provide a means to improve preventive health management on the farm and quality management in the netchain. For the first time actors responsible for the health management can jointly use one common information and communication system to improve their services.

Conclusion

Additional information does not necessarily lead to knowledge gain. Specific tools for pork chain actors are necessary. They are developed within this project and enable key actors of animal health management and food safety to extend their information base for more precise decisions on disease and zoonosis management, performance improvement and a risk based meat inspection. Benchmarking analyses between animal groups, suppliers and production methods become possible. Further data should be integrated in future. Especially information on the administration on pharmaceuticals is expected to reveal major optimisation potential.

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A DECISION MODEL FOR THE RISK-BASED MEAT INSPECTION

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Introduction

The European Union is reorganizing the European food safety system. Risk-based decisions and procedures play a major part in the new European food hygiene legislation. In the year 2006, the "Regulation of the European parliament and of the council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption" will be implemented. One of the key elements of this regulation is the transition from the "traditional" to a "risk-based" meat inspection. For the official veterinarian, this will offer the possibility to permit a mere visual meat inspection for batches of slaughter pigs, which were raised under controlled conditions and for which the information flow between farm and abattoir is regarded as sufficient for risk-based decisions. In case of epidemiological data indicating a risk, the official veterinarian can order a "traditional" meat inspection with incision and palpation for the pigs in question. To support this decision, one of the tasks of the official veterinarian will be the inspection and evaluation of food chain information. It is the task of the slaughterhouse to make the information available to the official veterinarian at least 24 h prior to the pigs' delivery to the abattoir.

Material and Methods

The project "Border-crossing quality assurance systems in pork chains" (GIQS) is a German- Dutch research and development project which develops solutions for a better information flow and better traceability in pork production chains and for the most efficient use of the information.

The GIQS solution for information management is an internet-based, inter-enterprise data-warehouse (called "GIQS Backbone") which provides a virtual integration in pork production chains on the basis of relevant food chain information (3). This information can be used by all members of the pork production chain in accordance with an access authorization.

Farmers as well as their veterinarians and consultants can get their own reports for the enhancement of farm management, abattoirs are able to collect information for every announced batch of slaughter pigs and the official veterinarian can use the "GIQS Backbone" for making risk-based decisions.

The Basis for the decision model are two EU regulations. In one of them relevant food chain information is defined (Regulation (EC) 853/2004) and the second one deals with specific rules for official inspections (Regulation (EC) 854/2004). The requirements are brought together and a "tailor-made" system for the German, heterogeneous pig production is worked out.

The decision model is tested in practice in a pilot chain near the Dutch German border. This pilot chain consists of a slaughterhouse, the regional official veterinarians and a farmers' cooperative. Real data are used to validate and optimise the decision model and to work out thresholds for each parameter of information.

Results

The report for the official veterinarian is put into a decision model which may be used when slaughter pigs are announced for slaughter and the food chain information is to be judged. The decision model is to support the official veterinarian in making risk-based decisions, it consists of a system for evaluating and using the very complex food chain information.

The decision model works like a cascade with three steps to evaluate relevant information about a batch of slaughter pigs (2):

1. On the first level it is judged, if the flow of information between the holding of provenance and the slaughterhouse is sufficient.
2. On the second level, information about the holding of provenance must be appraised.
3. On the third level, Information about the specific lot announced must be appraised.

To make this cascade work, data from farms, slaughterhouses and official veterinarians are brought together. At present, in Germany, information about eight relevant parameters is available:

1. Participation in a neutral certificated quality assurance system.
2. Housing factors and the farm's hygiene management.
3. The farm's Salmonella status.
4. Slaughter check data of previous slaughtered batches of pigs. To quantify this descriptive information, a slaughter check index has been developed (see figure 1).
5. Official findings and condemnations of previous batches of slaughter pigs.
6. The farmer's reliability of his delivery management.
7. The health status of the finishing pigs and medications.
8. The mortality in a group of finishing pigs.

Pneumonia Frequency within the last two years	Points	**Pleuritis** Frequency within the last two years	Points	**Liver** Frequency within the last two years	Pts	**Pericarditis** Frequency within the last two years	Points
<1%	0	<1%	0	<1%	0	<1%	0
1-10%	1	1-10%	1	1-10%	1	>=1%	1
11-30%	2	11-30%	2	11-30%	2		
>30%	3	>30%	3	>30 %	3		

All the Points are summed up, a farm can reach between zero and ten points in the slaughter check index.

0 Points: desirable low frequency of occurrence of lesions

1-3 Points: low frequency of occurrence of lesions

4-6 Points: medium frequency of occurrence of lesions, the herd health should be checked by a consultant or a veterinarian.

7-10 Points: high frequency of occurrence of lesions, indicates strong problems with the herd health on the farm

Figure 1: Slaughter check index (modified after (1))

In combination with the data-warehouse "GIQS Backbone", there is a good possibility to visualize the decision parameters for the every-day-use by the official veterinarian.
A "traffic light system" was developed to demonstrate the thresholds of every parameter. Before admitting a batch of pigs for slaughter in the framework of risk-based meat inspection, the official veterinarian will look up the visualized relevant information in the "GIQS Backbone". He or she will see eight different traffic lights, one for each parameter (see figure 2).
When the traffic light is green, it means, that there are no obvious problems with this parameter in that batch of slaughter pigs.
A yellow traffic light indicates a warning.
A red traffic light is shown, when the threshold for a parameter has been passed.
Detailed information behind the traffic light is available for the official veterinarian at a mouse-click.

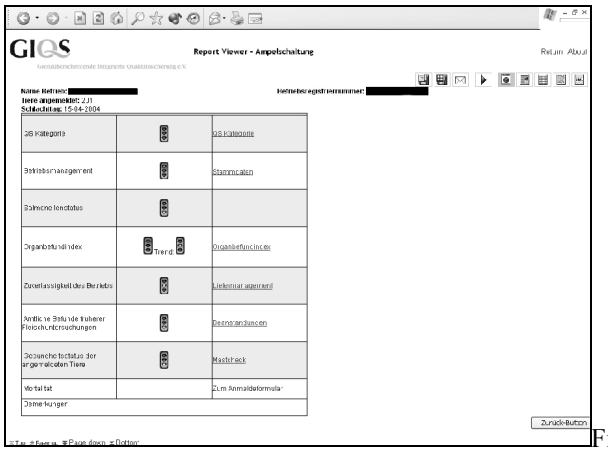

Figure 2 : traffic light system

Discussion
The decision model is to support the official veterinarian in making meaningful decisions, but still his or her expert knowledge is the most important instrument for the risk-based meat inspection. He or she makes the official and final decision, which meat inspection procedure fits best for an announced batch of slaughter pigs. The decision model makes use of modern information technologies and enables the official veterinarian to evaluate lots of information in a short period of time.

Conclusions
It is widely thought that risk-based meat inspection is only possible in strictly integrated systems. However, our findings indicate, that a well coordinated information flow is more important than the integration degree of a food production chain.

Acknowledgement
The GIQS Project is cofinanced by the EU Interreg III A Program of the Euregio Rhine Waal, as well as by the Dutch Ministry for Agriculture the Province of Gelderland, and by the Ministries for Economics of North Rhine Westfalia and Lower Saxony.

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IMPROVING HERD HEALTH THROUGH USEFUL INFORMATION

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Introduction

To improve herd health in a structured way the principal of a Deming circle can be used. In keywords a Deming circle can be described as What? How? Who? When? Where? evaluate, react, and adjust the process.
This system of continuous improvement is used in a joined Dutch – German research and development project of GIQS in which the Dutch Animal Health Service (AHS) and the University of Bonn are project partners. For this reason the Dutch-German initiative GIQS (trans-border quality assurance) develops a tool box consisting of six modules to support chain oriented quality- and health management in the pig production chain (1). One out of six tools is the **Module Herd Health Management.** The aim of this part of the entire project is to make a computer based, web enabled support system for swine farmers, their consultants and veterinarians and the pork production chain manager to gather information on herd health and give structured advice and background information to improve herd health. The work being done for one of three pilot-chains will be used as an example.

Materials and Methods

The principle of the Deming cycle will be assigned to the development of an information system supporting auditing and herd health management.
To develop the **Module Herd Health Management**, teams of experts were built:

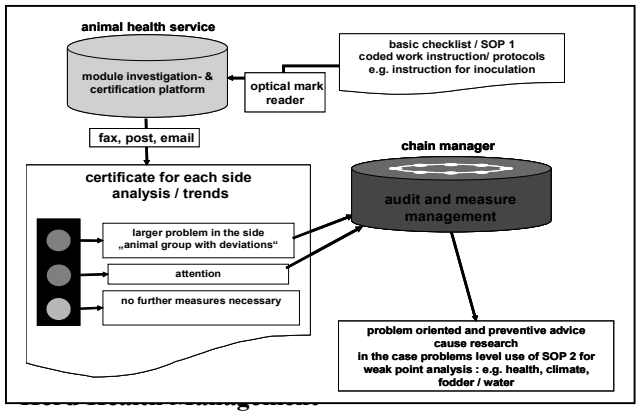
- pig health experts (veterinarians, advisors, agricultural scientists)
- system designer (software provider and developer)

These teams of experts defined, implemeted and validated the procedure of auditing farms in an integrated pilot chain, in which the value-added process is controlled by one hand. The technical tools supporting the procedure are:

- an audit management system and
- an investigation and certification platform

Results and Discussion

In the module animal health management an integrated audit management system is combined with an investigation and certification platform.
In the first step checklists for internal audits, herd management, visits of veterinarians and farm advisors supporting herd health management were developed. The second step deals with the construction of a "traffic light system" which is automatically able to assess the checklist data. In case of problems additional checks and measures can be induced (figure 1).



The developed checklists are divided into two levels:

- level 1 standard operating procedure (SOP 1) to gather basic data on a regular basis.
- level 2 standard operating procedure (SOP 2) to support analysis of identified herd problems.

SOP 1 is the basic checklist for regular farm checks for weak point analysis in the production chain which is applied twice a year. It collects data on general descriptive data of the herd and the inoculation program: vaccines and vaccination scheme, endo- en ectoparasite treatments and herd health certificates e.g. pseudorabies free status. Each item is evaluated as positive or negative or as "good", "reasonable" or "bad". Sow herd production data are entered and compared to the results of the last six months and to the chain average. The health situation in the farrowing house, boar house, gestation compartments, gilt introduction, piglet rearing and growing / finishing stages are evaluated on specific items where a percentage can be given as a measure for the size of the herd problem and can be evaluated by the person that fills out the checklist as "good", "reasonable" or "bad". All possible choices for the items like the vaccines, vaccination schemes, materials submitted for diagnostic purposes and the diagnostic tests applied are combined with codes in a reference lists that only a number has to be filled in..
The lay-out of the checklist and the codes which are filled in is adjusted so that it can be read by an optical mark reading system. The ASCII data file from the reader is than transferred to an Oracle database for storage and where the data are available for analysis. The image of the checklist is also stored as a PDF-file to retain the written text in the advice and therapy section of the form and the signatures of the farmer and the veterinarian for future reverence. Afterwards defined standard reports based on predetermined criteria are sent to the herd owner

and the chain manager, respectively. Depending on the number or seriousness of the identified problems herds can be categorised as "no intervention necessary", "attention needed" or "major problems in the herd". Based on the identified weaknesses, level 2 SOP's are used to analyse these specific problems. They contain specific relevant questions with regard to management, housing, climate, feed, feeding, and animal health etc. to help in the careful analysis of a herd problem. Subject for SOP2's are for example 'small litter size', 'return breeders', 'abortion/early farrowing', 'cannibalism', 'introduction of new gilts', 'diarrhoea', '(chronic) pneumonia', 'lameness', 'dead loss of suckling piglets', and specifically on Salmonella, but also general checklists on climate, feed and feeding, management and hygiene. To further help in the analysis, technical criteria are supplied on subjects of feed requirements, water, mycotoxins, climate, housing, and semen. Finally, summaries are made available on the major swine diseases and health problems as background information.
All this information, with the exception of the basic checklist on paper, is stored and transferred electronically. For this purpose a website is made where farmers, veterinarians, consultants and the chain manager have access by 'single sign on' identification. Once logged on to the website information becomes available on an "allowed to see" basis. Farmers have access to their stored checklists and the general information and announcements from the chain manager. Veterinarians and consultants have access to data from their herds and the chain manager has access to all data. An on-line analysis tool can be used by the chain manager to further analyse the available data beyond the standard reports.

This tool for integrated production chains allows a structured and chain wide storage of information on production status and health management and enables further analysis. It allows for a flexible system where changes can be made rapidly if necessary but it also prevents the build up of stacks of paper files that are very difficult to manage. Data analysis allows for better management information which allows for better funded decisions and support where necessary. The results are better herd health and production results leading to higher financial return on investment.
In the next phase of the project the herd health management and advice module will be integrated with other information sources in the GIQS Backbone (2) like information from identification and registration systems for pigs, abattoir information on production parameters (carcass weight, lean meat etc.) but also inspection results (e.g. lung-, liver-, pleuritis-scores), diagnostic laboratory results and the resulting herd health statuses (e.g. Salmonella, Pseudorabies, Scabies, Atrophic Rhinitis).

Conclusion

Effective health management in the pork production chain depends on knowledge- and information exchange between the various partners of the chain. The implementation and use of two software tools has benefits for all partners involved. Farmers will find that their problems are recognised sooner, taken serious and approached in a structured way, leading to a better herd health and with that an improved financial result. Veterinarians and consultants are handed tools to document the herd health situation and tools to analyse specific problems. Background information is provided in an easily accessible format. Finally, chain managers gain more insight in the herd health situation in their chain and can directly stimulate further analysis and intervention in specific herds but also get a long term overview in the development of the herd health and can initiate special health programs that focus on common problems or stimulate research in areas where the available knowledge is not sufficient. Overall this tool will improve the insight in the herd health situation and improve the herd health management leading to better financial results for all partners.

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CONTAMINATION PRIOR TO AND DURING SLAUGHTER

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Introduction

In Germany, a quality management and assurance program is being introduced for the entire food chain from feed production up to the retail (1). This "QS"-System (QS stands for Quality and Safety) is targeted at guaranteeing the compliance with the basic quality criteria throughout the production chain. The program has been running for 2 years. Participation in the QS-System is voluntary. However once a producer has decided to be a participant his participation in the QS Salmonella Monitoring System is mandatory. In 2004 about 20,000 (QS) herds are included. Random samples of meat juice from every participating herd (sixty per year, evenly distributed over the shipments from the herd in question) are tested for salmonella antibodies. After one year (i.e. after sixty sample results) every participating herd is assigned to the following categories:
Cat. I: < 20% of the samples are salmonella-antibody positive, i.e. the risk of introducing salmonella into the slaughterhouse is low
Cat. II: 20% to 40% of the samples are salmonella-antibody positive, i.e. the risk of introducing salmonella into the slaughterhouse is medium
Cat. III: > 40% of the samples are salmonella-antibody positive, i.e. the risk of introducing salmonella into the slaughterhouse is high (2).
The monitoring results (categorization) are to be the basis for continuously reducing the salmonella frequency in the pork chain. According to these results the following three major intervention measures are being implemented:
1.) Supporting owners and producers of pig herds to reduce the salmonella load of their herds
2.) Logistic slaughter through separating pigs from Cat. III from pigs from Cat. I and II at slaughter (4)
3.) Targeted measures for reducing salmonella cross contamination during slaughter of pigs from Cat. III herds (3)
The objective of this study was to identify risk areas for cross contamination in the slaughter procedure from lairage to chilling to be able to develop targeted intervention measures for minimizing cross contamination.

Material and Methods

The investigations took place in a slaughterhouse in the North-West of Germany with a capacity of 10,000 slaughtered pigs per week. At first, following the HACCP-Concept, a salmonella specific hazard identification took place resulting in a plant specific sample collection plan targeted at verifying potential high risk areas for the salmonella cross contamination.
The sampling focused on: lairage (before, during and after a week´s slaughter), slaughterline (direct and indirect contacts such as: intestine bowls, scalding tank, scalding water, surface areas with direct contact to the carcasses, surrounding areas such as walls and floors), tonsils and retained gut residues still attached to the carcass.
In the lairage swab samples were taken and 5 samples per pen were pooled and processed. Most of the samples at the slaughterline and their surroundings were taken by a wiping method. Pieces of cotton were moistened with buffered peptone water, packed into a plastic bag and sterilized. During sampling the bag was turned inside-out, was pulled over the hand to avoid contact between hand and cotton, the sample was collected by wiping the surface with the cotton, and then the bag was pulled back to cover the piece of cotton.
The tonsils were taken from the pluck with sterilized instruments. They were flamed to remove potential surface contamination and then some cuts were made using a sterilized scissor to expose the inner bacterial flora.
All samples were cultured according to ISO 6579.

Results

From 240 collected samples 39 were found positive. There were 96 samples taken in the lairage. 12 positive samples were found before and during slaughter, 8 positive samples after slaughter and 5 positive samples were found on Sunday in the totally cleaned lairage. Four water troughs were sampled and one of them was found positive. From 95 sampled tonsils 8 were found positive. Other positive findings were: two times an intestine bowl after cleaning and disinfection, surroundings of the splitter, piece of gut still attached to the carcass and the floor between the slaughter area and the cantine.

12.5.2003

13 swab samples lairage, before slaughter	1 positiv	
9 swab samples lairage, after slaughter	0 positiv	
20 tonsils	0 positiv	

19.5.2003

20 tonsils	4 positive	

20.5.2003

11 swab samples lairage, before slaughter	5 positive	
8 swab samples lairage, after slaughter	2 positive	

16.6.03

10 swab samples lairage, Sundays (15.6.)	5 positive	
9 swab samples lairage, after slaughter	6 positive	
20 tonsils	1 positive	

23.6.2003

8 swab samples lairage, after slaughter	2 positive	
16 tonsils	2 positive	

24.6.2003

7 swab samples lairage, after slaughter	4 positive	

30.6.2003

13 swab samples lairage, before slaughter	0 positive	
6 swab samples lairage, after slaughter	0 positive	
2 samples scalding water	0 positive	
19 tonsils	1 positive	

7.7.2003

1 sample gut attached to carcass	1 positive	
4 swab samples water troughs	1 positive	
2 wiping samples under the air filter in the lairage	0 positive	
10 wiping samples surrounding slaughterline	1 positive (surrounding splitter)	

28.7.2003

11 wiping samples surrounding slaughterline	2 positive (intestine bowl after cleaning)	
1 sample feces in carcass	0 positive	
2 samples gut attached to carcass	0 positive	

13.8.2003

7 wiping samples personnel rooms	0 positive	
7 wiping samples slaughterline before slaughter	0 positive	
3 wiping samples slaughterline during slaughter	0 positive	
1 wiping sample crossway canteen/ slaughterline	1 positive	
1 wiping sample washbasin at crossway	0 positive	

Discussion

Out of the identified "cross contamination areas" the lairage area has by far the highest impact on the cross contamination and on the salmonella load that is finally carried into the slaughter and processing area for pork. Even if the number of salmonella carrying slaughter pigs can be reduced by successful intervention measures at farm level, a salmonella cross contamination due to a poor lairage management can make all efforts of the producers and their veterinarians in vain.

Most intervention measures focus on faecal contamination. However, our study shows that the tonsils are of equal importance.

Another "problem area" for cross contaminating salmonella is the multitude of the daily minor violations of hygiene and separation rules such as unintended crossroads between "black" and "white" areas.

Furthermore, it is obvious that cleaning and decontamination of direct contact surfaces, such as intestine bowls for example, have to be improved!

Conclusions

Risk categorization of herds without strict separation of high and low risk herds for slaughter (logistic slaughter) is useless.

Targeted salmonella cross contamination reduction measures during the entire slaughter process need to be an indispensable part of overall salmonella reduction programmes!

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Quality in food chains - Pork

Posters

A FOLLOW UP STUDY TO SALMONELLA spp. ANTIBODIES IN A PIG UNIT

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Introduction

Current levels of occurrence of a number of pathogens, including *Salmonella*, pose potential threats to consumers and, in the longer term, to the viability of the pork processing industry. For example, surveys of retail outlets in Ireland have found pork products to be contaminated with *Salmonella* (9.9%, [1]). This report is generally in line with data from other countries that confirm the significance of pork as an important source of food-borne pathogens. The inadequacies and cost of conventional culture methods and the convenience of modern serologic techniques have spurred the development of serologic testing for *Salmonella* in pigs (3). A fundamental characteristic of serological testing is that serum antibody persists much longer than detectable faecal shedding. In Italy, *Salmonella* spp. infection and correlated diseases are submitted to control measures but a national control program is not present. Due to the relevance of damage related to *Salmonella* infection, voluntary control programs are in progress at herd level. The study of the antibody patterns to *Salmonella* within herd population is a useful tool at detecting critical points. The primary objective of this study was to provide baseline data on Salmonella infection involving swine population of an Italian farrow to finish herd.

Material and Methods

Antibody response to *Salmonella* spp. was detected in 54 pigs by ELISA (IDEXX HerdChek Swine Salmonella test kit) belonging to a farrow to finish 550 sow herd in Italy and experiencing a history of *Salmonella choleraesuis* infection. Serum samples were collected at 0, 21, 42, 63, 84, 112, 140 and 180 days after birth. In addition, *Salmonella* antibodies were checked in blood samples of 11 sows at farrowing and in their colostrum.

Results

The data concerning the antibody response to *Salmonella* spp. detected at different times are shown in Figure 1.

At time 0, after colostrum feeding, antibody response involved 98% of suckling piglets. These data are in accordance with seropositivity of sows (100%) and their colostrum samples (90%).

The trend of the seroprevalence showed a gradual decrease and at time 63 days the figure was 14%. This fact leads to the decline of maternal derived antibody (MDA). From 63 to 140 days we observed an increasing of antibodies from 14% to 95% as a consequence of *Salmonella* infection. Afterwards, the seroprevalence had a slight decrease to 78%. On clinical basis, no signs of disease referable to Salmonella spp. infection has been recorded during the observation period. In addition, the onset of respiratory disease was detected between 84 to 112 days of age. Laboratory investigations on lung samples did not allowed *Salmonella* to be isolated. In comparison with the standard productive performances, we have detected a 10% reduction of the average daily weight gain (ADWG).

Discussion

The study involved a typical Italian pig production unit so that it could be considered as a model. On epidemiological criteria, this was a longitudinal serological survey that, on the contrary of a single cross sectional sampling (2), was reliable estimate to monitor the follow up of *Salmonella* infection in a farrow to finish sow herd. Nevertheless, the lack of *Salmonella* isolation from faecal samples does not allow serological evidences and microbiological findings to be associated. In case of an infected herd, the infection involves 100% of sows as demonstrated by antibody response. So that suckling piglets result seropositive following colostrum feeding. Considering the pathogenesis of *Salmonella* infection has to be hypothesise a certain degree of faecal shedding from infected-healthy sows to piglets without clinical signs. Due to serological pattern, the time at major risk of *Salmonella* spp. infection starts from 63 days of age when MDA disappears and pigs are commingled at the beginning of the fattening period that triggers the shedding of the pathogen. On clinical basis, *Salmonella* infection did not elicit enteric signs but might contribute at the onset of respiratory disease by other pathogens and to cause a reduction of ADWG.

Conclusion

In the Italian production system the common use of wet whey acidifying liquid feed could control the facets of *Salmonella* infection. However this tool looks not able to control the infection. The use of antibiotic treatment at the beginning of *Salmonella* spread (about 2 months of age) could be suggested at controlling the infection in fatteners.

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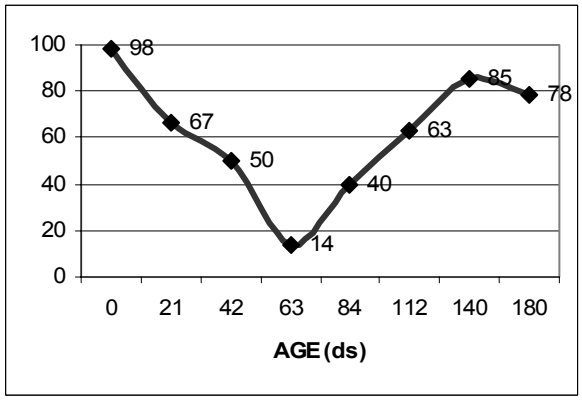

Figure 1 : Pattern of seroprevalence to Salmonella spp. detected in pigs at different times of observation.

ON FARM VETERINARY MANAGEMENT PROGRAMME (VMP) FOR THE PRODUCTION OF ENHANCED HYGIENIC QUALITY ANIMAL ORIGIN FOOD PRODUCTS: BIOSECURITY MEASURES AND ON FARM HACCP-COMPATIBLE SYSTEMS

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Introduction

The authorized farm veterinarian is responsible for the preservation of public health through the protection of the production "chain" that starts from the "stable'' and ends to the consumer's "table". He or she minimizes all possible risks on farm, in order for enhanced hygienic quality animal origin food products (meat, milk, eggs, fishes, honey) to be produced (5,7). The key for the hygienic assurance of all on farm production procedures is the implementation of an on farm VMP under full supervision of the authorized farm veterinarian (with the coordination and collaboration of the official civil servant Veterinary Authority) (7). Critical points of the VMP are: the monitoring of all on farm health economics, the application of the correct clinical nutrition programme and the judicious use of all pharmaceuticals substances in the farm (6,9). Moreover, major components of the above-mentioned programme are the appropriate "farm specific" biosecurity measures and the on farm Hazard Analysis Critical Control Points (HACCP)-compatible systems (1,3,4,10). Without the application of the VMP in all types of livestock production (porcine, ovine, bovine, avian, fish, shellfish, honey bees), it is impossible to fulfil the demands regarding the basic principles of food hygiene-safety in the E.U. (White Paper on Food Safety) for the on farm transparency and traceability of animal origin food products (that includes the food's origin, under which hygienic conditions it was produced and how did it reach the consumer's "table") (7,8,9).10

Authorized farm veterinarian and VMP

The authorized farm veterinarian in order to implement the VMP has to be well educated and certified at national level by specialized veterinarians in specific veterinary medicine specialties according to the European Board for Veterinary Specialization (EBVS) (5,9).

Regarding health economics in every farm the specialized veterinarian is responsible for improving all productive parameters (e.g. by declining the mortality rates), applying proper diseases surveillance schemes (e.g. with appropriate vaccination and metaphylaxis programmes) and preserving the animals' high health status (e.g. keeping the use of antimicrobial drugs to a minimum and only for therapeutics purposes while using alternative prophylactic or control substances such as probiotics, acidifiers, natural essential oils etc.) along with the application of welfare rules. In this way there can be protection of the consumers' health, the animals' health and welfare, the environment and the productivity of the farm (3,4,7).

Another major issue is the control of all incoming animal feed raw materials in the farm through their certification by an agronomist for plant origin products and/or a veterinarian for animal origin products along with the application of the appropriate clinical nutrition programme by the farm veterinarian. This programme as part of the VMP together with regular quality controls of long-term stored feeds and drinking water on farm and the obligatory application of HACCP systems in animal feed producing facilities (178/2002/E.U.) is the only way to prevent e.g. dioxins, mycotoxins and drug residues from getting into the food chain that leads to the consumers' "table" (2,8,9).

Furthermore, special care should be given in the veterinary drugs which are used in the farm. The use of these drugs must follow the authorised veterinarian's prescription, while the farm has to maintain all pharmaceutical substances in a specific pharmacy facility. Only officially approved products are used according to the register data sheet recommendations. It is important to maintain the labels of the veterinary drugs (including all data and prescriptions) for a period of at least 2 years in the farm's archive (3,4,6,8).

Moreover, the farm veterinarian is responsible for issuing all veterinary hygienic certificates of incoming and outgoing animals in the farm and for the strict control of all animals' movements. Full hygienic record is registered for all farm animals kept either for breeding or fattening purposes, until 24 months after their removal while computerized records are kept regarding performance parameters, health and treatments of every animal in the farm (5,7).

With the application of all above-mentioned measures as part of the on farm VMP it is possible to produce high health status slaughter animals, as well as improved health status animals that produce food for human consumption e.g. milk, eggs, honey etc. With the continuous preservation of the high health status of productive animals and the complete assurance of all on farm production procedures ("stable") it is possible to fully protect the consumer's "table" by producing animal origin food products which are absolutely safe for human consumption (6,8).

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Other Authorized Farm Veterinarian's responsibilities as part of the VMP:
Implementation of regular cleaning, disinfection programmes and control of rodents, insects and fomites (visitors, lorries). Continuous evaluation of these procedures e.g. by observing the incidence of common diseases in the farm and by regular sampling of concrete surfaces and measuring bacterial load (housing hygienic measurements).
Application of the essential rules for the protection of the health of farm workers and medical observation of their health. Human personnel are subjected to frequent health examinations for zoonoses such as leptospirosis, brucellosis, anthrax, and tuberculosis, and are vaccinated against inf1uenza
Correct operation of the farm's waste management programme and follow up of sanitary and other relevant legislative arrangements for their disposal.
Existence of appropriate alarm systems and use of back up emergency systems (e.g. electrical generator) in case of mechanical, electrical or other malfunctions in the farm.
Keeping record of notification of every problem regarding the final products for human consumption and implementation of the proper regular sampling protocol at all production stages for further laboratory examinations.

Quality in food chains - Dairy

Oral Communications

ANIMAL HEALTH AND WELFARE IN FREE RANGE CATTLE – A SURVEY OF FARMS IN WESTERN SWEDEN

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3 Swedish Animal Welfare Agency, Skara

Introduction

Keeping cattle in open pasture all the year round may be an inexpensive way of beef production, which also promotes the biodiversity of rural areas (Kumm, 2002). However, in order to maintain a good animal health, the management system must provide the animals with sufficient feed and water, as well as appropriate shelter. Such an extensive system may tempt the farmers to neglect necessary supervision of the animals. Furthermore, in the public debate in Sweden it has been questioned if shelters are needed or even used by the animals. Concern for poor animal welfare in such systems made the Veterinary administration in the County of Västra Götaland to initiate initiated an investigation of animal welfare in farms in the area.

The aim of the study was to investigate farms with free range cattle and evaluate their compliance to the animal welfare statutes.

Material and Methods

The study was carried out during the Winter of 2002. The local animal welfare inspectors were asked to inspect and score conditions at farms with free range animals, during the period of January to March 2002.

The questionnaire contained 55 questions; general information about the farm, housing conditions, water and feeding equipment as well as scoring of animal health (for details see, Gunnarsson et al., 2003; 2004). The questionnaire was designed to score how well the farmers were complying with the Swedish animal welfare legislation, as well as to get information about the further handling by the authorities (SFS:534, 1988a; SFS:539, 1988b; SJVFS:6, 2003).

In total 255 questionnaires were returned from 32 out of the 49 local administrations in the County. Seventy-five percent of the farms had cattle, 19% had sheep and 5% had both cattle and sheep. The median size of cattle stock was 13 animals (minimum 2; maximum 274) and for sheep 26 adults (minimum 4; maximum 549). This paper focuses solely on the animal health and welfare of free range cattle, and in total there were 204 questionnaires covering the conditions for cattle.

Results and discussion

In general the animal health was good (Fig. 1). In 11% of the farms occasional animals were lean and in 2% of the farms >50% were lean. In 5% of the farms >50% of the animals were dirty, and in these farms the animals had no access to an indoor lying area, the lying area was too small or the bedding was wet and dirty. In all farms where the indoor area was sufficiently large and had a dry, clean bedding the animals were using the area for resting.

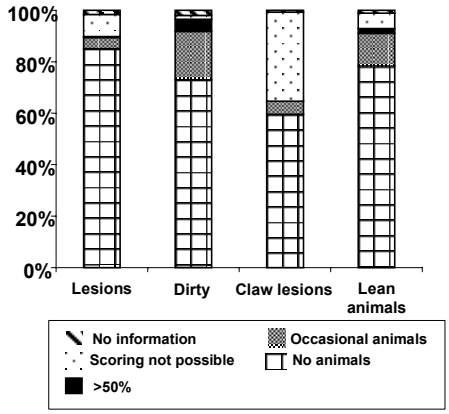

Figure 1. Scoring of animal health in 204 herds with free range cattle in the County of Western of Sweden.

Most farms were feeding animals using feed-racks or feed troughs, 13 % fed the animals on the ground outside with no other facilities. The animals had access to water in water troughs or bathtubs in 83% of the farms. In 17 % of the farms the animals could only find water at streams or a pond, and in 41 % of these farms the animals could not get water of acceptable quality.

One third of the farms did not have a separate pen for calving, and 50 % of the farms were lacking escape areas for the calves, which are compulsory according to the Swedish animal welfare legislation.

In 79% no pre-examination of the farm building plans had been carried out commissioned by the regional authorities, although this is compulsory according to the Swedish animal welfare legislation.

The animal welfare inspectors reported that 38 % of all farms were found to be in full compliance to the legislation, and in 40 % some kind of action were taken; 31 % got oral remarks, 30 % got written remarks and 4 % got injunctions (Fig 2). The most common reasons for injunctions were to improve the housing and to give the animals sufficiently access to feed and water.

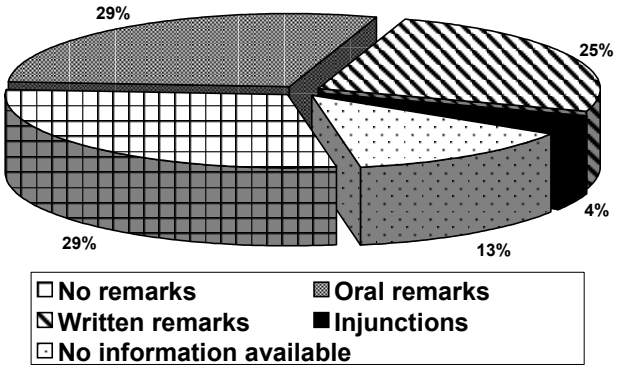

Figure 2. Remarks at animal welfare inspections 204 herd with free range cattle in the County of Western of Sweden. Please notice that more than one option was possible for each farm.

The results of this study were similar to previous reports from other parts of Sweden and from the UK (Askerblad & Jonsson, 2002; Gunnarsson et al, 2002; Sandberg, 2003; Pritchard et al., 2003).

Conclusion

In general the animal health was good, but a few farms had animals that were lean and/or dirty. At farms that had dirty cattle, the animals did not have access to an appropriate indoor lying area. Furthermore it was found that in farms where the indoor area was sufficiently large and clean, the animals were resting indoor. This means that appropriate shelter during the Winter is necessary to maintain acceptable animal health and welfare.

Thirteen percent of the farms were offering feed solely on the ground. In 17% of the farms the animals had no other water supply than streams, often with unacceptable water quality. In 79% of the farms no pre-examination of the building plans had been carried out, although this is compulsory in Sweden.

Only one fifth of the farms had pre-examined their building plans, which is compulsory according to Swedish animal welfare legislation. The inspectors reported that 40% of the farms did not fully comply with the Swedish animal welfare statutes.

Acknowledgements

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AUTOMATIC MILKING: LESSONS FROM AN EU PROJECT

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Abstract
Since the first commercial systems appeared in 1992, automatic milking systems (AM-systems) have been installed at an increasing rate. No other new technology since the introduction of the milking machine, has aroused so much interest and expectations among dairy farmers and the periphery. Reduced labour, a better social life for dairy farm families and increased milk yields due to more frequent milking are recognised as important benefits of automatic milking.
Automatic milking changes many aspects of farm management since both the nature and organisation of labour is altered. Manual labour is partly replaced by management and control, and the presence of the operator at regular milking times is no longer required. Visual control on cow and udder health at milking is, at least partly, taken over by automatic systems. Facilities for teat cleaning and separation of abnormal milk are incorporated into the automatic system and several adaptations are needed to accommodate continuous milking. Cow management including routing within the barn, the opportunity for grazing and the use of total mixed rations is altered. A high level of management and realistic expectations are essential to successful adoption of automatic milking.
Results from commercial farms indicate, that milk quality is somewhat negatively effected, although bacterial counts and somatic cell counts remain well below penalty levels. In terms of quality control, AM-systems offer extra means to assure milk quality and food safety. No adverse effects of the transition have been found for body condition, lameness or teat condition. A potential risk is that fertility of the herd may decline faster than the current trend for conventional dairy farms. The only obvious change was that milk cell count, often an indicator of the prevalence of mastitis, increased overall.
Automatic milking systems require a higher investment than conventional milking systems. However increased milk yields and reduced labour requirements may lead to a decrease in the fixed costs per kg milk. Automatic milking is gaining widespread acceptance and is estimated to be in use on more than 2500 farms in over 20 countries worldwide.

Introduction

Interest in fully automated milking began in the mid-seventies, and was initially driven by the growing costs of labour in Europe. Since machine milking, and automatic detaching, and teat spraying were already in common usage, automatic cluster attachment became the focus of European work. Although various prototypes demonstrated this capability, it took a decade before fully integrated and reliable automatic milking became a reality.
The term "Automatic Milking System" refers to a system that automates all the functions of the milking process and cow management undertaken in conventional milking, by a mix of manual and machine systems. In contrast to conventional milking, where humans bring the cows to be milked at regular times (usually twice a day), automatic milking places emphasis on the cows motivation to be milked in a self-service manner several times a day by a robotic system without direct human supervision.
In modern society consumer concern about methods of food production include food safety, as well as ethical questions related with animal welfare, animal health, housing conditions and access to grazing. Because unsupervised, automatic milking, raised a number of questions, an extensive EU research project was started at the end of 2000 (www.automaticmilking.nl). This project focussed on farm-level adoption determinants of automatic milking, on-farm social-economic and environmental implications, societal acceptance, impact on milk quality, impacts on animal health and welfare, including the combination of automatic milking with grazing and requirements for management information systems. Other research groups around the world have also contributed substantially to progress in our understanding of automatic milking and related management considerations.

Automatic milking systems

AM-systems include single stall systems with integrated robotic and milking functions and multi-stall systems with a transportable robot device, combined with milking and detachment devices at each stall. Single stall systems milk 55-60 cows, while multi-stall systems with 2 to 4 stalls milk 80 to 150 cows up to three times per day. Automatic milking strongly relies on the cow's motivation to visit the AM-system voluntarily. The main motive for this is the supply of concentrates dispensed in a feed manger in the milking box during milking. An automatic milking system has to take over the 'eyes, ears and hands" of the milker. Such a system includes electronic cow identification, cleaning and milking devices and computer controlled sensors to detect abnormalities in milk, in order to meet international legislation and hygiene rules from the dairy industry.
Teat cleaning systems include brushes or rollers, inside teat-cup cleaning or a separate 'teat cup like' cleaninge device. Several trials showed that cleaning with a device is better than no cleaning (Schuiling, 1992, Knappstein et al, 2004), it is not as good as manual cleaning by the herdsman.
AM-systems are also equipped with sensors to observe and to control the milking process. Data are automatically stored in a database and the farmer has a management program to control the settings and conditions for cows to be milked. Attention lists and reports are presented to the farmer by screen or printer messages. The AM-system also provides remote notification to the farmer if intervention is required.

Farms with Automatic Milking Systems

The first AM-systems on commercial farms were implemented in The Netherlands in 1992 primarily in response to expensive labour and the farm structure of family farms. Increasing costs of inputs while milk prices decreased, forced farmers to increase their output per man-hour. After the introduction of the first AM-systems, adoption went slowly, until 1998 (Figure 1). From that year on automatic milking became an accepted technology in the Netherlands and other European countries, but also Japan and North America. At the end of 2004, worldwide some 2500 commercial farms are expected to use one or more AM-systems to milk their cows. More than 85% of the world's automatic milking farms are located in north-western Europe.

<Figure 1. Number of farms using an automatic milking system at the end of 2003>

Automatic Milking and Management Aspects

Switching from a milking parlour to automatic milking results in big changes for both the herdsman and the cows and can cause stress to both. Although with AM-systems immediate supervision of milking is eliminated, new labour tasks include control and cleaning of the AM-system, twice or three times a day checking of attention lists including visual control of the cows and fetching cows that exceeded maximum milking intervals. Generally, a 10% labour saving is reported (De Koning & Rodenburg, 2004).

However, the biggest change is the nature of labour. The physical work of machine milking, is replaced with management tasks such as frequent checking of attention lists from the computer and appropriate follow up. This work is less time bound than parlour milking the input of labour is more flexible. This is attractive on family farms. But because milking is continuous, and system failures can occur anytime there must be a person "on call" at all times. System failures and associated alarms typically occur about once in two weeks although this varies with the level of maintenance and management.

In terms of the impact on cows, the AM-system is not suitable for all cows. Poor udder shape and teat position may make attachment difficult and some cows may not be trainable to attend for milking voluntarily. In new installations, the number of cows found to be unsuitable is generally reported to be less than 5-10%. In the transition from conventional to automatic milking, cows must learn to visit the AM-system at other than traditional milking times. Training and assistance in the first weeks should involve quiet and consistent handling, so they adapt to the new surroundings and milking system.

Milking frequency

In practice, the average number of milkings per cow day varies from 2.5 till 3.0, but rather big differences in milking intervals are reported by commercial farms. A typical figure is presented in figure 2 (De Koning and Ouweltjes, 2000). Almost 10% of the cows realised a milking frequency of 2 or lower over a two year period milking with an single stall AM-system. This occurred even though cows with a too long interval were fetched three times per day.

<figure 2. Frequency distribution of milking intervals in hours over a 2-year period (De Koning & Ouweltjes, 2000)>

Such cows will not show an increase in yield and may even show a production loss. By changing the milking parameters of the AM-system, it is quite easy to prevent cows from being milked at low yields or short intervals. But it is much more difficult to prevent cows from being milked with long intervals. This means it will be necessary to manage the intervals by fetching cows that have exceeded a maximum interval. Usually this is done several times per day at fixed times around the cleaning procedures of the AM-system. In a large study on 124 farms in the Netherlands, Van der Vorst & Ouweltjes (2003) found that in farms where cow numbers were 25% or more under the capacity of the system, cows were rarely fetched. On almost 50% of farms cows were fetched twice a day when the interval exceeded 12 hours and on 35% cows were fetched 3 or 4 times per day. These studies also showed that too long intervals cannot be prevented completely. Fetching cows three times per day that have exceeded an interval of 12 hours, means that the maximum interval will be 20 hours. Fetching cows with intervals shorter than 12 hours is time-consuming and moreover may lead to some habituation of the cows.

Increase in milk yield

One of the benefits of automatic milking is increased milk yield from more frequent milking. An increase from 6 to 25% in complete lactations has been shown when milking frequency increases from two times to three times per day (Erdman & Varner, 1995). French data show an average 3 % increase in milk yield and up to 9% for farms that utilized the AM-system for more than 2 years (Veysset et al, 2001). In the study of Van der Vorst & Ouweltjes (2003) an average increase of 5% with a range of – 16% to + 35% was reported. In many larger herds with highly automated conventional parlours, 3 times daily milking is commonplace. For 3x herds adopting automatic milking, a production decrease of 5 to 10% would be expected.

Attitude and expectations

One important factor in successful implementation of an AM-system is the attitude and expectation of the dairy farmer (Hogeveen et al, 2001, De Koning et al, 2002, Ouweltjes& de Koning, 2004). While there is considerable variation in level of satisfaction with different types of systems, an estimated 5-10% of owners have switched back to conventional technology. In some cases expectations were not realistic, in others farmers were unable to adapt to the different management style, and in some cases a high rate of failures on the AMS resulted in ongoing high labour input for manual intervention. During the start up period, automatic milking requires a high input of labour and management. Key factors of a successful implementation of AM-systems are:

- Realistic expectations

- Good support by skilled consultants before, during and after implementation
- Flexibility and discipline to control the system and the cows
- Ability to work with computers
- Much attention to the barn layout and a good functioning cow traffic
- Good technical functioning of the AM-system and regular maintenance
- Healthy cows with good feet and 'aggressive' eating behaviour

Grazing

In most European countries, grazing during summer time is routine (Van Dooren et al, 2002) or in some Scandinavian countries even compulsory. Moreover, from an ethological point of view, many consumers in North Western Europe believe grazing is essential for cows and one Dutch dairy pays a premium for milk from grazed herds. In the Netherlands grazing is common practice (>80%). However, about 52% of the farms with an AM-system apply grazing, showing that grazing in combination with AM is less common, but still possible (Van der Vorst & Ouweltjes, 2003). Grazing is critical to low cost milk production in New Zealand, and while there is no commercial use of AMS in that country at this time, the "Greenfield" project uses automatic milking in a 100% grazing system under very different circumstances than those found in Europe.

Milk quality

Milk quality is a critical concern on modern dairy farms because milk payment systems are based on milk quality and consumers expect a high level of quality and safety from the milk products they buy. Although automatic milking uses the same milking principles as conventional milking, there are major differences. Results from commercial farms in Europe (Klungel et al, 2000, Van der Vorst & Hogeveen, 2000. Pomies et Bony, 2001, Van der Vorst et al, 2002) and North America (Rodenburg and Kelton 2001) indicate, that milk quality is somewhat negatively effected after introduction of automatic milking. In general data show an increase in bacteria counts, although the levels are still relatively low and well within the penalty limits. Helgren and Reinemann (2003) determined that SCC and bacteria counts in the US were similar to conventional milked herds. Both the cleaning of the milking equipment and milk cooling are critical factors in controlling bacteria counts. Also cell counts are not reduced after the change to automatic milking, despite the increased milking frequency.

With increasing milking frequency a small decrease in fat and protein percentage and an increase in the free fatty acids levels has been reported (Ipema and Schuiling, 1992, Jellema (1986),Klei et al, 1997). Van der Vorst et al (2003) found both technical and management factors influencing FFA levels. Wiking and Nielsen (2003) found relations with FFA levels and fat globule size and showed that feeding and cooling strategies affect FFA levels. In studies from Van der Vorst et al (2002) and Svennersten & Wiktorsson (2003) increased FFA levels were also found with increased milking frequencies using conventional milking methods.

The general conditions of hygiene in milk production in the EU are currently defined by the Commission Directive 89/362/EEC (1989) but not all elements apply to automatic milking (Rasmussen, 2004). The following text is proposed to be included in the coming EU Hygiene Directive: "Milking must be carried out hygienically ensuring in particular, that milk from an animal is checked for abnormalities by the milker or by a method achieving similar results and that only normal milk is used for human consumption and that abnormal, contaminated, and undesirable milk is excluded".

AM-systems have accurate cow identification and this also means less chance of human errors than in conventional milking, which might have a positive effect on lowering the presence of inhibitors in milk, as reported from North America. In this way automatic milking also potentially enhances food safety and quality.

Animal Health

Within the EU project Automatic Milking, special attention was paid to animal health. In Denmark, The Netherlands, and the UK, 15 herds each were recruited for monitoring the impact of transition to automated milking on animal health (Hillerton et al, 2004). The herds recruited represented the types of AMS marketed in each country. Each farm was visited at least twice before installation of the AMS and a minimum of twice, but often up to six times, after installation. On these visits assessments were made of at least half of the cows or fifty animals on body condition and locomotion, and forty cows for teat condition (on some farms in the Netherlands and UK only). Farm data including milk production, milk quality, animal records on individual cow cell count, fertility, animal treatments, animal movements, veterinary purchases were collected.

The body conditions varied more between countries than in response to the introduction of AM (Hillerton et al, 2004). In Denmark and the UK there was no change in body condition between 3-6 months prior to AM installation and 6 months post installation. A slight but not significant drop occurred with the Dutch cows (Dearing et al, 2004). On the Dutch farms the range of body condition narrowed significantly from 1.35 to 0.98 points score suggesting that the farms are managing body condition better.

No change in locomotion was seen one month after AM installation. The scores in Denmark and UK increased slightly by 3 months after installation, but not significant. In the UK the average score increased on seven farms whilst unchanged on 6 farms. Scoring was continued on 12 of the UK farms. Twelve months after installation of AMS the lameness has increased significantly. Prior to installation eleven of fourteen UK herds were grazed but only six after installation. The poorer locomotion may reflect the increase in constant housing (Hillerton et al, 2004).

The overall impact of conversion to AM was assessed by comparing how each individual farm handled the main indicators of animal health during and after the transition to automatic milking. Comparing 12 Dutch farms only

one farm improved in locomotion, body condition as well as cell counts. Overall, little change was apparent. Locomotion improved in five herds and deteriorated in five herds. Body condition score decreased in eight herds but only by a small amount. It increased in two herds but not making the cows any fatter, just more typical (Hillerton et al, 2004). The only major deterioration was in average milk cell count and the proportion of cows with a cell count above a threshold, where only two of the herds produced better quality milk. Average milk yield in the Dutch herds decreased in continuation of a trend starting up to 12-months prior to installation of the AMS and the cows became thinner with only a small reduction in DIM. Overall there is little evidence of major changes occurring in the common measures of fertility. None of the changes were statistically significant but all suggestive of poorer fertility, at least in the transition period from conventional milking to AM.

Hillerton et al (2004) conclude that no major problems in converting from conventional milking to AM have been identified but equally none of the 44 farms has been found to achieve a substantial improvement in any aspect of cow health. The transition period to AMS comprises a period of higher risk to health that extends from weeks before installation when resources start to be diverted from cow management. The length of the transition will vary on individual farms related to many unique factors. Several potential problems may develop in the longer term and anticipation of these is necessary. Clearly AMS succeeds but its longer-term promises for animal welfare and milk quality are unfulfilled to date (Hillerton et al, 2004).

Economical aspects

Investment required for AM-systems are much higher than for conventional milking systems and thus the fixed costs of milking are higher. However more milk with less labour means that the costs of milking per kg of milk will decrease. Theoretically, with an AM-system more cows can be kept with the same labour force than with conventional milking, but this may involve additional investments in buildings, land or feed and perhaps milk quota. On a farm with more than one full time worker the possibility exists to reduce labour input and thus costs. Quite often that does not happen and the time saved as a result of lower labour requirement is used for personal activities. Mathijs (2004) reported that two third of AM-farmers state social reasons for investing in automatic milking, such as increased labour flexibility, improved social life and health concerns. On average total labour was reduced with 20% compared with the conventional twice daily milking.

Little economical information is available from commercial herds using an AM-system. The high-tech farm at Waiboerhoeve experimental station realised a cost price, which was approximately € 1,50 per 100 kg higher compared with a the cost price of a reference group of farms using conventional milking. The small plus on the cost price is mainly due to increased machinery costs per kg of milk, despite the decreased costs of labour (Van der Kamp et al, 2003). An extra 10% more milk harvested per year would lead to a reduction in cost price of approximately 3 € per 100 kg milk.

Several simulation models have been developed to calculate the economic effect.

The “Room for Investment” model computes the amount of money that can be invested in an AMS, without a decrease in net return compared with conventional milking (Arendzen & van Scheppingen, 2000). The RFI-value calculates the annual accumulated return from increased milk yield, savings in labour, and savings in not investing in a milking parlour and divides this by the annual costs of the AM-system. The model can use farm specific factors and circumstances to calculate the RFI-value. Figure 3 shows the results of a combined sensitivity analysis illustrating that increased milk yield and labour savings are essential factors regarding the economy of automatic milking. The RFI-value for the basic farm with 500 kg per cow yield increase, 0,75 hour net labour saving per day (~10% labour saving), compared with a highly automated milking parlour and 25% annual costs of the AM-system amounts € 136,942. Both labour saving and yield increase have a large effect on the RFI value.

<figure 3>

Since capital costs tend to decrease while labour costs tend to increase, more widespread adoption of automatic milking in nearly all areas of the developed world would appear to be only a matter of time.

Conclusion

The number of farms milking with automatic milking has increased significantly since 1998. In areas where labour is expensive labour or in short supply, automatic milking is a valid alternative to traditional parlour milking. However if labour is available, and particularly where herd sizes are large conventional milking, often with rotary or rapid exit parlours equipped with features to increase throughput per man hour will remain popular.

The introduction of automatic milking has a large impact on the farm and affects all aspects of dairy farming. Because milking is voluntarily there is large variation in milking intervals. Both farm management and the lifestyle of the farmer is altered by automatic milking. AM-systems require a higher investment than conventional milking systems but increased milk yields and reduced labour may lead to lower fixed costs per kg milk. Successful adoption of automatic milking depends on the management skills of the farmer and the barn layout and farming conditions. Animal health and well-being is not negatively affected by automatic milking, but on the contrary till now no particular benefits for the health of the cows have been found.

A better understanding of the characteristics of automatic milking systems will help farmers to make the right decision. Both conventional and automatic milking will be used on dairy farms in modern dairy countries in the foreseeable future.

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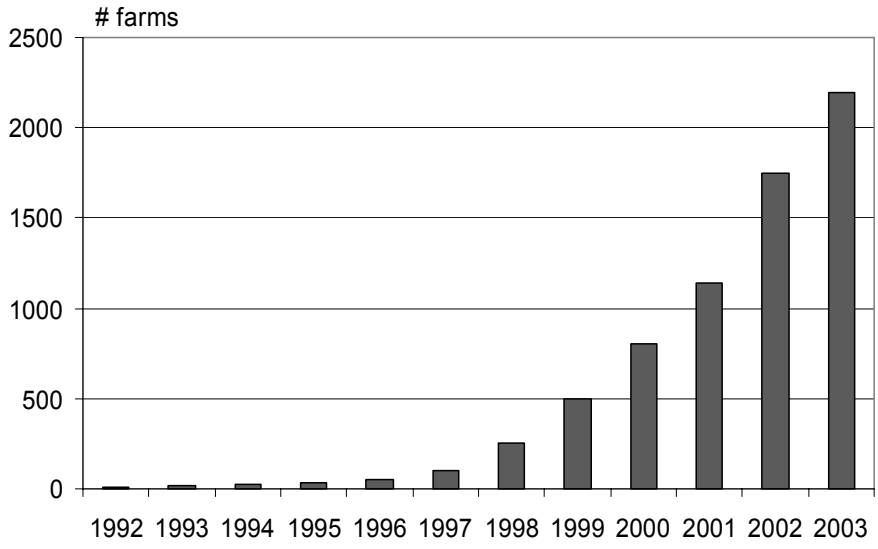

Figure 1. Number of farms using an automatic milking system at the end of 2003

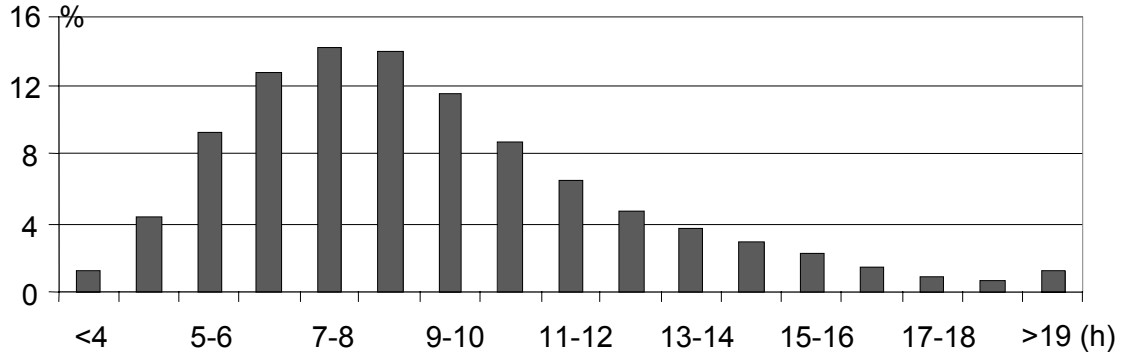

Figure 2. Frequency distribution of milking intervals in hours over a 2-year period (De Koning & Ouweltjes, 2000)

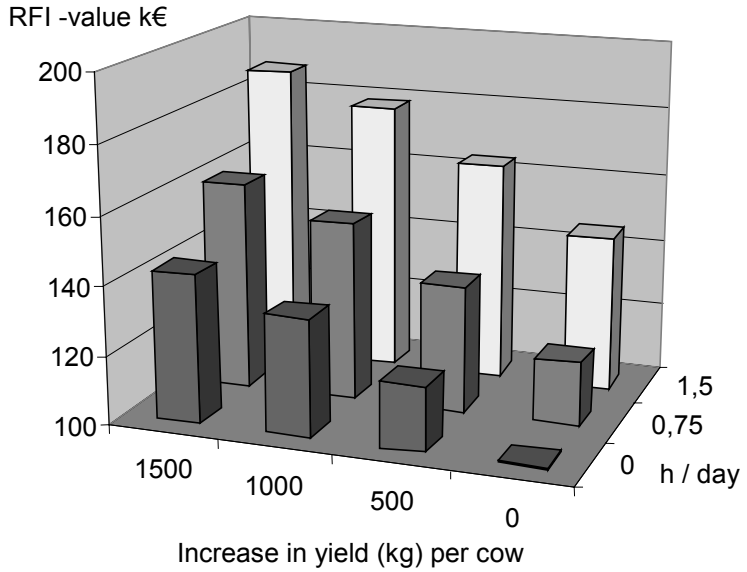

Figure 3. Room for Investment (RFI) due to labour saving and milk yield increase with annual costs for AM-system of 25% of investment. Comparison made with an highly automated milking parlour.

SURVEILLANCE AND MANAGEMENT IN DAIRY CATTLE HERDS AFTER IMPLEMENTATION OF AUTOMATIC MILKING: IS THE HACCP CONCEPT USEFUL?

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Introduction

The transition from traditional milking to automatic milking (AM) causes dramatic changes in management procedures in a dairy cattle herd (4). In many new AM-herds there have been problems with mastitis and somatic cell count (8) and further problems with water contamination and hygienic quality in the milk (3). Also animal welfare may be compromised in new AM-herds due to less efficient health surveillance. Lameness is found to be a general problem in AM-herds (2). Thus, there is a need for a surveillance and management tool in new AM-herds.

At the Danish Institute of Agricultural Sciences a three-year project was initiated in January 2004 aiming at the development of a tool for surveillance and management in new AM-herds. The purpose of this paper is to suggest a concept for such a tool.

The HACCP-concept

A generally accepted method for systematic surveillance is a HACCP concept (Hazard Analysis Critical Control Points). This concept was developed in the food industry to cover food hazards (human health risks). However, it has been suggested to be relevant also for dairy cattle herds (5, 1, 9, 10). A HACCP-system for a primary production, such as a dairy cattle herd, will fit well into a whole product chain surveillance system (from stable to table). However, it need to cover a range of quality issues and will not be confined to human health risks.

The idea is to prevent specified problems by continuous control of critical control points (CCP's) indicating an increase of a certain risk of the problem. If an alarm value (AV) for a certain control point is reached then a predefined action should be taken in order to decrease the risk.

Development of a HACCP concept follows 7 steps:

1. Identification of potential hazards
2. Description of critical control points
3. Definition of alarm values for each control point
4. Description of a programme for measurements of each critical control point
5. Description of appropriate actions to take when alarm occurs for a critical control point
6. Making a test procedure validating the surveillance system
7. Documenting all procedures and actions

How to develop a HACCP concept for an AM-herd

The surveillance system should be focused on problem areas and at the same time be system oriented. We have initially chosen to focus on six problem areas for a new AM-herd, namely:

- Milk quality
- Cow health
- Udder health
- Reproduction
- Milk production and feed management
- Animal welfare

Milk quality is assumed to include parameters such as bacteria counts, freezing point and free fatty acids. Cow health is likely to include factors such as lameness, body condition score and skin lesions. Udder health may include new infections, electric conductivity and teat lesions. Reproduction may be reduced to heat detection and milk production may include milking interval and incomplete milkings. Animal welfare may include queuing time, lying time, and lying behaviour.

Risk factors for each problem area will be identified through literature review. Besides, the identification of risk factors, CCPs and AVs must be specified.

Many parameters would be candidates to be included. Each parameter need to measurable in a robust way and need also to be controllable by the farmer. We need an operational system with relatively few CCP's. Therefore, not all parameters will be covered by CCP's. Some of the parameters may be controlled by a description of Good Farming Practises (7).

Since AM is still a new technology little relevant knowledge on how to quantify the risks is published. Across Europe there are several advisors and applied researchers with valuable experiences in robotic milking systems that can be used for the setting of CCPs and AVs. Therefore, we have chosen to use an expert panel analysis to establish CCPs and AVs. CCP's and AVs will be identified using a Delphi method, comparable to the methods described and applied by Sørensen et al. (11).

The expert panel will be asked to suggest CCPs individually. Then the expert panel will be asked to score each CCP on a scale from 1 to 5 (no relevance to mostly relevant). Based on the evaluation a full set of CCPs will be established. The expert panel will then be asked to suggest alarm values for each CCP. Based on their opinion a set of alarm values will be established.

How should we evaluate the prototype?

A surveillance and management tool needs several qualities in order to be relevant to the farmer. The system should be operational, efficient and economically beneficial.

An on-farm evaluation is necessary to test if the developed HACCP prototype is operational.
The developed prototype will be applied on 16 AM-herds during a year. A control group of 16 AM-herds with a production system (housing, herd size, milk yield) and AM-experience similar to the HACCP-herds is set up as well. The HACCP system will be implemented and all alarms and implemented actions will be recorded. The effect of the HACCP system will be evaluated by means of epidemiological methods, semi-structured qualitative interviews and systems analysis.

The effect of implementing a HACCP –system on milk production, milk quality and herd health will be analysed by means of epidemiological methods using the cohort-design with HACCO-herds and control herds.

The applicability of the HACCP-system will be evaluated using a semi-structured qualitative interview technique (12). The farmers will be interviewed about their experience using the HACCP-system and their opinion of the possibilities and limitations in the HACCP-system for new AM-herds.

The economic consequences of implementing the HACCP concept will be analysed by means of simulation models. The analysis will be conducted by the SimHerd III-model (6). The long-term economic effect of application of the HACCP-system is predicted and the overall economic consequences in terms of costs and benefits can be analysed.

Acknowledgement
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TRACEABILITY OF THE MOVEMENTS OF LIVING CATTLE TO DAIRY HERDS IN BRETAGNE

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Introduction

To manage animal health, it is of importance to determine the risks of introduction of a transmissible pathogen in a herd free from this pathogen. The between-herd spread of pathogens is related to the between-herd contact structure. On the one hand, this structure depends on the spatial distribution of the herds in a region and on the neighbouring relationships. On the other hand, it depends on the between-herd movements of living animals. The management of the spread of infectious pathogens then needs generally that collective control measures are applied. For dairy herds, large scale screening methods, such as the antibody testing on the milk tank, are available for the surveillance of pathogens. Therefore, control programmes for several infectious diseases have been implemented (e.g. IBR, BVD). Control actions aiming at limiting the risk resulting from animal movements should be defined according to the prevailing risks, which depend on number and types of introductions.

The objective of this study was to describe and quantify the movements of living cattle resulting in introductions of animals in dairy herds in Bretagne.

Material and Methods

With more than 800,000 dairy cows and 20% of the French milk production, Bretagne is the main breeding region in France for dairy cattle. In this region dairy and beef cattle are bred under several management systems in relation with the farmer's productive aims and with the breeds.

All animals are registered to the French identification register from 7 days of age. Available data are the identification numbers of herds, the identification number of animals, the sex, the breed and the birth date, the date and reason for entry in the herd (birth, purchase) and the date and reason for exit from the herd (mortality, sale, cull). More than 8 million cattle were registered between 1998 and 2001 in Bretagne, and 15,632 dairy herds (out of 35,326 cattle herds).

Dairy herds were defined as herds without any beef reproductive cow. Only herds with more than 10 animals were considered. Four types of dairy herds were distinguished according to their size and composition: strictly dairy herds – with more than 15 adult cows and no fattening alternative activity–, mainly dairy herds – with more than 15 adult cows and an alternative activity, such as calf or bull fattening -, small dairy herds – with less than 15 adult cows -, and herds of heifers – with only dairy females aged less than 20 months.

Movements of cattle exist between all types of herds for animals of variable age and destination (reproduction, fattening). In the case of dairy herds, introduced cattle come not only from other dairy herds, but also from herds with another main farming activity (beef herds, fattening). A movement was defined here to the exit of one cattle from his herd of origin and its entry in a dairy herd. Herds were characterised by their principal productive activity and by the possibility of an alternative fattening activity. Herd productive aims were taken into account because they are influent on the within-herd contact structure and on the decision making in terms of sales and purchases.

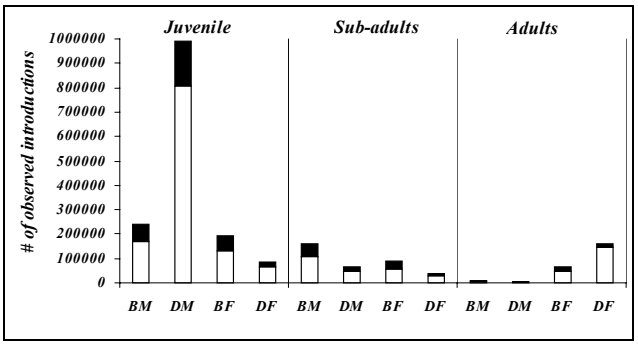
For an average year, we evaluated the proportion of dairy herds that introduced at least one animal (open), the number and type of introduced cattle, and the number and characteristics of the herds of origin when known.

Results

Among strictly dairy herds, 35% were open, whereas mainly and small dairy herds were half open. Per se, Heifers herds were almost ever open, except if they did not rear any new heifer in a given year.

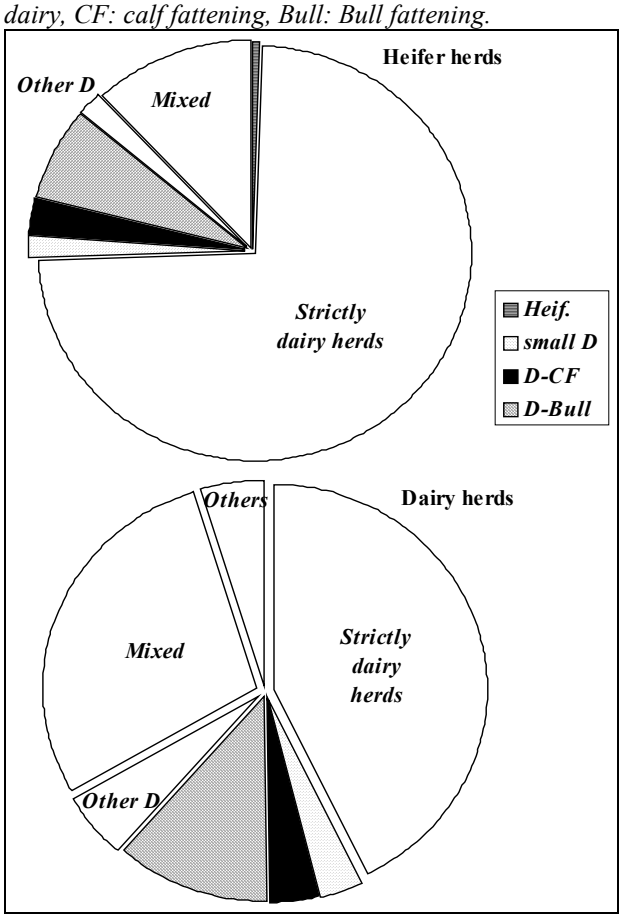
The origin of the introduced animals was known in 77% of the introductions. Availability of information depended on the type of animals (Fig. 1).

Fig. 1: Numbers of introductions in Bretagne (France, 1998-2001) with a known (white) vs. unknown (black) herd of origin, per type of animal. M: male, F: female, B: beef cattle; D: dairy cattle, Juveniles: 0-1 month old, Sub-Adults: 1-20 month old, Adults: ≥ 20 month old.



For Heifers herds, < 1/8 of the cattle did not come from dairy herds. This proportion was > 1/4 for Strictly dairy herds and > 1/3 for Mainly and Small dairy herds (Fig. 2). Cattle came from herds located in Bretagne in 88, 43, 56 and 96% of the between-herd movements for Strictly dairy, Mainly dairy, Small dairy and Heifer herds. When open, strictly dairy herds introduced in average less animals than other types of herds (6.0, 41.5, 27.7, 21.8 animals per year in strictly dairy, mainly dairy, small dairy and heifer herds, respectively). Moreover, a few number of source-herds provided animals to strictly dairy herds compared to other types of herds (3.0, 28.4, 14.2 and 8.5 herds provided animals to strictly dairy, mainly dairy, small dairy and heifer herds, respectively).

Fig. 2: Proportions of the types of herds of origin for heifer herds (top) and strictly dairy herds (back). D: dairy, CF: calf fattening, Bull: Bull fattening.



Discussion

The results given here are average numbers and proportions of movements of cattle to dairy herds. The variability in the direction and intensity of movements are also of importance for evaluating the risk of introduction of an infectious animal in a herd. We confirm here that the type of herds must be accounted for in order to define precisely the movements of animals.

Strictly and Mainly dairy herds are the two main types of dairy herds. Strictly dairy herds are often closed. When open, they buy animals from only a low number of herds. On the contrary, Mainly dairy herds introduce more animals, coming from several different herds. This potentially increases the risk of introducing an infectious animal.

Less frequent types of herds (Small dairy and Heifers herds) are not negligible in terms of between-herd infectious disease transmission because they highly contribute to the movements of animals.

Only 77% of the herds of origin were known in 1998 to 2001 despite the implementation of a national identification register. Efforts in registration are likely to have improved completeness in the data.

Conclusion

In the perspective of studying the between-herd spread of transmissible pathogens with a modelling approach, movements of living cattle have been described and quantified. The type of the herd was an important factor of variation, which influenced the type of introduced animals, their number and the origin of the introductions. The type of herds was defined on a yearly scale. The stability of the herds in a given type would be interesting to study, as well as their inclination to move from open to closed and opposite. Moreover, movements could be seasonal, which has to be further studied. Based on descriptive data, probability distributions will be defined and their parameters calibrated in order to model between-herd transmission of pathogens.

VETERINARY QUALITY MANAGEMENT: THE DUTCH TOTAL TOUCH

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Introduction

The Dutch dairy quality assurance program KKM was initiated in 1998 as a voluntary program, instigated by Dutch dairy industries, NZO, and the Dutch LTO, the farmers' association. It comprises 6 modules: health & welfare, hygiene & desinfection, milk harvesting & storage, medicinal drugs, water &waste management, nutrition. In 2000, KKM became compulsory for dairy farmers. Non-compliance meant that the milk was collected but at a price of 10 eurocent/l. Between 1998 and 2000 emphasis was on the formation of a certain quality attitude and mentality, Good Farming Practice. The veterinarian had to comply by issuing a statement of functioning according to Good Veterinary Practice; moreover, he had to be formally certified to have access to KKM-approved dairy farms. In 2002, a compulsory clinical herd health inspection was introduced. Inspections have to be conducted 4 times per year and following a pre-set protocol. The protocol originates from the legal requirements regarding the delivery of milk for human consumption. There are 7 categories of disorders distinguished in this protocol:

1. Zoonoses by agents that are transferred by milk
2. Disorders causing organoleptic changes in milk
3. Diseases with generalized signs and weight loss
4. Diarrhoea with fever and intestinal disease
5. Disorders of the genital tract; vaginal discharge
6. Udder and teat skin lesions
7. Highly contagious (list A) notifiable diseases

In this presentation, the outlines of the KKM program are addressed, as well as the results of the 6 herd health inspection rounds. Furthermore it is discussed how this KKM program can be integrated with veterinary herd health and production management programs and how the developments will be, given the recent EU policy.

KKM, Dutch dairy quality assurance at farm level

The 6 basic modules of KKM refer to the "do's and don'ts" on the dairy farm. It is e.g. stated what is and what is not allowed in the bulk tank room. The farmer conducts a self-evaluation to find out to which extent he complies with the rules. In principle the modules regard elements of a good farming practice code. KKM-inspectors conduct unannounced audits on the 25.000 dairy farms regularly The clinical herd health inspection is performed 4 times yearly by certified cattle veterinarians. The veterinary profession has been involved from scratch in preparing this inspection, the dairy industry being their client.

Basically, one looks for disease prevalences, meaning that chronic cases will be found together with prevalent cases and accidental incidents. Veterinarians had to follow a compulsory course to do the herd inspections.

KKM, results of the herd health inspections

The first 6 rounds of herd health inspections were performed between the end of 2002 and in 2003. More than 125.000 farm inspection records were available for statistical analyses (24.295 farms). About 99% of the herd inspections with cows listed as attention cows referred to less than 6% of cows; and 1% of all visits showed numbers of attention cows higher than 6% (= about 200 farms). About 25 farms had the highest numbers of attention cows, up to 15% of the cows on conventional farms (50-200 cows per herd) and up to 45% on small farms (< 15 cows per herd). The median percentage of attention cows per visit is 1.25% (skewed distribution). The number of farms with repeatedly high numbers of attention cows was about 120. The median number of attention cows per category per visit was from 1% (cat. 7) to 1.56% (cat.3). One visit showed more than 10 attention cows; no repetition occurred. On 752 farms more than 5 attention cows were listed during at least one visit. Highest ranking categories were 2, 3 and 4. Statistically considered, is the role of the veterinarian in the outcome of the analysis relevant in categories 2 (15% variation attributed) in category 3 (10%) and category 4 (7%). There is no effect of herd size or season on the outcomes.

The forenamed results point to the fact that relative few dairy farms can be classified as showing an excessive number of attention cows or showing high numbers repeatedly.

So, it should be feasible to identify criteria for a further classification of farms in classes Green (only a few attention cows), Orange (increased number) or Red (too high number). The last class could lead to withdrawal of the license to sell milk to the market.

This procedure is currently under study and will be installed in 2005 probably.

Next phases in KKM

First of all it should be stated that prevalences give less reliable pictures of the herd health situation than a continuous monitoring of incidences would. In a pilot-study it was found that the incidence figure between successive inspection visits was 5 to 10 times higher than the prevalence figures. But the herd inspection is the first step; more steps will follow.

One of these is the monitoring of cow welfare. Based on the Five Freedoms (Webster, 2001) and the derived Biological Needs (Bracke et al., 1999) a clinical welfare scoring tool was designed.

The primary Biological Needs refer to:

- Feed quality & availability
- Drinking water availability
- Safety & Resting place
- Health & Locomotion
- Grooming
- Social interactions

Furthermore, secondary needs have been listed: Respiration, reproduction, thermoregulation, excretion, orientation/exploration, pain experience related issues.
The clinical welfare scoring tool was tested on 100 farms in the south and the center of the country. Scoring was by giving 1 (poor), 3 (average) or 5 (good) points to the different items. Results were obtained at farm level, the level of clusters (eg housing) and items within clusters (eg bedding material). The results were as follows:
On average 77% of max score was achieved; while 14% of farms showed scores 1 in clusters or items. Scores 1 were most seen in Housing (maintenance, quality of slatted floor, manure removal, cubicle bedding, cow density), in Pasturing (absent, long paths, path quality) and in Health management (lameness, body condition, disease control planning, herd claw trimming).
Highest scores 5 were achieved in items like access to feed, feed availability, pasturing, general behaviour, herd health programs, cubicle beddings, light regimes, barn climate, lying and resting, space per cow, bacteria count in milk.
The advantage of this type of scores is that not only deviant cows are detected but the farmer can also show on which items he scores best (motivation). Moreover, next to scoring cows also cow surroundings are scored, meaning that one searches for risk conditions. The latter is paramount when one strives after risk identification and risk management (prevention) instead of disease combat and disease control. The step of continuous monitoring of health incidences, by eg the farmer is an issue of debate. Although it is accepted as a highly relevant issue it will not be implemented within 2 years time.
In the meantime it has been determined that the average duration of a herd health inspection takes 30 min on farms of between 50 and 150 cows; shorter and easier on farms where a routine veterinary herd health program is running. This might be relevant for implementation of EU directive 97-12 when veterinary herd inspections have to be executed. It is more valid to have an inspection report based on insight knowledge of farm obtained during monthly visits than based on a prevalence estimation of 4 times a year.
KKM is currently adapting the procedure documentation to work instructions and flow diagrams, not in the least to reduce the variation between observers. These KKM work sheets, diagrams, and audits can be considered elements of a HACCP-like approach of the dairy farm. Maybe that is the direction that developments of the KKM program will go. It would comply to the statements made in the EU regulation 178-2002 and in the discussion about the harmonisation of hygiene directives. It was stated that a HACCP-compatible program on dairy farms in the 4 areas of food safety, public health, animal health, animal welfare is indicated if the farmer has the responsibility and liability regarding the control of products/processes on his farm, and show that to third parties (authorities, retailers, consumers).

It has been evaluated earlier that HACCP-concept would be best applicable to dairy farms as compared to ISO systems or GFP (Noordhuizen & Welpelo, 1996; Cullor, 1997) but not as a panacea. This means that Critical Control Points have to be complemented with Critical Management Points both as part of a on-farm monitoring plan to detect risks in the production process and control them. Examples of this type of approach will be given at the meeting.
HACCP-concept is elaborated via 7 principles:
- make a production process diagram;
- identify the hazards on the farm;
- find the associated risk conditions;
- select CCPs and CMPs; standards; SDs;
- design an on-farm monitoring plan;
- determine the measures of control;
- set the documentation and validation.

It appears that structured, formal veterinary herd health programs and the HACCP-compatible plans can rather easily be merged; they have much in common. The most relevant issue in both regards the prevention of problems and failure costs via risk identification and risk management, thrue preventive actions.

Concluding remarks

The main question for farmers these days is where we all are heading for. The EU policy, the retailer strategies, and the general public opinion all point to the farmer's need of safeguarding or providing best certainties. Certification of farms as first link in the dairy food quality assurance chain seems obvious in the near future, especially in exporting countries. It would be in the interest of the farmer when the legal requirements (EU 97-12; EU 178-2002) could be coupled to quality requirements (HACCP-like) and to operational issues (herd health programs).

This would be the best way to motivate the farmer and achieve all targets at the same time. The role of the veterinarian hence might also change in the near future: from a solely sick animal consulting to more advisory - consultant type.

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DAIRY COWS FEEDING CHANGES THE BIOCHEMICAL COMPOSITION AND THE SENSORY PROPERTIES OF THE DAIRY PRODUCTS, BUTTER AND CHEESE.

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Introduction

The characteristics of dairy products depend of a large number of factors, in particular dairy cow feeding practices. After a short recall of the feeding factors that can modify the composition of milk, we will evoke the impact of feeding and in particular forage on the quality of milk and the sensory properties of butters and cheeses. The concept of forage implies at the same time its nature, its mode of conservation and its floristic diversity. The mechanisms explaining the sensory differences between dairy products in relation with feeding of dairy cows will be studied.

Feeding factors can modify the composition of milk

Milk protein content

The increase in energy supply increases milk yield and milk protein content. Energy level is most often increased by higher concentrate supplementation, which induced great changes: increased volatile fatty acid production especially propionic acid, larger amount of starch digested in the small intestine, higher synthesis of microbial proteins….(Rulquin and Hurtaud, 1994). The source of energy supply can also have an effect on milk protein content. For example, lipids usually induce a decrease in protein content (Chilliard et al, 2001).

Improving nitrogen nutrition, in terms of crude protein content of diets, has been thought to have a beneficial effect mainly on milk yield, and to slightly increase protein content (Rulquin and Hurtaud, 1994). Optimizing the amino acid composition of protein supplements can constitute a means of increasing the protein content of milk. With diets based on maize silage alone, or mixed with grass silage, using a mixture of soya and rapeseed meal, induces higher milk protein production than with peanut meal, maize gluten or cotton meal. It's due to the supply of Lys and Met, most limiting amino-acids with maize silage (Rulquin and Hurtaud, 1994).

Milk fat content

Compared to maize silage diets, conserved grass as hay or grass silage, or pasture induced a decrease in milk fat content. Milk fatty acid composition is also modified. The main fatty acids in milk fat from cows on pasture are palmitic acid (23-28%) and mono-unsaturated fatty acids (23-32%), the main one being oleic acid. Diets containing more than 60% of maize silage result in 30-34% palmitic acid. Fat supplementation of dairy rations generally decreased milk fat content except encapsulated or protected fat (Chilliard et al, 2001).

Impact of dairy cow feeding on the quality of milk and on the sensory properties of butter and cheese

The specific effects of dairy cow feeding on the sensory characteristics of the dairy products have been studied following the requests of the AOC cheese producers who want to have objective references on the effect of dairy cow feeding. The trials consisted in comparing the characteristics of products resulting from animals receiving different feeding rations. They tested the effect of maize silage compared to grass and for grass, various types of conservation and various floristic compositions.

Effects of type of feeding and conservation of grass

Various work comparing dairy products obtained from milk of cows fed with maize silage or grass (grazed or conserved as hay or as silage), shows that the maize silage leads to whiter, firmer and generally less appreciated butters or cheeses because of their less developed flavour (Carpino et al., 2002; Coulon and Priolo, 2002; Houssin et al., 2002; Hurtaud and al, 2002a, 2002b). The differences seem to be reduced when grass is used in the form of hay.

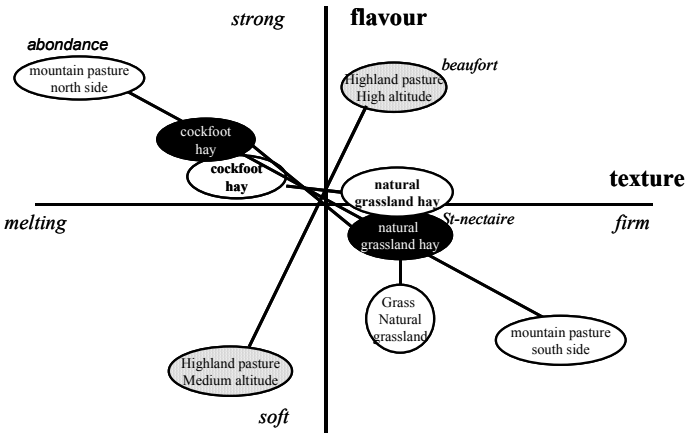
For the feeding only based on grass, the conservation of grass can have a significant impact on the characteristics of cheeses and butters; the cheeses and the butters obtained with the pasture are yellower, less firm and the cheeses have a more developed flavour than those obtained with feeding containing hay and grass silage (Coulon and Priolo, 2002, Verdier-Metz et al., 2002). For cheeses, the differences in flavour seem to be reduced when milk is pasteurized (Martin et al., 2004).

The conservation of grass as silage is for a long time a subject of debate within AOC cheeses producers. Some specific defects can be observed with badly preserved silages, in particular in manufacture of cooked pressed paste where the presence of butyric spores in the silage and milk is responsible for late swellings and bad tastes and odours. On the other hand, when the silages are good, the mode of conservation (hay vs ensilage), has only a limited effect, apart from the colour of the paste (yellower with the silage) on the sensory characteristics of cheeses. These experimental results have been confirmed by observations in farm cheese producers but it is however possible that these effects are variable according to the type of cheese. Indeed, according to Martin et al. (2003), grass silage compared to hay induces more significant sensory differences on Cantal cheeses than on Saint Nectaire cheeses.

Effect of the botanical nature of forage

The effects of the botanical nature of the meadows, in particular of the permanent meadows of mountain have been studied on various cheeses with pressed paste or cooked and pressed paste. In all the trials concerning cheeses, the differences of texture and flavour could be shown when the animals received grass with different floristic composition, whether the grass is grazed or preserved in the form of hay. The cheeses coming from altitude grass and/or more diversified meadows had a more various and more intense flavour (Figure 1).

Figure 1 : Sensory characteristics differentiation of cheese according to grass botanical composition (Coulon et al, 2004)



Mechanisms explaining the sensory differences between dairy products

Some cheese sensory characteristics are due to certain components directly derived from forages. Cheese colour is dependent of carotene content of forages. Carotene is destroyed during forage drying and conservation. Cheeses made with spring grass are much yellower than cheeses made with maize silage, containing very little carotene. Terpenes have also recognized aromatic properties. But even if their concentration increases in cheeses with specific plants, its appears that changes in their concentration in cheese is not sufficient to have a direct effect on sensory properties (Coulon et al, 2004).

Some of the effects of the feeding factors on cheese properties are due to modifications in the milk protein and fat composition. Milk fat composition, closely dependent on animal feeding is at the origin of differences in texture and flavour of butter and cheese. The C16:0+C18:0 /C18:1 ratio, an indicator of butter spreadability decreased with conserved grass compared to maize silage (Hurtaud et al, 2004) and with pasture (Hurtaud et al, 2002a), inducing less firm cheeses and butter. The native milk fat globules affect physico-chemical properties and sensory properties of Camembert cheeses such as meltability, elasticity and colour of the curd (Michalski et al, 2003). Reduced disruption of the protein matrix, probably accounts for the observation that a higher percentage of small native fat globules are transferred to cheese than larger globules (Hill, 1995). Plasmin, implicated in cheese proteolysis, is involved in the texture and flavour (Bugaud et al, 2002). Differences in the rate of proteolysis, due to different feeding systems can be responsible for different cheese flavour as ammoniac flavour (Hurtaud et al, 2004). The intake of certain plant species, as buttercup, could also induce an increased cellular permeability of mammary gland followed by an increase of plasmin in milk. Lastly, as some of the differences induced by feeding are only observed with raw milk, (Martin et al., 2004), it can't be ruled out that the type of forage influence the microbial ecosystem of milk or its activity. It has also been recently suggested that terpenes, whose plant-specific origin has been documented, may have an indirect impact on cheese sensory properties by modifying the dynamics of the microbial ecosystem activity during cheese making and ripening (Coulon et al., 2004).

Conclusion

Dairy cow feeding has a relatively great impact on cheese and butter. But the effects may vary according to the type of cheese. Some studies have to be conducted to compare the effects of feeding treatments of dairy cows on different types of cheeses.

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Quality in food chains - Dairy

Posters

COMPARATIVE STUDY OF ELECTRICAL CONDUCTIVITY AND CMT OF QUARTER MILK SAMPLES IN DAIRY FARM IN MEXICO

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Introduction

Mastitis is an important problem for the dairy industry. Clinical mastitis can be detected by the farmer. Subclinical mastitis can only be detected by measurement of inflammatory components and pathogens in the milk.

The diagnostic methods for subclinical mastitis preferably are inexpensive, easy to use, and accurate.

In milk production is very important the detection subclinical mastitis caused by pathogens most commonly associated with mastitis in dairy cows.

Wide range farmers used CMT, but electrical conductivity method to detect sublclinical mastitis, few framers they used it.

Material and Methods

The sample of milk was squirted directly from the teat into the opening of the Mas-D-Tec®. Electrical conductivity based on 25 degrees C was measured in milk from each quarter the operator presses a button on the front of the instrument. The result appears instantaneously and the entire measuring procedure can be done in less than five seconds per quarter. The instrument provides a reading ranging from 0 to 9. Readings between 0 and 4 are acceptable and indicate low electrolyte content in the milk and a healthy quarter. Any reading of 5 or higher was interpreted as a positive indication of subclinical mastitis in that particular quarter.

Measurements of the conductivity of quarter milk samples were made in 89 cows in an 89-cow herd in northeast Mexico City, for a period of 15 weeks.

Eigth quarters were nonfunctional resulting in a total of 348 used in the analyses. We tested one time a week we used CMT and Mas-D-Tec ® (MDT) immediately prior to milking, milk samples from each quarter were tested, using a hand-held EC meter and CMT.

We have required two or more positive milk samples to define infection mastitis.

Results

The data were analyzed and compared statistical analysis with the T student, since with we will know it the significant among tests. The statistical analysis by means of the T student throw results ($P > 0.01$) in all the compared quarters meaning highly significant differences among the tests.

Discussion

According to CMT 33.7% of the cows in the herd was infected and 59.5% was in the test of conductivity. Tests were standardized with Somatic Cell Count SCC. Also we estimate the milk production and economics losses in the farm for mastitis. Biggadike, (1) report the positive predictive value of the individual quarter milk conductivity is insufficiently accurate to be used as the sole criterion for the selection of quarters for early antibiotic treatment.

Conclusion

Related to CMT and MDT. Electrical conductivity can be utilized as indirect tests of subclinical mastitis with better results than CMT.

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Quality in food chains - Poultry and Fish

Oral Communications

CAMPYLOBACTER IN POULTRY; ONGOING ACTIVITITES WITHIN EU AND EXPERIENCES FROM THE NORWEGIAN ACTION PLAN

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Introduction

Campylobacteriosis is an important public health problem in most areas of the world. According to the EU reporting of zoonoses, the incidence of campylobacteriosis is significant throughout EU and there has been a general trend towards an increase in the incidence over the last decade. In several Member States, the incidence of campylobacteriosis has surpassed that of salmonellosis in recent years and has become the most commonly reported bacterial gastrointestinal disease (1).

The epidemiology of thermophilic *Campylobacter*, the etiological agents of campylobacteriosis, is complex. The principal reservoirs are the alimentary tracts of wild and domesticated mammals and birds. *C. jejuni* is predominantly associated with poultry, but can also be isolated from cattle, sheep, goats, pigs, dogs and cats, while *C. coli* is predominantly found in pigs, but can also be isolated from poultry, cattle and sheep (1,2,3). Typically, about 90% of the isolates from human campylobacteriosis cases are identified as *C. jejuni*, while *C. coli* accounts for most of the remaining cases. *Campylobacter* may be spread to humans by direct contact with contaminated animals or animal carcasses, or indirectly through the ingestion of contaminated food or water. Campylobacteriosis in industrialised countries is primarily a foodborne disease, with poultry as a principal source (4,5). Several case-control studies have identified handling and consumption of poultry products as important risk factors for human campylobacteriosis (5,6). However, the contribution of the various food and non-food sources to the incidence of campylobacteriosis within EU will be country and time dependent due to various factors such as climate, consumption patterns, drinking water distribution, food production systems, degree of implementation of control measures, etc.

Poultry as source of *Campylobacter*

Campylobacter spp. are commonly isolated from poultry and poultry products. Prevalences vary, however, as a function of country, time period of sampling, type of birds (broilers, other type of poultry, free range, organic) and products (fresh, frozen, heat treated), methodology of detection (direct plating *vs.* enrichment), and type of sample (caecal contents, faeces, litter) (1). In many countries a seasonality of the prevalences is common, with a peak at the end of summer and the early autumn (1). When a flock is infected, contamination will within a short time involve most if not all birds.

Transmission of *Campylobacter* to the birds from environmental sources (poultry house surroundings), including drinking water, is the most likely route of infection. Split slaughter (thinning) is a risk factor for introducing *Campylobacter* to a flock. The most efficient measures for controlling *Campylobacter* contamination of broilers at farm-level concern biosecurity measures and farm practices aimed at preventing the introduction of *Campylobacter* in the flocks (7). Cross-contamination from positive to negative flocks at slaughter and processing is a common problem. The application of good hygienic measures and introduction of techniques to reduce fecal spread are important preventive measures. Consumer exposure to *Campylobacter* is inevitable if poultry meat is not handled hygienically, i.e. if cross-contamination from raw poultry meat to ready-to-eat food products is allowed to happen, and/or if the meat is not properly cooked before consumption. Studies show that unhygienic food handling procedures are commonly practiced. Thus, proper and targeted risk communication is critical in the prevention of campylobacteriosis.

EU activities in regard to *Campylobacter*

On November 17, 2003 the European Commission adopted a new Regulation on the control of *Salmonella* and other specified food-borne zoonoses (No. 2160/2003) as well as a new Directive on the monitoring of zoonoses and zoonotic agents (2003/99/EC). The central idea of the Regulation is the setting of pathogen reduction targets along the food chain, mainly for animal populations, and the establishment of national control programmes in order to meet these targets. In this context, targets for *Campylobacter* could be established. The Directive contains a possibility to harmonise the monitoring of certain zoonotic agents, when it is necessary to make the data collected easier to compile and compare. This option may be used to gather more detailed information on *Campylobacter* spp. in order to fill data gaps. The Commission has also started a revision of the microbiological criteria in Community legislation. Criteria would be set for food products at different stages of the manufacturing process as well as for products on the market. During the discussions with the Member States and the consultation of the stakeholders, wishes to set criteria for *Campylobacter* spp. in raw milk, poultry carcasses as well as live bivalve molluscs have been expressed.

In late 2003, the Commission asked the European Food Safety Authority (EFSA) to deliver a scientific opinion on *Campylobacter* spp. in animals and foodstuffs, and in particular:

- identify categories of foodstuffs where *Campylobacter* spp. represents a significant risk to public health;
- identify possible control options to reduce this risk along the food chain, and evaluate their effectiveness, with special reference to the measures taken at the primary production and the setting of microbiological criteria;
- identify the gaps in available data together with the best means of collecting this information.

This work is still going on, and an opinion is expected to be adopted before the end of 2004.

Experiences from the Norwegian action plan against *Campylobacter* in poultry

Campylobacteriosis is the most commonly reported bacterial gastroenteritis in humans in Norway. The incidence increased substantially during the 1990s and peaked in 2001 with a total of 64 reported cases per 100.000 inhabitants. For close to half of the cases, the infection is acquired in Norway (8). Consumption of poultry meat purchased raw has been identified as a significant risk factor together with the drinking of undisinfected water, eating at barbecues, occupational exposure to animals, and eating undercooked pork (6). The action plan against *Campylobacter* spp. in Norwegian broilers has the objective to reduce human exposure to *Campylobacter* spp. through Norwegian broiler meat products. The action plan regarding *Campylobacter* in Norwegian broilers was implemented in the spring of 2001 (9). The objective is to reduce the human exposure to *Campylobacter* through Norwegian broiler meat products. The action plan is a joint effort involving several stakeholder groups from "stable-to-table". The Norwegian Zoonosis Centre developed the action plan in cooperation with the Norwegian Food Safety Authority, the National Veterinary Institute, the Norwegian Institute of Public Health, the Norwegian School of Veterinary Science, the Centre for Poultry Science, and the poultry industry. The Norwegian Zoonosis Centre coordinates the programme, and is responsible for collection and analyses of data and dissemination of results.

The action plan consists of three parts; a surveillance program including all Norwegian broiler flocks slaughtered before 50 days of age, a survey of broiler meat products, and a follow-up advisory service on farms with flocks positive for *Campylobacter* spp. In the surveillance, pre-slaughter sampling of a flock is performed eight to four days before slaughter by the owner and consists of ten swabs from fresh faecal droppings. Positive flocks are slaughtered at the end of the day, and the carcasses from these flocks are either heat treated or frozen for a minimum of five weeks before being marketed. All flocks are tested again upon arrival at the slaughter plant by sampling of ten cloacal swabs per flock at the slaughter line. Broiler farms that deliver *Campylobacter* positive flocks are subject to a follow-up visit by advisors from the poultry industry or the district veterinary officer. The visit should result in interventions on the farm aimed at reducing the risk of flocks being contaminated with *Campylobacter* in the future. In the product survey approximately 100 samples from retail are analyzed each month.

In the three years period from May 2001 through April 2004, a total of 10396 flocks from 569 farms were examined, of which 578 (5.6%) flocks were positive, approximately 90 % of these with *C. jejuni*, the remaining with *C. coli* or *C. lari*. There is a pronounced seasonal variation in the proportion of positive flocks, with a distinct peak in late summer. Of the positive flocks, 47.4% were identified at the pre-slaughter sampling and thereby subject to sanitary slaughter and freezing/heat treatment of carcasses, while the remaining flocks were identified at slaughter only. The positive flocks originated from 270 (47. %) of the farms. A total of 142 (52.6%) of the positive farms had only one positive incidence during these three years, whereas 66 farms (24.4 % of the positive farms and 11.6% of all the farms) had three or more positive incidences accounting for 51% of the positive flocks. Of these 66 farms, 28 had positive flocks all the three years, and these 28 farms accounted for 26% of the positive flocks. Thus, a considerable proportion of the positive flocks origins from the same few farms. A substantial decrease in flock prevalence from the second to the third year was observed, from 6.5% to 4.8 % positive flocks. The product surveys from May 2002 to April 2003 detected 8.8% positive samples of 1080 tested, while from May 2003 to April 2004 only 4.2% out of 1106 samples tested were positive. The seasonal variation in prevalence of positive products corresponded well with the prevalences of positive flocks (9).

The Norwegian Action Plan against *Campylobacter* spp. in broilers has been a successful cooperation between the various stakeholders. During the first three years, approximately 3.5 million positive carcasses have been prevented from entering the market fresh and thus significantly reduced human exposure to *Campylobacter* spp. through Norwegian broiler meat products. The significant reduction in the proportion of positive flocks from the second to the third year can probably partly be attributed to general improvements of hygienic practices in the Norwegian poultry industry stemming from the action plan. There are indications of a positive public health effect, but due to the complex epidemiology of *Campylobacter* and campylobacteriosis, it is difficult to assess this effect.

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Merete Hofshagen, Norwegian Zoonosis Centre

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OFFICIAL SYSTEMS OF QUALITY IN FRANCE

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Introduction

The official systems of quality have been created to promote our food products and agriculture and to develop products of higher quality. In France, this quality is related to the origin, the good taste, the landscape respect. Moreover, when a food crisis occurs, confidence must be reinforced and consumers are searching for products, the safety of which is guaranteed by official controls, although these products are more expensive.

Material and methods

1. Official systems of quality

These official systems of quality are divided in official signs for products and a global system including all uses in farms. All these systems of quality are optional. Official signs also exist in the EEC, such as "appellation d'origine protégée" (AOP) and "indication géographique protégée" (IGP) or "attestation de spécificité" (AS). When a French operator wants to use AOP, IGP and AS he must also ask concomitantly for a "label rouge" (LR) and "certification de conformité de produits" (CCP).

1.1. Official signs

The aim of the law of the 9th July 1999 on the agriculture strategy is to safeguard quality and origin to consumers. The decree n°96-193 of the 12th March 1996, introduced four official signs of origin and quality for products, “appellation d’origine contrôlée” (AOC) which identifies a product that draws its authenticity and specificity from its geographic origin, “certification de conformité de produits” (CCP) with specifications of constant quality, “label rouge” (LR) which guarantees prime quality, “agriculture biologique” (AB) which is an organic farming production that uses cultural and farming practices that aim to respect natural balances. These signs are respectively dated from 1965 for LR, 1966 for AOC, 1980 for AB, 1990 for CCP. Since the beginning of the nineties, these signs are not strictly limited to wines for AOC and to fowl for label and even vegetables, beef, pork...or fishes are certified.

For CCP and LR, operators write their own specifications and present their system of reference to the referential section of the "Commission nationale des labels et des certifications de produits agricoles et alimentaires" (CNLC). This section may approve these systems of reference provided they are conformed to the standards of the section.

For organic farming, in the vegetal sector, operators must respect the European regulation n°2092-91 whereas, in the animal sector, operators must respect the French specifications "CC REPAB F". The organic farming section helps operators to interpret these systems of reference.

1.2. Global system

Now, citizens are also interested by other aims such as environmental problems, security for persons working in a farm, animal welfare or traceability of all treatment on animals or vegetables. The integrated farming is a concept newly regulated in France with the decree n°2002-631 of the 25th April 2002, which takes into account all these subjects. The reference for farmers is national, and all described in the rule of the 30th April 2002. It is divided in 98 requirements grouped in the following parts : knowledge of the farm and farming through vocational training, traceability, health and security of employees, ground management, fertilisation, sanitary products for plants, irrigation, animals identification, animal health, animal feed, animal welfare, hygiene, waste management, landscape and biological diversity respect. Some of these items are limited to the farms producing plants and others to the farms producing animals or their products.

The referential section of the "Commission nationale de l'agriculture raisonnée" (CNAR) gives farmers some advice for implementing the 98 items.

2. Official controls

Whatever is the official system considered, products certification for quality or origin signs or the farm qualification for integrated farming, the ministry of agriculture and the ministry of consumption are in charge of a two-steps control.

The control bodies guarantee the first step of the official control by regularly inspecting all operators in the production chain.

On the second step these control bodies must be accredited by the COFRAC (Comité français d'accréditation) on the basis of the NF EN 45011 standard in order to check criteria such as independence, impartiality and competence.

Moreover, control bodies must present their inspection plan and penalty table to the agreement section of the CNLC for quality signs and the agreement section of the CNAR for integrated farming.

These consultative agreement sections give then their opinion on the agreement of the control bodies. On the agreement section's (of the CNLC) advice, the French ministries in charge of agriculture and consumption deliver an agreement to the control body for the certification of food products (for quality or origin signs). If the agreement section of the CNAR is favourable to the agreement the French ministries in charge of agriculture and consumption may deliver the agreement to the control body for the qualification of farms (for integrated farming).

AOC products are linked to the ground, climate and people's abilities and these AOC are delivered by the Institut national des appellations d'origine" (INAO).

Results

1. Number of control bodies

In France among 23 control bodies : 15 are implicated in LR and CCP for fowl and eggs whereas only 5 of them control LR and CCP for fishes , molluscs or crustaceans.

Five control bodies have an agreement for organic farming control.

At this time, 13 control bodies have already sent their inspection plan and penalty table to the agreement section

of the CNAR. The first agreements for integrated farming have been promulgated the 28th of march 2004 on the *Journal Officiel de la République française.*

2. Production
In 2001, the turnover for AOC is the highest of all quality signs (see table 1, references 1 and 2).

Table 1 : Number of farms concerned and turnover in 2001
(1) number of companies
(2) in milliard €

Signs	AOC	AB	CCP	LR
N° of farms	113000	10364	218(1)	39816
N° of products	About 550	/	About 300	About 450
Turnover	17(2)	6	2.7	1.7

In 2001, CCP mainly concern beef and pork with 37% of the CCP whereas fowl represents 26% and sea products 2%.
Concerning label rouge, avian products count 54% of the red labels and 45% of the total turnover for red labels whereas sea products reach only 1,8% of this turnover. Thus, the main sector of LR remains the avian sector. The LR represents 10,9 of the slaughtered fowl and 17% of all the chickens slaughtered in France.
Concerning AB production fowl and eggs production represent respectively 8.8% and 10.4% of the organic farms.

Discussion
1. Evolution of the systems
The role of the agreement sections is to harmonize the inspection plans and penalty table of all control bodies so as all the operators must have the same chance to have certified products and / or qualified farms. In France, French authorities want to keep this two-steps control which gives the same chance to everybody. Thus, impartiality is guaranteed by a large representation in each section of producers, members of food industry, consumers, research institutes, control bodies, associations of employees, official authorities...
The referential sections take into account the technical improvements in order to review the national systems of reference or instructions.
2. Relation between private brands and official quality systems
Official signs have been created to propose different products to the consumers. When consumers prefer a quality product they are able to pay more for them than for conventional products. But through the law of the 9th July 1999, official authorities tend to limit the official signs so as consumers must have a clear message on higher quality products. Now private brands such as "produit de l'année" and "saveur de l'année" are used and the role of the French authorities is to keep the message as clear as possible. Concerning these private brands they are not checked by agreed control bodies. Moreover these private brands are not informing about an higher approved quality as they are only tested by a small group of consumers.
3. Relation between the official systems of quality
Official signs and integrated farming may exist in the same farm as products concerned by signs may be produced in one farm respecting the global system of integrated farming. Otherwise different labels informing consumers are used for official signs, whereas the mention "product issued from a qualified farm" is used for integrated farming.
Organic farming and integrated farming are quite different; organic farming is just looking for cultural practices respecting environment whereas integrated farming includes various items such as security, hygiene, animals welfare and health and so on, as previously described. Moreover, the use of artificial products is forbidden by the organic farming and permitted but at a reasonable dose for integrated farming.

Conclusion
These systems give official guarantees to the French consumers and citizens, as far as control bodies check operators are respecting their specifications for origin, quality signs or integrated farming. Moreover, all labels or mentions are inspected by the Fraud Squad and all sanitary measures are controlled by the Veterinary Services such as for all conventional products or farms.

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EPIDEMIOLOGY AND BEHAVIOUR OF *LISTERIA MONOCYTOGENES* STRAINS ALONG THE FISH CHAIN PRODUCTION

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Introduction

The microbial quality is one of the most important aspect of the quality of food from animal origin. The lack of applying this concept can result in a rapid deterioration or in the presence of potentially human pathogens at a high level in the product. In this field, *Listeria monocytogenes* is one of the major problem due to the severity of human listeriosis. Actually listeriosis is a rare but very serious foodborne disease affecting preferentially pregnant women, new-born infants and immunocompromised patients (Rocourt *et al.,* 2000). Nevertheless the relationship between *Listeria monocytogenes* and fishes and fish products still remains enigmatic. In fact, *Listeria monocytogenes* occurs widely in different environments including fish production farms and slaughter plants and consequently can contaminate the processing line and finished products (Johansson *et al.,* 1999). But in an other hand, in recent years, fishes and fish products were not really associated with human listeriosis cases. Only 2 cases in relation with vacuum-packed gravad and cold-smoked rainbow trout preparations were described (Ericsson *et al.,* 1997, Miettinen *et al.,* 1999 and Tham *et al.,* 2000). During the production, fishes are submitted to different environmental conditions, from a rearing period in an opened place in the water, to a slaughter then a processing plants where products could be salted and smoked. All these steps can promote, or not, the presence of bacteria and specially of *Listeria monocytogenes.*

In this context it seemed interesting to follow the epidemiology and the behaviour of strains isolated in different steps.

Material and Methods

Two fish farms (A and B) and one processing plant (C) situated along a river were investigated for the presence of *Listeria monocytogenes*. In total, 7 series of sampling (swabbing equipment, water from upper and down river, fishes…) were realised as follow : one on site A and 3 on sites B and C. All samples (102) were analysed for *Listeria monocytogenes* detection using an enrichment (Fraser broth) and isolation (ALOA medium) procedures, then typical colonies were identified using Microgen-Listeria-ID test. *Listeria monocytogenes* strains were characterised by Pulsed Field Gel Electrophoresis (PFGE) technique using *Apa*1 and *Asc*1 enzymes.

Results and Discussion

Listeria monocytogenes was present in different sites (table 1), not only on the surface of fishes but also on the equipment and in the water sampled in the river and in farms. The environment of premises and notably where conditions are in favour of creating biofilms can justify this presence of *Listeria monocytogenes.*

During the first series of sampling in the slaughtering plant, it can be noted a very high level of contamination, specially on the equipment (conveyor belt, boxes….). Nevertheless the occurrence was lower during the second and the third series, due to the improvement of the cleaning and disinfecting procedures and of the hygienic conditions applied during slaughtering.

Combination of *Apa*1 and *Asc*1 results allows classifying isolates into PFGE types. Seven types were identified among 44 strains tested (table 2). Two types are predominant : the first one (type 15) corresponding to the serotype 4b, was present in the 2 farms, not only on the surface and in the intestines of fishes, but also in the environment and in the water. The second one (type 18) was present only in the processing plant. These results confirm that strains present during the primary production do not get over the other during the processing step (Dauphin *et al.,* 2001). The presence of the same genotype 18 in the processing plant after several weeks and whether the improvement of hygienic procedures confirms the ability of different resident strains to survive and colonize surfaces of equipment.

It was interesting to compare the behaviour of these strains, and others isolated in different fish productions environment (salmon and trout), specially with different concentration of salt (NaCl) in the medium. These results confirm that the "resident" strain (type 18) seems to be very well adapted to a high concentration of salt (10 %) and that the growth of strain isolated from a marine environment is not very strong in the absence of salt. In general the different phases of microbial growth (lag. time and growth rate) were affected by the concentration of salt added in the medium. Interestingly it can be noted that there is no relationship between the behaviour of these strains and the genotype obtained by PFGE.

Conclusion

The characterisation of *Listeria monocytogenes* strains isolated in different places along a river, confirms the diversity of these bacteria in the fish farms and the ability of one type to colonise the equipment of the processing plant, despite the improvement of hygienic conditions. Therefore the study of these strains in contact with different concentrations of salt shows difference during the growth phase and no relationship between the genotype and this behaviour.

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Table 1 : Results of *L. monocytogenes* from different sites and samples.

Sites	A		B			C	
Series	1	2	3	4	5	6	7
Fishes*	8/17	1/4	1/4	0/4	2/7	2/3	0/10
	(4/4)	(1/1)	(1/1)	(0/1)	(1/2)	(1/1)	(0/3)
Environment	5/9	2/2	1/1	1/2	9/12	1/15	2/11
Water	1/4	1/2	0/1	1/4	-	-	-
Total	14/30	4/8	2/6	2/10	11/19	3/18	2/21

* ( ) : Number of positive fishes out of number of analysed samples

Table 2 : Number of isolates of *L. monocytogenes* according to PFGE types.

PFGE Types	N° of isolates
11	1
15	10
17	5
18	22
19	1
30	2
32	3

Table 3 : PFGE types of *L. monocytogenes* isolated from different sites.

Sites	A		B			C	
Series	1	2	3	4	5	6	7
Fishes	11-15-30-32	15	15-19	-	18	18	-
Environment	15-17-30-32	15-17	17	15	18	18	18
Water	30	15	-	15	-	-	-

SALMONELLA CONTROL IN BROILER FLOCKS

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Introduction

In the Dutch broiler industry Salmonella paratyphi B biovar. Java (S. java) is the predominant serovar. From all poultry meat Salmonella isolates, S. java increased from approx 17% in 1999 to over 50% in 2002 (van Pelt et. al., 2003). Although the Dutch Salmonella intervention program succeeded in a decrease of Salmonella incidence in broiler meat from 35% in 1998 to <10% in 2003, the emerging S. java prevents a further decrease.
In a research project on a number of Dutch broiler farms a guided intervention program was carried out in order to control and eradicate S. java.
Although S. java is important as a zoonosis agent, its developing antibiotic resistance pattern is of concern. The resistance against flumequine increased from 19% in 2001 to 39% in 2002 (van Pelt et. al, 2003).
The origin of S. java is not known, but feed ingredients or broiler parents in 1997-1998 may be involved. S. java was neither isolated from a Dutch parent flock, nor from compound feed recently.

Materials and Methods

S. java appears to be very persistent in broiler houses, so the project started with a risk analysis and an inventory of critical points after cleaning and disinfection at a broiler farm.

- Farm hygiene (Good Farming Practice) of all farms was of high standard
- The cleaning and disinfection process was carried out very thoroughly and checked by swab sampling
- The buildings, floors and equipment was closely monitored and repaired, when necessary
- The operation during broiler raising was intensively monitored,
- Extra sampling during every round was carried out, in order to know when S. java was introduced in the house.
- The internal part of the feeder system in the broiler house was disinfected
- Every farm increased the eradication of insects and rodents.
- Water and feed were treated with organic acids (short chain or medium chain)
- Antibiotic resistance pattern was estimated from every S. java isolate
- Etc.

This inventory suggested that not a general strategy could be followed for every broiler farm. On basis of this risk analysis a specified intervention was designed for every single farm. The eradication of S. java by application one single intervention proved to be not successful. When a number of different interventions were applied they acted as a hurdle system and stimulated one another.

The hygienic standard on farms was very high, as was illustrated by most farms where the Salmonella infection was not present in all broiler houses but limited to a small number. On these farms often the infection occurred in the same broiler houses in successive rounds.

Results and Discussion

From weekly monitoring on the farms, it appeared that at most of the farms broilers were S. java positive during the first week of life. In some farms S. java could be isolated at three or four week of age. Vertical transmission could be excluded in these flocks by intensively monitoring hatchery debris and paper inlays of transportation crates of one-day-old chicks. Feed samples taken from silos proved to be Salmonella negative. At a small number of farms a lot of *Alphitobius diaperinus (A.d.)* beetles and larvae (lesser mealworm or black beetle) were observed from week 2 or 3 onwards, and appeared to be Salmonella positive. Since the broilers were already Salmonella positive at that time, A.d. was not held responsible for the present infection.
Salmonella could be isolated from feed of the feed pans, but this infection was obviously taken from the direct environment such as faeces.

The results of this monitoring led to the suggestion that the infection was present in the broiler house at the time that the broilers arrived. Swab sampling of the broiler house after cleaning and disinfection showed in some cases residual Salmonella especially in cracks and joints, sewer systems but also in the feeder system and roof ventilators that could not be dismantled during cleaning. Moreover, from joints between floor and walls after cleaning and disinfection, beetles and larvae entered the broiler house. They appeared occasionally to be S. java positive.
Long periods without animals in a broiler house without additional cleaning, disinfection or reparations does not eliminate the infections, as was observed in The Netherlands after the Avian Influenza crisis.

In the broiler farms where S. java infection at week one was observed, the feeder system was disinfected internally with organic acids and cracks and joints filled with flexible pasta. Ventilators were dismantled when necessary.
The following round either S. java had disappeared from the farm or from a number of houses on an infected farm. During the next rounds interventions were increased when necessary, i.e. in case S. java was not eliminated on the particular farm.

These additional interventions were chosen from the following list.

- Sewer systems in broiler houses were additionally disinfected and closed
- Air inlet valves were extra cleaned externally

- Application of Competitive Exclusion flora at day one.
- Use of one-day-old chicks of vaccinated parent flocks, so maternal immunity would offer additional protection.
- Medication on basis of resistance patterns of S. java.
- Application of detergents for cleaning and different disinfectants (preferably formaldehyde), although no resistance against these agents was found.

In total approx. 25 farms were monitored intensively and applied a specific intervention program. About 70% of those farms are S. java free now for about one year or even longer. On a small number of farms interventions were applied right after the first infected flock was slaughtered. In these farms the successive flock was Salmonella negative again. In one occasion not S. java was isolated, but S. infantis appeared to be persistent on that farm. After application of a similar intervention program this farm produces Salmonella negative broilers again. Unfortunately not all farms succeed, but in many cases the problem is that technical state of the houses is such that thorough cleaning and disinfection is impossible with traditional systems. In these farms, application of steam disinfection could be a solution, although this is very expensive and may cause damage to the plastic equipment of the broiler house.
This type of farm often suffer from insect problems, which can contribute in continuation of the infection. Additional eradication programs are required here in order to destroy the insects that enter the broiler house.

Conclusions

The eradication of Salmonella on frequently infected broiler farms is not very easy and requires a complicated approach, thorough coaching and frequent sampling. There is no generally applicable package of interventions and every farm should be considered individually so an adequate package can be designed.
Salmonella could be isolated most frequently from the feeder system, from cracks and joints, insects that stay in the house and ventilators. In many farms chicks were infected with Salmonella during the first week of life and I these farms feeder system often was Salmonella positive.
Eradication of Salmonella from frequently infected broiler farms is often a matter of applying an increasing number of interventions, which in the end may be successful.
Farms that are free of Salmonella have to apply an elevated hygiene standard for a number of successive rounds, and probably need a number of additionally applied interventions such as acidification of drinking water during the first two weeks.

Acknowledgements

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PREVALENCE OF *SALMONELLA* IN EGGS AND OTHER SAMPLES FROM LAYING FLOCKS VACCINATED FOR *SALMONELLA* ENTERITIDIS

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Introduction

The global spread of *Salmonella enterica* serovar Enteritidis (*S.*Enteritidis) in chickens (Rabsch et al, 2001) has resulted in an international food poisoning pandemic. The special facility of *S.*Enteritidis to cause prolonged infection of the avian reproductive tract has been a major factor in vertical transmission of the organism from breeding flocks and internal contamination of eggs is thought to have been the major factor in its spread (Guard-Petter, 2001). *Salmonella* Enteritidis localises in glandular parts of the reproductive tract, such as the magnum and isthmus and ovarian granulosa cells (De Buck et al, 2004). Chickens infected at day old can remain as life-long carriers, with infection increasing during stress, especially the onset of lay. Vaccination is an important aid. This paper describes the results of testing for *Salmonella* eggs, spent hens and poultry house faecal and environmental samples from flocks which were previously infected with *Salmonella* Enteritidis despite vaccination with a tested bacterin.

Material and Methods

Laying farms where *S.*Enteritidis was present were identified from statutory notifications or traceback investigations. A commercial killed *S.*Enteritidis vaccine ('Salenvac', Intervet) was used or introduced on all the flocks, administered by intramuscular injection given as two separate 0.5 ml doses at approximately 4 and 16 weeks of age during the rearing period. Faecal and environmental samples from farms were taken directly into 225ml of buffered peptone water (BPW) using gauze surgical swabs and consisted of approximately 25 g faecal material or 10 to 15 g dust or other dry environmental samples or surface swabs.

Eggs were returned to the laboratory under ambient conditions and stored overnight at room temperature. Chickens at the end of their productive life ('Spent Hens') were humanely killed, returned to the laboratory and stored at 4^{o}C for up to 16 hours before aseptic post mortem and culture of separate 25 g pools of caeca and liver/spleen/ovary/oviduct. Tissue samples were thinly sliced with sterile scissors before culture.

Eggs were cultured in batches of six. Eggs were carefully cracked and contents released into 500 ml BPW supplemented with 7 g/litre beef heart infusion and this was incubated for 48 hours at 37^{o}C. Shells were placed in 225 ml BPW. Samples in BPW were incubated at 37^{o}C for 18 hours. This was followed by selective enrichment in Diasalm (41.5^{o}C/24 & 48 hr) and plated in Rambach Agar (37^{o}C, 24 hr). Suspect isolates were serotyped.

Results

The table shows the results of culture of samples from all the 12 houses. 24 batches of 6 eggs shells from the 13,652 tested (0.18% [0.11 - 0.26 CI ^{95}] single egg equivalent) were positive for *S.*Enteritidis and 54 (0.40% [0.30-0.52 CI ^{95}] single egg equivalent) for other serovars. Six batches of 13,640 egg contents (0.04% [0.02-0.10 CI ^{95}] single egg equivalent) contained *S.*Enteritidis and three batches contained other serovars. In addition (data not shown) three further batches contained *S.*Enteritidis in both contents and shells and two other batches contained other serovars in both. The total level of contamination by *S.*Enteritidis of both contents and shells found in vaccinated flocks was therefore 33 batches/13,682 eggs (0.24% [0.17-0.34 CI ^{95}] single egg equivalent). The total of contamination for any *Salmonella* serovar was 92 batches/13,682 eggs (0.67% [0.55-0.84 CI ^{95}] single egg equivalent). These results contrast with the findings of testing of eggs from three unvaccinated flocks prior to this study (data not shown) where 21 batches of egg shells from a total of 2,101 eggs (1.0% [0.63-1.56 CI ^{95}] single egg equivalent) and six batches of contents from 2,051 eggs (0.29% [0.11-0.64 CI ^{95}] single egg equivalent) were contaminated with *S.*Enteritidis. *S.*Enteritidis was found in 67/699 (9.6%) of vaccinated spent hens and 64/562 (11.4%) of bulked fresh faecal samples taken from laying houses. Serotypes other than *S.*Enteritidis were found in 0.4% of spent hens and 14.4% of bulked faeces samples, but the presence of *Salmonella* in the flocks was most readily detected by testing environmental samples such as spillage from egg belts, beneath cage stacks and dust, of which 25.6% and 19.0% of 961 samples contained *S.*Enteritidis or other serovars respectively.

In a separate investigation of 13 cage layer flocks before and after introduction of vaccination, the incidence of *Salmonella* Enteritidis in bulked faecal samples was reduced from 70/173 (40.5%) in non vaccinated flocks to 32/539 (5.9%) in vaccinated flocks (p=<0.001). Results of *Salmonella* isolation from range of environmental samples, such as egg belt spillages, dust and spillages under cages, were also lower [228/1410 (16.1%)] in vaccinated than in non-vaccinated flocks [196/664 (29.5%)], but this difference was not significant (p=0.110). 49/634 (7.7%) of spent hens from vaccinated flocks harboured *S.*Enteritidis and this was particularly pronounced in one flock where 29/75 (38.7%) of birds were carrying *S.*Enteritidis at post-mortem.

In six barn egg flocks *S.*Enteritidis was found in 16/42 (38.1%) bulked faeces samples and 89/223 (39.9%) of other environmental samples compared with 16/226 (7.1%) (p=0.062) and 23/362 (6.3%) (p=<0.05) of 11 vaccinated flocks.

In four non vaccinated free-range flocks *S.*Enteritidis was found in 97/152 (63.8%) of faeces or litter samples and 199/427 (46.6%) of other environmental samples, including soil and pooled water in paddocks. The mean for the vaccinated flocks was 2/302 (0.7%) (p=<0.001) for bulked faeces and 0/402 (p=<0.001) for other

samples. Only 2 of 17 previously infected flocks showed evidence of infection after vaccination and this was on a farm where infected unvaccinated cage birds were housed adjacent to the free-range flocks. Both of these flocks became *Salmonella* negative subsequently.

Discussion

The results of this study suggests that vaccination has a beneficial effect on egg contamination but that there is still some contamination risk associated with the presence of *S.*Enteritidis in infected vaccinated flocks. Vaccination also significantly reduced the prevalence of post mortem tissue, faecal and environmental samples containing *S.*Enteritidis and was associated with elimination of infection on free-range farms, and the majority of barn units.

Although vaccination of laying flocks for *S.*Enteritidis is to be recommended for all situations where risk cannot be fully controlled by other means the current study has confirmed that flock infection and production of contaminated eggs may still occur, albeit at a lower frequency than would be expected in unvaccinated flocks. Currently the majority of the UK laying flock has changed to a live *S.*Enteritidis vaccine so the effect of this change on the incidence of infection in chickens and people will be interesting to follow. It is essential, however, to combine vaccination with good husbandry, which should include all in-all out production, effective cleaning and disinfection between flocks and a high standard of pest control. It is also important to adequately monitor laying flocks so that persistently infected farms can be identified and *Salmonella* eliminated as houses are depopulated.

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Total summary of prevalence of Salmonella in eggs and other samples from vaccinated cage laying flocks

No. samples positive for *Salmonella* Enteritidis [other serotypes]/No. samples taken
(% positive for *S.*Enteritidis)[% +ve other serotypes]

	Shells (6 egg batches/total no. individual eggs)	**Contents (6 egg batches/total no. individual eggs)**
TOTAL	24 [54]/13652(0.18-1.05)^{1,2,4,5}[0.4-2.37]^{acde}	6 [3]/13640(0.04-0.26)^{4} [0.02-0.13]^{cd}

	Spent hens	**Bulked faeces**	**Environmental samples**	**Post C & D Samples**	**Mouse droppings**	**Rat droppings**	**Flies**
TOTAL	67[3]/699 (9.6)^{1,2} [0.4]^{ac}	64[81]/562 (11.4)^{1,2,4} [14.4]^{adefh}	246[183]/961 (25.6)^{1,2,4,5} [19.0]^{ade}	231[338]/1365 (16.9)^{1,4,5} [24.8]^{adeg}	16[7]/26 (61.5)^{1,4} [26.9]^{ade}	1/1^{4}	4[1]/10 (40.0)^{1,4,7} [10.0]^{a}

Key

S.Enteritidis Phagetypes : ^{1} PT4, ^{2} PT6, ^{3} PT7, ^{4} PT21B, ^{5} PT35, ^{6} RDNC, ^{7} PT5a
Other Serotypes: ^{a} *S.*Typhimurium DT104, ^{b} *S.*Typhimurium DT204B, ^{c} *S.*Newport, ^{d} *S.*Infantis, ^{e} *S.*Livingstone, ^{f} *S.*Anatum, ^{g} *S.*Tennessee, ^{h} *S.*Yoruba

QUANTIFYING THE RISK OF *SALMONELLA* CONTAMINATION OF TURKEY MEAT : SPECIFIC ROLE OF PREHARVEST CONTAMINATION AND POSSIBLE CONTROL MEASURES

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Introduction

Salmonella remains the first foodborne disease in France (2) like in many countries and the contamination of poultry products by this microorganism is a major concern. Consumption of contaminated raw eggs remains the first cause of *Salmonella* outbreaks in France. Contamination of turkey raw products is most of the time located to the surface of the meat or the skin, and regarding that, easily eliminated by cooking. Nevertheless, *Salmonella* foodborne diseases could occur in humans by cross contamination with heavily contaminated raw meat. Considering that, a study was undertaken in order to evaluate i) the amount of *Salmonella* present on the surface of the turkey skin after slaughter and on the breast fillet after cutting, ii) the relationship between caecal contamination at the age of slaughter and meat surface contamination, iii) some pre-harvest control measures that may be implemented in order to lower the risk of *Salmonella* on turkey raw meat.

Material and methods

Risk assessment : On the basis of pre harvest swabs realised 15 days before slaughter, *Salmonella* positive turkey flocks were selected. For each flock entering the abattoir, cleaning and disinfection of the plant was assessed by swabbing, and then 60 caeca, 60 neck skin and 60 breast meat were sampled. *Salmonella* monitoring was done by a classical pre-enrichment, enrichment, isolation procedure, using buffered peptone water (BPW), Modified Semi Solid Rappaport Vassiliadis (MSRV) and Xylose Lysine Tergitol 4 (XLT4) agar. *Salmonella* numeration was performed with the Fravalo *et al.* (1) method based on miniaturised Most Probable Number using BPW, MSRV, and XLT4. Sensitivity of the miniaturized MPN method was 1,3 CFU/g of sample.

Evaluation of pre-harvest control measures : day old turkeys (strain BUT9) were inoculated by 10^4CFU/bird of a *Salmonella* Typhimurium rifampicine resistant suspension. The birds were raised in an isolator during 14 days. Two days after *Salmonella* inoculation, they received through drinking water or through the feed a treatment supposed to be efficient to lower *Salmonella* excretion. Ten birds were sacrified at day 6, day 10 and day 14 and were checked for caecal contamination by *Salmonella* Typhimurium rifampicine resistant with brilliant green agar containing rifampicine. The following treatments were evaluated :

- -organic acid mix
- -acetic acid 0,1%
- -lactic acid 0,1%
- -ascorbic acid 0,1%
- -nisin 7 ppm
- -essential oils mixes (4 different formulations) 0,05%, 0,2%, 0,3%
- -*Saccharomyces boulardii* 50 ppm
- -Lactobacilli 2%
- -Alpha tocopherol 2500 ppm
- -Competitive exclusion (CE) treatment 0,1%.

Results

The results of the experiments are reported in tables 1 to 4.

Risk assessment experiments :

Table 1 : Salmonella contamination of the batches coming from positive flocks

	Flock1	Flock2	Flock3	Flock4	Flock5	Flock6
Caeca	25%	25%	0%	0%	95%	72%
Neck skin	20%	37%	0%	3%	93%	100%
Breast meat	0%	3%	0%	0%	41%	12%

Table 2 : Numeration of Salmonella in 36 positive samples coming from lightly contaminated flocks (flocks 1 to 4)

	N>100/g	10/g<N<100/g	N<10/g	N<nl
Caeca	0	0	1	3
Neck skin	0	0	11	19
Breast meat	0	0	0	2

nl : numeration limit

Table 3 : Numeration of Salmonella in 216 positive samples coming from heavily contaminated flocks (flocks 5 and 6)

	N>100/g	10/g<N<100/g	N<10/g	N<nl
Caeca	38	28	43	19
Neck skin	2	26	47	11
Breast meat	0	0	1	1

nl : numeration limit

As it can be seen on tables 1 to 3, *Salmonella* contamination resulting from the slaughtering of a pre-harvest positive flock is tightly depending on the contamination level of live birds as measured by caecal contamination. When the contamination of the gut was lower than 10 CFU/g of caecal content, all the *Salmonella* positive neck skins were contaminated by less than 10 CFU/g and most of them by less than 0,1 CFU/g which was the detection limit. As a consequence, breast meat taken from such carcasses was contaminated by less than 0,1 CFU/g when positive. On the contrary, heavily contaminated guts (N> 100 CFU/g) were responsible for more heavily contaminated neck skins even if most of the samples were contaminated by less than 100 CFU/g. In the meantime, contamination of the positive breast meat samples remained low (less than 10 CFU/g).

Evaluation of pre-harvest control measures :

Table 4 : results of the most significantly efficient treatments (expressed in log10 Salmonella reduction)

Product	1st trial	2nd trial	3rd trail
α tocopherol	-3.5	-1.8	Not done
Lactobacilli	-2.7	-2.4	-2.4 -1.1
CE treament	-1.6	-1.0 ns	Not done
Essential oil PO96A	Not done	-1.7	-0.8 ns

ns : not significant

Organic acids, nisin, ascorbic acid and yeasts did not prove their efficiency during these trials. Among the 4 tested formulation of essential oils, only one was significantly efficient in one trial.
As it can be seen in table 4, some treatments able to lower *Salmonella* excretion were significantly efficient even if their efficiency could vary from a trial to the other. The most efficient treatments able to significantly lower *Salmonella* contamination in the gut were high doses of α–tocopherol and the strain of *Lactobacillus* tested. This strain of *Lactobacillus* is able to decrease the contamination of more than 2 log10.

Discussion

Using an original method for *Salmonella* numeration, we were able to quantify the risk of meat contamination linked to gut content. As it could be seen, the proportionality between caecal bacterial content and neck skin contamination existed and is relevant. These results also showed that even if contamination of the caecal content is high (more than 100 CFU/g), breast meat samples remained lightly contaminated and did not present a high risk of foodborne disease for the consumer.
In order to lower the risk of neck skin and meat contamination, the potential of some "natural" molecules, probiotics or competitive exclusion treatment was evaluated. The purpose of the work was to screen some active products in experimental facilities using a one day old turkey model, and then to validate the results with field trials. The first part of the experiment enabled the authors to screen α tocopherol and a strain of lactobacillus which both proved their regular efficiency in *Salmonella* exclusion. These two products will be now evaluated in a field trial. Concerning the mechanisms that determine such an efficiency, the role of probiotics is well known and could be described as a combination of some of the following effects : direct production of *Salmonella* efficient bacteriocin, nutrient competition or acid production (3) or competition with mucosal adhesion sites. Few elements are available in this study to explain the observed effect with the *Lactobacillus* strain. It should be noted that a specific antimicrobial or non dissociated form of acid production did not explain alone the effect of the strain tested as organic acid and nisin tested separately did not have any effect. A synergistic effect between these two molecules was not tested but perhaps this particular strain had another property which is still to be investigated. α tocopherol is well known for its antioxidant properties and has recently be involved in lowering the virulence of *Listeria monocytogenes* in turkey (4). This could partly explain its efficiency against *Salmonella.* The mechanisms of the efficiency of both products have to be further investigated regarding their impact on the composition and equilibrium of the gut and caecal flora.

Conclusion

This preliminary work is reassuring as it confirms that the amount of *Salmonella* that might contaminate turkey meat are low, and the associated risk for the consumer consequently low as well, even in potential cases of cross contamination in the kitchen.
The use of molecules or probiotics able to decrease the excretion of *Salmonella* before slaughtering is also promising as the proportionality between the contamination of the gut content and those of the skin existed. If confirmed by field trials, this treatment might be used for the treatment of the turkey flocks detected positive 2 weeks before slaughter.

The authors wish to thank CIDEF (French committee of turkey producers) and Ministry of Agriculture for their support.

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CONJUGATED LINOLEIC ACID (CLA) AND THE RATIO OF Omega6:Omega3 FATTY ACIDS ON THE LIPID CONTENT OF CHICKEN MEAT

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Introduction

Conjugated linoleic acid have been shown to have favorable physiological effects in the lipid metabolism, including a reduction of body fat (West et al., 1998) The use of CLA in association with oils rich in Omega3 fatty acids or in diets that have a balanced ratio of Omega6:Omega3 has optimized the CLA effect (Aydin et al., 2001) showing that the CLA effect depends upon the amount of fatty acids Omega6 and Omega3 in the diet. The purpose of these studies was to evaluate the dietary supplementation of CLA and the ratio of Omega6: Omega3 on the lipids content in the thigh and breast meat of broiler chickens.

Material and Methods

Two studies were conducted simultaneously using 100 male or female Ross broiler chickens with 21 days of age at the start of the experiment. The experimental design was a completely randomized, in a factorial arrangement 2 x 5 (two oil sources, i.e., soybean or canola oil and five levels of CLA supplementation, i.e., 0.0, 0.25, 0.50, 0.75 and 1.00%). The oils used were supplied by Bünge Foods and CLA (Luta-CLA 60) by BASF. The control diet had 4% soybean or canola oil. CLA levels were obtained by isometrically replacing soybean or canola oil in the control diets. The lipids contained in the meat were extracted using the technique of Folch et al. (1957). The F test at 5% of significance was used to compare results between sources of oils when interactions were not detected. When there was an interaction (P<0.05), it was used the SNK test to compare results between sources of oils. Regression analysis was used to report the effects of CLA levels.

Results

The total content of lipids breast meat was consistently lower than that of thigh meat (Table 1). An interaction, oil source vs CLA levels was observed on the total lipid content of thigh and breast meat. The use of canola oil and growing CLA levels resulted in a linear reduction (P<0.05, Figure 1) on total lipids of the breast meat. These results can explain a linear reduction (P<0.05) observed in the malonaldehyde content of refrigerated and frozen meat of birds receiving canola oil. However, without CLA, total lipid content of breast meat (1.34%) of birds receiving canola oil was higher (P<0.05) than that of birds on soybean oil (0.89%). This difference in lipid content did not influence the oxidative stability of the breast meat (P>0.05) values obtained for refrigerated (0.140 vs 0.149 mg of malonaldehyde/Kg of meat) at 3 days of storage or frozen (0.193 vs 0.190 mg of malonaldehyde/Kg of meat) breast meat at 75 days of storage from chickens receiving canola or soybean oil, respectively. Birds receiving soybean oil and supplemented with CLA had an abrupt reduction of total lipids on breast meat from 0.89% at 0% CLA to 0.36% at 0.5% CLA followed by a small increase at higher levels of CLA. These results were explained by a quadratic response (P<0.05, Figure 2). These observations may help to explain the reduction (P<0.05) of oxidation on refrigerated and frozen breast meat at 50 and 100 days of storage. For the thigh meat the results of total lipid content were similar to that of breast meat. Birds receiving canola oil without CLA had higher (P<0.05) fat content in the thigh meat (4.12%) compared to soybean fed birds (3.20%). The TBARS (thiobarbituric acid reactive substances) values of refrigerated thigh meat go along with the fat content, where thigh meat of birds fed canola oil had TBARS value of 0.214 compared to 0.158 of birds fed soybean oil. It is hypothesized that this effect observed on the thigh but not on the breast meat was due to the variation on the heme pigment on the two meats. The oxidation of the pigment may have catalyzed the lipid oxidation which corroborates Akamittath et al. (1990) and Monahan et al. (1994). On the other hand, feeding soybean oil with growing levels of CLA produced a linear increase (P<0.05, Figure 3) on thigh meat total lipid content what may be responsible for the lack of difference between oil sources.

Table 1 – Lipid content of broiler meat fed diets with canola or soybean oil and CLA

LIPID CONTENT ON THIGH MEAT (%)			
CLA (%)	**SOYBEAN OIL[1]**	**CANOLA OIL[3]**	**$\bar{x}$ CLA**
0.0	3.20b	4.12a	3.66
0.25	3.64b	5.17a	4.40
0.5	3.73a	4.45a	4.09
0.75	3.80a	3.69a	3.74
1	4.29a	3.75a	4.02
$\bar{x}$ OIL SOURCE	3.73b	4.24a	
LIPID CONTENT ON BREAST MEAT (%)			
CLA (%)	**SOYBEAN OIL[2]**	**CANOLA OIL[1]**	**$\bar{x}$ CLA**
0.0	0.89b	1.34a	1.11
0.25	0.66b	1.33a	0.99
0.5	0.36b	1.04a	0.70
0.75	0.46b	1.09a	0.78
1	0.60a	0.48a	0.54
$\bar{x}$ OIL SOURCE	0.59b	1.05a	

[a,b] Averages values within the same line with no common superscript differ significantly by the SNK test (P<0.05)
[1] Linear effect (P<0.05)
[2] Quadratic effect (P<0.05)
[3]Cubic effect (P<0.05)

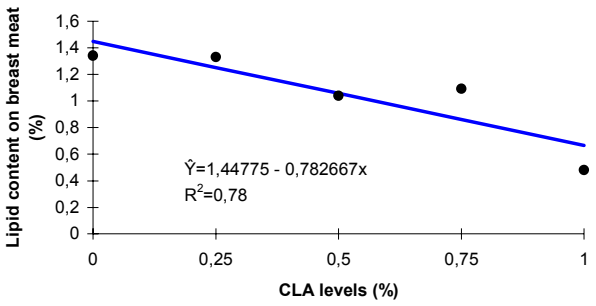

Figure 1. Lipid content on breast meat (%) of broilers fed diets with canola oil and CLA supplementation.

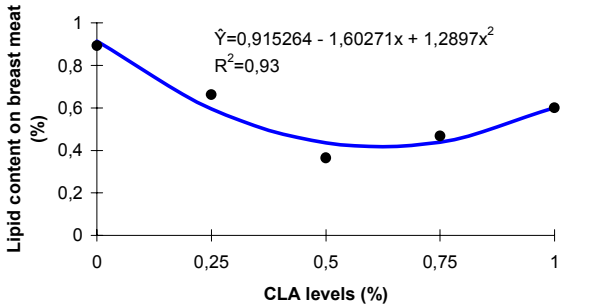

Figure 2. Lipid content on breast meat (%) of broilers fed diets with soybean oil and CLA supplementation.

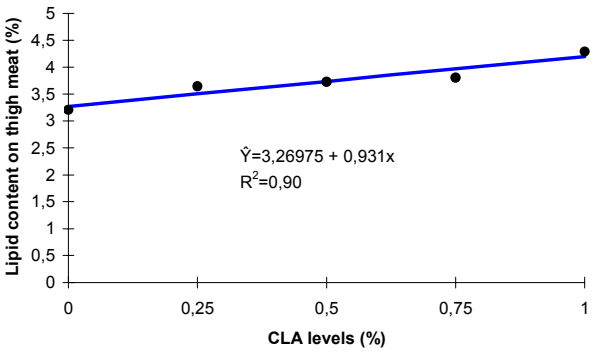

Figure 3. Lipid content on thigh meat (%) of broilers fed diets with soybean oil and CLA supplementation.

Discussion

The lipid content on breast meat is represented by intramuscular lipids such as cell membrane phospholipids while that the thigh meat is characterised by both intramuscular and intermuscular lipids containing much more triglycerides (Leskanich & Noble, 1997). Dietary supplementation with CLA may change the composition of lipids produced by the liver (Belury & Kempa-Steczko, 1997) as well as reduce the total lipid concentration in rats (West et al., 1998) by alteration of the genetic expression of the lipogenic enzymes (Bauman, 2001). In this study it was shown a synergic effect between CLA and canola oil on the lipid metabolism demonstrated by reduction of thigh and chest lipid content. However, it was observed adipogenic effect on the lipid content of thigh meat with increased levels of CLA in association with soybean oil, showing that the anti-adipogenic effect of CLA can be reversed. Brown et al. (2001) reported that culture of pre-adipocytes supplemented with CLA and sunflower oil (rich in omega 6 fatty acids) resulted in higher content of triglycerides when compared to the cultured treated with only CLA. Therefore, it is reasonable to think that studying the CLA effect on the lipid metabolism it is important to take into consideration the fatty acid composition of the diet as well as the ratio of omega 6 to omega 3 fatty acids. Also, these alterations on the lipid content by CLA supplementation vs oil source resulted in changings in the oxidative stability of meat.

Conclusion

The lipid content of thigh and breast meat is influenced by the oil source. The CLA response on lipid content in the meat depends upon the source of fat added to the diet.

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Quality in food chains - Poultry and Fish

Posters

THE MEASUREMENT OF TOTAL VOLATILE NITROGEN (TVN) IN QUALITY CONTROL OF SOME BONY FISH IN THE RETAIL MARKETS OF THE CITY OF SHAHREKORD, IRAN.

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Abstract
In the spring and summer of 2003, the Total 90 samples from three species of fishes, which are available in south sea and are called: (Chirocentrus dorab ,Teuthis siganus and Hilsa kanagurta) were collected, from retail markets. The samples were examined by kjeldahl method for measurement of TVN in the meats. The results showed that in 5.55% of samples, TVN were more than normal rate. The mean value of TVN in Chirocentrus dorab,Teuthis siganus And Hilsa kanagurta were 20.3, 19.37 and 17.69 mg/100 g of meat respectively.
The rate of TVN in the central districts of the city of Shahrekord was lower than out skirts. And it is because of better supply, keeping of the fish and specially the better quality of non-frozen fishes.

Key words: Total Volatile Nitrogen (TVN), Quality control, and Bony fish, Retail markets.

Introduction

With regards to the importance of fish and fish products as an important available resources of animal proteins and with attention to their rapid spoilage, it is necessary to open a new window in rapid and economic control of these products. Therefore we have carried out a study on 90 samples of bony fish in the retail markets sale in Shahrekord, IRAN, in 2003, with macro kjeldal method for determination of Total Volatile Nitrogen (TVN).

Material and methods

In the spring and summer of 2003, the Total 90 samples from three species of fishes, which are available in south sea and are called: (Chirocentrus dorab ,Teuthis siganus and Hilsa kanagurta) were collected, from retail markets. The samples were examined by kjeldahl method by A.O.A.C methods for measurement of TVN in the meat. 10 g from meat bony fish was obtain and to place in kjeldahl distillation system, then Volatile Nitrogen in glass balloon (to contain Boric acid 2%, methyl red, Bromocresol green), was collected and to titration by sulfuric acid (0.1 N) for measurement of TVN by mg / 100g of fish meat (1,2).

Results

Out of 90 meat bony fish samples, 5(5.55%) of samples, TVN were more than normal rates (25mg/100g meat).

Discussion

In comparison with previous study in Iran, for examples in Tehran, IRAN in 1999, in 85.7% of samples, TVN were more than normal rate(1). So in other study in Tehran, in 2000, in 3.4% of samples TVN were more than normal rate (1). The rate of TVN in the central districts of the city of Shahrekord was lower than out skirts. And it is because of better supply, keeping of the fish and specially the better quality of non-frozen fishes.

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Table- 1) Characteristic of three species of bony fishes to measurement of TVN, in Shahrekord , IRAN(2003).

Species of bony fishes	Mean value	S.E	S.D	Max.	Min.
Chirocentrus dorab	20.3	0.55	3	28	15.4
Teuthis siganus	19.37	0.66	3.615	28	15.4
Hilsa kanagurta	17.69	.045	2.45	23.8	15.4

THE MATRESA PROJECT – TREATMENT STRATEGIES FOR LIVESTOCK MANURE FOR SUSTAINABLE LIVESTOCK AGRICULTURE

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The EU-funded MATRESA project was concluded in 2003 with the publication of a detailed reference book* that sets out a thorough review of the management and treatment of agricultural wastes across Europe. The objective was to raise awareness among European agriculturalists (including farmers, advisors and local authorities) of the current research and technology available within Europe to facilitate better management of livestock wastes to (a) minimize environmental and health hazards and, (b), gain the maximum benefit. Information was drawn from the contributions of project partners representing 24 countries - engineers, agronomists, vets and scientists were chosen for their involvement in national and European programmes.

A central finding of this review was that good management of livestock wastes (*eg*, the collection, storage, mixing, pumping and spreading of livestock manures) following existing guidelines can alleviate problems in *some* circumstances, but it is rarely a complete solution. Some livestock farms simply lack enough suitable land to safely receive the manures produced. The application of excessive quantities of livestock manure (and/or mismanagement) is already leading to a range of pollution problems. These include water contamination (by nitrates, phosphates and organic matter) air emissions (including ammonia, nitrous oxide and methane) and soil residues (including phosphates and heavy metals). Poor manure handling can also lead to disease risks to farm animals, the general public and food production in general.

Sustainable agriculture in Europe

Today, agricultural production systems in Western Europe are highly developed with individual farms tending to specialize; resources are used very efficiently and output is high. Nonetheless, as a consequence, local and regional surpluses are generated; supplies and products are transported over increasing distances. For the manures and effluent produced, local land disposal remains the main option but they often become regarded as waste streams and treated accordingly. However, the more sustainable situation essentially involves greater recycling and reduced losses to the environment; input of inorganic fertilizer can then be reduced as a result In order to reach such a situation, changes in approach will be needed from those in the agricultural business as well as from the authorities and the public in general.

Manure and effluent management

Water management issues

One of the key difficulties with handling many liquid animal manures lies with their relatively low concentrations of dry matter. For some dairy waste-waters (or dirty waters) this value can be well below 10 kg/m^3. The implications are threefold; (i) there is a need for larger storage capacity, (ii) the application to meet crop requirement is more difficult and (iii) large quantities of water are being used implying increased transportation. Reduction of manure volume by using less water thus has clear benefits and there are various guidelines for efficient water use on a farm. There has been some research on re-using partly treated slurry for flushing channels in buildings. This has the benefit of both reducing water requirement and increasing the solids concentration in the slurry. The treatment implied may be simply a physical clarification process or it may include some biological activity as well to degrade the dissolved organic matter. The limitation of this strategy lies with the cost and efficiency of the treatment process involved balanced against the penalties of the alternatives; *eg* using more water and needing to deal with larger volumes.

Transportation of livestock manures

Moving manures from region to region represents a seemingly simple solution to the environmental problems of those areas with excess nutrients. However, this approach is fraught with problems based on the scale of the operation, nutrient monitoring and in some cases, disease risks. The problem is mostly attributable to the volume of liquid slurry; in many cases, the solid wastes (eg, the farmyard manure) could be beneficially used without problem on the farm or locally. However, slurries often contain more than 95% water hence pre-concentration is important if the exercise is not to become one of moving water. Such an approach will require low cost concentration systems if it is to be viable; the implication is some form of physical process with a very dilute waste water being irrigated locally.

The relatively low concentration of dry matter in most slurries does enable transport by pipeline which may be a more practical option for shorter distances. Some pre-screening is necessary to remove suspended matter that may lead to blockage. Otherwise, the issue comes down to the question of investment in pipeline systems as much of the technology already exists. Concern over disease spread may yet be the greatest hurdle to large scale redistribution of livestock slurries.

Treatment systems in agriculture

It is unlikely that complete abatement of pollution and the other problems associated with livestock manure can be achieved by improved farming practice alone. In some situations further measures including treatment will form part of the solution. Even where there is adequate land available and a good nutrient balance, some form of treatment may still be appropriate e.g., for odour abatement or to minimize disease risks These can be physical, biological, chemical or a combination of all these processes.

Treatment has a clear role in the overall management package, but only some of systems emerging are both practicable and effective at the farm level The broad theme behind good manure management is proposed as one based on aiming for a more balanced farming system to avoid the

release of excess nutrients into the environment. This implies greater targeting of nutrients in manures to meet the crop need and a subsequent reduction in the applied level of inorganic fertilizers. However, improved monitoring in the application of the nutrients in raw and treated manures is necessary to reduce the uncertainty on the subsequent interaction with the soil and crop uptake. Aerobic treatment can remove unwanted nutrients or stabilize them to enhance plant utilization; it is also effective in odour abatement. Information is lacking though to enable an objective comparison and evaluation of such processes and although effective, the general cost is still too high for many farms. Reducing the manure burden of a farm lacking enough land implies the export of surpluses. Even with improved transportation systems, some pre-concentration is desirable. The implied volume reduction can have an additional benefit in enabling improved water use in and around the farm. Conversion of solid manure and livestock slurries to a range of saleable products is an attractive option but quality and consistency are important. This may involve the co-processing with other organic wastes to gain a balanced blend. Separate from farming, manure surpluses may yet be a resource for industry in the future owing to the wide range of chemicals it contains.

Process equipment design and verification

There is a wide range of technology and related machinery available now for the use of processing the various livestock manures. Much of this originates from designs used in other industries especially sewage treatment and water supply. However, the satisfactory application to the much stronger effluents from agriculture does not necessarily follow; the objectives for treatment are not necessarily the same and available funds are usually much less. A key problem lies with a systematic evaluation of the individual machine or complete process; what is it achieving, what are the costs and how does it compare with the alternatives? The response to this is in part a matter of policy making, ie, setting specific environmental standards, but this is not so simple when it comes down to objectively scoring a piece of equipment. A typical claim that a process "reduces water pollution" is obviously vague and clearly much will depend on other agricultural factors. However, a more precise standard can often be identified such as aerator performance in kg oxygen dissolved per kWh of electricity consumed. Likewise, a process may be rated in terms of the percentage of nitrogen removed (or conserved as the organic form) - the full benefit of the process will still depend on other agricultural factors (*eg* spreading method and timing) but they will be the same for any process chosen.

Conclusion - are there any "best" options?

One of the first issues to arise from the workshop meetings that gave rise to this publication is the wide range of farming scenarios across Europe. Factors such as farm size, local geography and land type, climate and production method all give rise to farms with highly individual features. It is not surprising then that there are no universal solutions to the manure problems experienced on livestock farms. Rather, the many methods are likely to be as highly individual as the farms themselves. However, the situation can be rationalized to some extend by the grouping of farms according to farm type and dominant manure problem(s) - each such group would then suit a manure management strategy and for each there may be one (or more) best options.

A second general theme to arise from this collaboration was that treatment should not be as the first choice in dealing with the perceived problems on a farm. Indeed, owing to the relatively high costs often involved, treatment should only be considered when existing methods of good manure management have been implemented and found to be inadequate. However, when a problem persists despite running a good farm operation and action is required, then the treatment option is necessary.

The key message is one of correctly identifying the problem and setting out an effective and verifiable strategy to deal with it. This involves being specific on what is required of the waste management plan thus enabling the selection of effective technology that meets the requirements.

** BURTON, C.H.; TURNER, C. (editors) (2003) Manure management - treatment strategies for sustainable agriculture; second edition Silsoe Research Institute, Wrest Park, Silsoe, Bedford, UK. 490 pages.*

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