Infectious Diseases // Human Health and Zoonoses // Emissions // Disinfection // Waste Management // Horse Session

Proceedings of the

XIV ISAH Congress 2009

International Society for Animal Hygiene

19th to 23rd July
Vechta, Germany



Volume II

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XIV ISAH Congress 2009

Proceedings of the 14th International Congress of the
International Society for Animal Hygiene

"Sustainable Animal Husbandry: Prevention is Better than Cure"

Innovations in Hygiene, Nutrition and Housing
for Healthy Food from Healthy Animals

University of Veterinary Medicine Hannover, Foundation

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Preface

On behalf of the Organizing Committee, the Scientific Committee and the Executive Board of ISAH, I cordially welcome you in Vechta, Germany to participate in the XIV Congress of the International Society for Animal Hygiene (ISAH).

The ISAH (www.isah-soc.org) was founded in 1970 and has today members in 50 countries throughout the world. The initial incentive was the fact that the problems of hygiene in animal production had surpassed their local character and solutions required international co-operation. The first Congress was held in Budapest in 1973 followed by 12 main congresses (see list next page) and 13 in-between conferences.

Animal Hygiene is the third pillar of veterinary medicine besides curing and care taking. It represents the science of preventing diseases in a broad sense. This concept encompasses the knowledge of all relevant aspects of the abiotic and biotic environment of animals in a housing and keeping system and the understanding of the principles of the interactions of animals with their technical, physical (thermal), chemical and biological surrounding in order to improve their health, welfare and performance without the unnecessary use of drugs being the preconditions for the production of safe and healthy food. Furthermore, it requires a profound knowledge of management, handling and behaviour of animals on individual and herd level, and the understanding of the effects of animal husbandry systems on the surrounding environment by airborne emissions, manure disposal and spread of diseases (environmental hygiene) in order to define prevention strategies including bio-security measures and disinfection. The concept of hygiene integrates in this way the relevant aspects of animal health and welfare in livestock production, ethics, ecology and consumer demands on a sound economic basis.
The International Society for Animal Hygiene can therefore be considered as a group of veterinarians and non-veterinary academic scientists (animal science, agricultural economics, engineers, microbiologists, public health professionals, epidemiologists etc.) and respective professionals in animal husbandry, who work and/or do research and education in the field of animal hygiene.

The present XIV ISAH Congress in Vechta, Germany, in July 19-23, 2009 is organized under the device "**Prevention is better than cure**" which meets the EU animal health strategy for the coming years until 2013.
The scientific programme follows the scope of the ISAH and concentrates on the following subjects: interaction between the environment and health and welfare of the individual animal and herds; managing animal health on farm level; ensuring animal welfare during transportation and slaughter; considering economical implications of animal health care; demonstrating the potentials of precision livestock farming in maintaining good health and welfare of animals; preventing the development and spread of diseases and pathogens including those posing a health risk for humans (zoonoses); food safety relevant infections and contaminations such as residues in food derived from animals; influence of the animal production on the environment and public health, practical bio-security including cleaning and disinfection.
The Proceedings from the XIV ISAH Congress present papers of lectures from invited speakers, oral and poster presentations held in 26 parallel sessions. A special section is reserved for the FAO Side Symposium on Monday afternoon devoted to hygienic and production problems in the Developing parts of the World. The introductory plenary talks of David Byrne, former EU Commissioner of DG SANCO, Walter Winding, President of the Federation of Veterinarians

of Europe and Prof. Dr. Hans-Wilhelm Windhorst, University of Vechta, lead us the way to a broad understanding of the hygiene related problems in Europe and the world and the role of the veterinarians to find the appropriate answers.

The organisation of such a congress requires the help and input of many people, and I hereby would like to express my deep thanks to all who contributed to realise ISAH2009. My most sincere gratitude goes to the ISAH2009 Organising Committee, the Scientific Committee and the Executive Board of ISAH. My special thanks are reserved to our host, the University of Vechta, namely Professor Marianne Assenmacher and her team around Mr. Johannes Wilking. Equally important was the support by our University of Veterinary Medicine Hannover (TiHo-Han), namely President Dr. Gerhard Greif, and the Unit for Epidemiology in Bakum, Prof. Thomas Blaha. Our gratitude goes also to the Mayor of Vechta Uwe Bartels and the Vechta Tourist Office helping with the hotel booking and organisation. A big thank goes also to our supporters from industry and business who helped with ideas and some of them opened their premises for our technical tours. An excellent job was done by the Tribun EU s.r.o. company, Brno which printed the proceeding in high quality.

Last but not least, it is my privilege to thank all participants, contributors, chairpersons, organisational and technical assistants for their considerable efforts and inputs. Now, we wish you all an interesting and pleasant ISAH Congress and an enjoyable time in Vechta t the heart of farm animal production.

J. Hartung
Chairman of the ISAH-2009 Organizing Committee

List of ISAH Congresses

ISAH founded 20th November 1970 in Budapest, Hungary (founding members: Prof. Cena, Poland, Prof. Ivos, Jugoslavia/Croatia, Prof. Kalich, Germany, Prof. Hojovec, Czechoslovakia)

		Place of Venue	**Organizer**
I.	Congress 1973	Budapest,Hungary	F. Kovacs
II.	Congress 1976	Zagreb, Yugoslavia	J. Ivos
III.	Congress 1980	Vienna, Austria	H. Willinger
IV.	Congress 1982	Strbske Pleso, Czechoslovakia	J. Rosocha
V.	Congress 1985	Hannover, Germany	H.G. Hilliger
VI.	Congress 1988	Skara, Sweden	I. Ekesbo
VII.	Congress 1991	Leipzig, Germany	G. Mehlhorn
VIII.	Congress 1994	St. Paul, Minnessotta, U.S.A.	S.L. Diesch
IX.	Congress 1997	Helsinki, Finland	H. Saloniemi
X.	Congress 2000	Maastricht, Netherlands	M. Tielen
XI.	Congress 2003	Mexico City, Mexico	J. A. Saltijeral
XII.	Congress 2005	Warszawa, Poland	A. Krynski
XIII.	Congress 2007	Tartu, Estonia	A. Aland
XIV.	Congress 2009	Vechta, Germany	J. Hartung

In addition 13 in-between-Conferences were organised in different countries.

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Part VII

Infectious Diseases

Chapter 17

Block 9C

Prevalence and burden of helminths in laying hens kept in free range systems

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Summary

A total of 144 laying hens, from 5 different genotypes, was examined for the presence of endoparasites. Four different nematode and 3 cestode species were identified. The most prevalent species were *Heterakis gallinarum*, *Ascaridia galli* and *Capillaria spp.*.

Introduction

In October 2001 conventional cages were banned in Germany by law. Therefore an increasing number of farmers keep their animals in so called alternative production systems. The number of animals kept in such systems increased in the period of 2001 to 2007 from 15 to 34 % [1]. Chickens in free range production systems are kept under worse hygienic conditions and physical environment, such as ambient temperature and humidity, when compared with pure barn systems. These factors increase the risk of parasite infections [2]. Therefore the change of production systems accumulates the importance of helminth infections in the future [3,4]. Direct and indirect losses caused by helminth infections are economically important. Indirect losses occur as lowered feed utilization rate, which may reduce egg production as well as weight gain. Furthermore helminths can act as carriers of pathogenic agents that cause other diseases [5,6].

To improve and maintain the performance and productivity, and plan strategies against parasitic diseases, it is essential to determine the prevalent agents.

Therefore, the aim of the present study was to estimate the prevalence and burden of helminths in free range layers in Germany.

Animals, Material and Methods

Animals. 144 laying hens of 5 genotypes (Isa Brown, Tetra Brown, Lohmann brown, Bovans Black, Lohmann Tradition) were collected at different ages from 11 farms in Germany. The animals were slaughtered and examined for the presence and number of helminths.

Parasitological examination. The gastrointestinal tract from esophagus to the rectum, including both caecal tubes, was removed and opened longitudinally with scissors and washed in tap water. Content of each organ was poured into a sieve (100ym mesh size), flushed and the retained material transferred into petri-dishes. The mucosa of the small intestine was scraped and the keratinized layer of the gizzard was peeled off [7].

To remove the cestodes from mucosa, the attachment point were located, the intestine was cut around the attachment point and refrigerated (-20 °C). After thawing the cestodes, the scolexies could be removed out of the mucosa using fine tweezers to identify the species.

Individual faecal samples were taken and analyzed using a modified McMaster technique [8] to estimate the faecal egg count (EpG) and to detect coccidial oocysts. Saturated NaCl and a sensitivity of 50 eggs per gram faeces were used.

Parasite identification. Visible parasites were collected immediately after detection. The content of each organ was examined under dissecting microscope (20x). The parasites were identified using the helminthological keys of SOULSBY [9], SCHMIDT [10], KAHLIL et al. [11] and McDOUGALD [12].

Statistical analyses. The prevalence (P,%) and mean worm burden (WB) of individual helminth species as well as correlations between WB and EpG were calculated. Differences between WB according to genotypes were analyzed with ANOVA. Due to the number of available animals, only 3 genotypes (Tetra Brown, Lohmann Brown, Isa Brown) were compared. All statistical analyses were performed with SAS (1999).

Results

The prevalence, the mean worm burden for the identified helminths are shown in the table.
92,4% of the birds were parasite positive with a total mean worm burden of 147,7 158,3 (SD). The following nematodes were found (P; WB): *Heterakis gallinarum* (84%; 97,6), *Capillaria spp.* (71,5%, 45,7), *Ascaridia galli* (66,6%; 16) *Acuaria hamulosa* (1,4%; 1); cestodes identified were: *Raillietina cesticillus* (24,3%; 41,3), *Choanotaenia infundibulum* (2,8%; 26,8) and *Hymenolepis cantaniana* (2,1%; 11,3). 7% of the examined faeces samples were positive for coccidial oocysts. There was a significant correlation between number of nematodes and EpG ($r = 0{,}48$; $p < 0.0001$). TETRA hens had higher worm burden when compared with ISA and LB hens ($221{,}1^a$; $123{,}9^b$; $85\ 4^b$; $p < 0.0001$).

Discussion

In layers kept in free range systems 7 helminth species were identified of which 4 were nematodes and 3 cestodes. *Capillaria* species were not differentiated and thus they were all classified as *Capillariaspp.*
The most prevalent species were the nematodes *Heterakis gallinarum*, *Ascaridia galli* and *Capillaria spp.* These results are in argreement with results from PERMIN et al. [13,2], POULSEN et al. [14], MAGWISHA et al. [15], MARTIN-PACHO et al. [16] and ABDELQADER et al. [17] who found that *A. galli*,*H. gallinarum* and *Capillaria spp.* are among the most prevalent species in such systems. However the prevalence and worm burdens in the current study were on a higher level. This was maybe the case because most of the animals were sampled from organic production systems, where treatments against endoparasites are very limited. In addition, in 7% of the layers coccidia oocysts were found. TETRA hens had higher total worm burden when compared with ISA and LB hens. Genetic variation for parasite resistance has been described earlier in different parasite and host systems [18,19,20,21]. Therefore it could be possible to select chickens for worm resistance.

Table 1: Prevalence (%), mean worm burden, standard deviation and range of infections with adult helminths and prevalence (%) of coccidia in 144 laying hens

	Prevalence	Mean WB ± SD	Range
Nematodes			
Ascaridia galli	66.6	16,0 ± 24,3	1 - 149
Heterakis gallinarum	84.0	97,6 ± 127,9	1 - 736
Capillaria spp.	75.1	45.7 ± 60.6	1 - 244
Acuaria hamulosa	1.4	1.0 ± 0	1 - 1
Cestodes			
Raillietina cesticillus	24.3	41.3 ± 82.3	1 - 350
Hymenolepis cantaniana	2.1	11.3 ± 17.9	1 - 32
Choanotaenia infundibulum	2.8	26.8 ± 34.4	3 - 76
Coccidia	7.0	-	-
Total	92.4	147.7 ± 158.3	2 - 775

This approach may be of importance in poultry husbandry relying on alternative and organic housing systems.

Conclusion

A broad spectrum of helminths were found in layers kept in free range systems. The high prevalence of helminths indicates that the majority of the chickens are subclinically infected, which may cause economic losses. The economic and animal welfare impact of helminth infections remain to be investigated. Losses due to a high morbidity might be considered of greater economic importance than the high worm counts that may cause mortality in a few birds.
Genetic differences in parasite resistance were described earlier. This should be taken into account, when birds are selected for such production systems.

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Relationship between hygiene management and endoparasite infections in 20 sow herds

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Summary

The objective of the study was to assess the hygienic measures used on organic pig farms and to evaluate their effectiveness in reducing endoparasite infections on 20 organic pig-breeding farms in Germany. Management factors related to helminth infections of the herds were recorded in personal interviews. In total, 382 faecal samples were collected from gestating sows and examined for nematode egg counts per gram of faeces. The majority of the farrowing units (n = 15) were cleaned wet, whereas most of the farmers did not clean the gestation pens at all and if so only mechanically by removing dung. Chemical disinfection was only performed in at most 20% of the farms in farrowing units. Strongylid, *Trichuris suis* and *Ascaris suum* eggs were detected in 78.5%, 2.8% and 1.3%, respectively. Regarding the worm control and hygiene management, there were no differences between strongylid free or infected farms. Thus, those farms who used comprehensive hygiene measures were not necessarily gaining the best results with respect to endoparasite infection. Without the implementation of strategic control and feedback mechanism within the production process, effectiveness of hygiene measures related to worm burden can not be assessed sufficiently.

Introduction

Studies in different European countries have shown that infections by endoparasites are a prominent health problem in swine herds [1,4,6]. Differences among farming enterprises can be considered as high. The variation in parasite burden among the farms is likely to be caused by different management routines in the herds. The aim of the study was to assess the prevalence of helminth infections, the hygienic measures against endoparasites used on organic pig farms and to evaluate their effectiveness in reducing endoparasite infections.

Animals, Material and Methods

The study was carried out in 20 organic sow herds in Germany with a herd size >10 sows each. Management factors of the herds were recorded by using a standardised questionnaire in a personal interview. Closed and open-ended questions regarding cleaning and disinfectant measures, documentation of production data, anthelmintic treatment and diagnostic measures on farm-level were included. Furthermore, 10 faecal samples per group, which were taken twice during Spring and Autumn, were collected from gestating sows, weaners and fatteners. They were examined within 12-24 h after collection for nematode egg counts per gram of faeces (EPG) and coccidian oocysts using the 'Concentration McMaster technique' with saturated sodium chloride solution (detection limit: 20 EPG;[8]). A total number of 382 sows was sampled. Data analyses were performed using SPSS (Version 17.0). Data were categorised to evaluate the relationship between hygiene management and parasite infection using the Fisher-Yates-Test.

Results

The herd size varied from 18 to 680 sows per farm with a median of 48. 50% of the farmers raised their own gilts, and 35% received the replacement animals from other farms. The mean number of litters and weaned piglets per sow and year was 2.01 (0.14) and 19.03 (1.02), respectively. The mean weaning age of the piglets was 43 days. All of the farms provided straw bedding for the sows.

Hygiene management. The hygiene management measures varied between the gestation and farrowing units of the farms (Tab. 1). The majority of the farrowing units (n = 15) were cleaned wet, whereas most of the farmers did not clean the gestation pens at all and if so only mechanically by removing dung. Chemical disinfection was only performed in at most 20% of the farms in farrowing units, mostly using peracetic acid.

Tab. 1: Distribution of implemented measures within hygiene management in gestation and lactation units in 20 organic sow herds in Germany

Hygiene measures	Gestation units		Farrowing units	
	n	%	n	%
No cleaning of pens	4	20	0	0
Dung removal only	10	50	2	10
Wet cleaning	4	20	11	55
Wet cleaning and disinfection	2	10	4	20
Move huts	0	0	3	15

Tab. 2: Prevalence of gastro-intestinal infections in 20 organic sow herds

Parasite	Sows (n = 382)		Farms (n = 20)		Within-farm prevalence (%)	
	Number affected	%	Number affected	%	Average	Range
None	136	35.6	3	15	-	-
Strongylides	171	44.8	12	60	78.5	10-100
Trichuris suis	11	2.8	7	35	8.3	5-20
Ascaris suum	5	1.3	4	20	6.3	5-10
Coccidia sp.	192	50.3	17	85	61	5-100

Strongylid infections were detected in 60% of the sow herds with a mean within-farm prevalence of 78.5% (Tab. 2). *Trichuris suis* and *Ascaris suum* eggs were only marginally detected in faecal samples of sows (2.8 and 1.3, resp.). Coccidia infections were diagnosed in 85% of the farms, whereas 6 of them showed a prevalence rate 85%. Sows were treated with anthelmintics on all farms. A 'targeted' deworming based on reproductive cycle was performed on 11 farms whereas 9 farms performed a deworming at least twice a year. Anthelmintics most commonly used in sows were (pro)benzimidazoles (75%).

In eight of the 20 sow herds (40%) no strongylid egg shedding was detected, while wet cleaning and disinfection was performed in only 25% of their lactation units (Tab. 3). Regarding the worm control and hygiene management, there were no differences between strongylid free or infected farms.

Tab. 3: Anthelmintic treatment and hygiene measures in 20 organic sow herds, depending on strongylid within-farm prevalence

Parameter		Farms with prevalence of strongylid egg-shedding (n)	
		0% (n = 8)	10-100% (n = 12)
Routine anthelmintic treatment		8	12
Gestation units	no cleaning	1	3
	dry cleaning	6	4
	wet cleaning	0	4
	wet cleaning and disinfection	1	1
Lactation units	dry cleaning or moving huts	3	2
	wet cleaning	3	8
	wet cleaning and disinfection	2	2

Discussion

In the present study, farms varied to a high degree with respect to the implementation of hygiene measures and the prevalence of gastrointestinal parasite infection. Data show that 20% of the farms did not clean their gestation units; half the number of farms performed dung removal only. Gerwert et al. [4] found the same level of inconsequent hygiene management in conventional pig-breeding farms in Germany. Furthermore, wet cleaning and disinfection using a chemical disinfectant was insufficiently implemented on sow farms (20%). In a Danish study, it was found that 80.7% of the farms were performing regular disinfection measures [3].

Strongylid infections were most frequent in adult swine [5], irrespective of production system [7]. The rate of strongylid egg-shedding of sows in the present study (44.8%) was comparable with those from Gerwert et al. [4] detecting 55% of sows infected with strongyles. Only the study of Carstensen et al. [2] showed a lower prevalence of strongylid egg-shedding (20%) resulting from strict improvement of housing and pasture conditions in the past. In our study, *Trichuris suis* and *Ascaris suum* were found rather sporadically confirming results of previous studies [2,4,7].

Dangolla et al. [3] found a positive relationship between anthelmintic treatment and the prevalence of strongylid infections of sows. Other studies dealing with strongylid or *Ascaris suum* infections showed neither influence of treatment strategy nor of different cleaning methods in stables on endoparasite infections [4,9]. In the current study, no relationship was found between the hygiene measures and the prevalence of strongylid infections on the farms. Thus, those farms who used comprehensive hygiene measures were not necessarily gaining the best results with respect to endoparasite infection.

Conclusion

Without the implementation of strategic control and feedback mechanisms within the production process, including reasonable documentation, effectiveness and efficiency of hygiene measures related to worm burden can not be assessed sufficiently.

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Effect on the Endoparasitic Burden in Cattle and Sheep by Pasture's Re-wetting

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Summary

The aim of this study was to gather information on the year-round egg and larval contamination of pastures and to determine effects of the re-wetting on the prevalence of important endoparasites over the course of four years. The study was conducted on the Eiderstedt peninsula at the German North-Sea Coast. Faecal samples were analysed with routine diagnostic methods for the occurrence of *Eimeria* spp., nematode and trematode eggs and lungworm larvae three times per year: before turning out, in the middle of the grazing season and at the end. A total of 940 samples were used for statistical analysis. The overall prevalence was 26.6% for *Eimeria* spp. and 42.1% for nematodes. Eggs of the liver fluke and lungworm larvae were only detected at very low levels. Statistical analyses were performed with a generalized linear model including the fixed effects farm, re-wetting status, test day, and if it was the first summer on pasture for the animal. Significant influences on nematodes were detected for the farm, the stage of re-wetting, and the test day, while *Eimeria* spp. were in addition significantly effected by the grazing period. These findings indicate that the important parasites liver fluke and lung worm have not hitherto been affected at all by re-wetting measures and the prevalence of nematodes and *Eimeria* spp. did not increase, but even longer observations are essential to evaluate long-term consequences.

Introduction

The health of cattle, especially grazing cattle, can be considerably decreased by gastrointestinal parasites and liver flukes. The impact of nature conservation, mainly re-wetting, and the associated possible changes on infection risks in animals have not yet been described. The peninsula Eiderstedt, located in the northern part of Germany at the North Sea coast, is an area characterized by land reclamation by embankment and hence, wet grasslands. In the 1970s, together with the call for strategic control programs to abate the existing endoparasites, mainly *Fasciola hepatica*, pastures were drained on a large scale. In combination with anthelmintic treatment, the prevalence of endoparasites was reduced from 80% prevalence in 1969 to 0.005% in 1992 [6]. Because Eiderstedt is an important nature reserve for various birds breeding on wetlands, several nature protection programs have been implemented in the last decade and are still scheduled. Consequently, as part of extensification, drainage measures have been partly countermanded. Therefore, one main concern regarding cattle health is the re-emergence of endoparasites due to improved living conditions in this environment. The present study compares the excretion pattern in cattle reared on drained or re-wetted areas over four years and takes different parameters into consideration, in order to determine effects of the land-management on the prevalence of gastrointestinal endoparasites.

Animals, Material and Methods

This examination was conducted over four years on six farms. During the summer season, animals were kept on areas included in a nature protection program differentiating between three states of re-wetting according to a traffic light principle: Green areas were in an unaltered, conventional state of draining, while yellow and red areas were characterized by an increasing process of re-wetting. In all sampled animals, a short examination for clinical signs of endoparasitic diseases like diarrhea or cachexia was performed. Animals were randomly selected from the herds and sampled three times per year before turning out to graze, in the middle of the grazing season in July and at the end of it in October. After the end of the grazing season, anthelmintic treatment was conducted once a year at all farms. For sampling, the study distinguished between calves in their first grazing period (FGP) and older animals in their second or more grazing period (SGP). A total of 940 samples were analyzed in the laboratory with qualitative routine diagnostic methods for the occurrence of *Eimeria* spp., strongylid nematode eggs and *Fasciola hepatica* eggs. If a sample contained eggs of the respective species, the sample was classified as an "event"; otherwise it was classified as "no event". Data were described by relative frequencies and standard errors. According to the structure of the data, the statistical analysis was done with a generalized linear model using the procedure GENMOD of the SAS statistical software. Either the trait occurrence of nematodes eggs or the occurrence of *Eimeria* ssp. oocysts was included as dependent variables. As independent variables, the effects farm, status of the wetted area, grazing period (FGP or SGP) and the test day were included in the model.

Results

During the whole study, no clinical signs of endoparasitic diseases were detected in the examined animals. In all samples examined for the occurrence of *Eimeria* spp. oocysts, nematode and *Fasciola hepatica* eggs, and lungworm larvae, mainly the former were detected, while *Fasciola hepatica* (7/940, 0.7%) and lungworm larvae (34/940, 3.6%) were only very rarely found. Consequently, these parasites were not considered in the statistical analysis. For nematodes, 396 samples were positive regarding egg occurrence (42.1%). *Eimeria* spp. oocysts were present in 250 samples (26.6%). Taken the sampling years into consideration, the prevalence of nematodes and *Eimeria* spp. differed between the years (Fig. 1).

Animals in their first grazing period showed a higher prevalence of both nematodes and coccidian parasites compared to animals in the second or higher grazing period. Especially for *Eimeria* spp., the difference between FGP with 60.0% and SGP with 40.0% was significant; for nematodes it was less with 45.5% for FGP and 54.5% for SGP.

The prevalence of nematodes and *Eimeria* spp. was highest on areas classified as yellow regarding the stage of re-wetting with 58.8% and 73.2%. On green areas, the prevalence was lowest for both traits.

In the model, the effects farm, status of the wetted area, and test day influenced the prevalence of nematodes significantly. Neither FGP nor SGP significantly affected the occurrence of strongyles. For *Eimeria* spp., the effects farm, test day and grazing period had significant influences upon the prevalence; the status of the wetted area did not have a significant effect upon the prevalence.

The transformed estimated Least-Square-Means for the prevalence of nematodes with regard to the stage of re-wetting differed significantly, showing highest estimated prevalence on yellow areas with 58.9% and lowest with

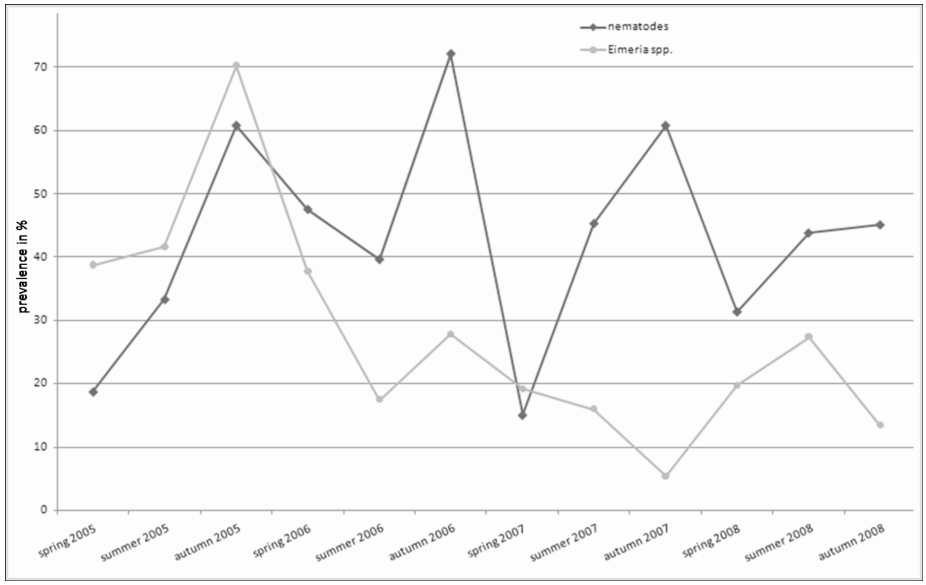


Fig. 1: Prevalence for nematodes and Eimeria oocysts in all samples (n=940) over the whole sampling period

22.6% on green areas; conditions were similar for *Eimeria* spp. The transformed estimated Least-Square-Means for nematodes were not significantly diverse with 37.1% (FGP) and 37.0% (SGP), but for *Eimeria* spp. with 24.7% (FGP) and 9.7% (SGP) the difference was significant.

Discussion

Over the four-year sampling period, *Fasciola hepatica* eggs were detected only in very low numbers; as were lungworm larvae. Machatschek et al. [5] reported an increased liver fluke occurrence after destruction of drainage systems at alpine pasture. Accordingly, the risk of a liver fluke infection might be increased by re-wetting, especially on red areas. This thesis was not corroborated in our study. The regular medical treatment against liver flukes, such as that performed on the examined farms, is the best way to maintain the status quo. Besides gastrointestinal nematodes, lungworms, especially *Dictyocaulus viviparous*, are the most important nematode parasites of cattle in temperate regions. On permanent pastures, where cattle are kept for the entire grazing season, as in this study, dictyocaulosis is expected to be an endemic disease [3]. However, in our study, the prevalence was low and animals showed no clinical signs. Nematode eggs were detected on a level comparable to other studies analyzing endoparasites in cattle herds of different age structure in a moderate climate [1]. In their second grazing year, animals showed lower prevalence of all parasites, because of the developed immunity. The excretion follows seasonal patterns with a peak in autumn at the end of the grazing seasons. This is consistent with several previous studies in temperate regions of northwest Europe, for instance Borgsteede et al.[2]. Furthermore, the assessed prevalence of coccidial parasites is in agreement with other studies in extensively reared cattle [4].

Conclusion

These findings indicate that the important parasites liver fluke and lung worm have not hitherto been affected at all by re-wetting measures. Moreover, the prevalence of nematodes and *Eimeria*

spp. did not increase. In conclusion, no clinical signs for parasitic diseases and no significantly elevated infection levels were shown after the third year of re-wetting, but even longer observations are essential to evaluate long-term consequences.

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Notes on the Prevalence and Control of Psoroptic Mange in Buffaloes at Upper Egypt

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Summary

A total of 1659 buffaloes at Various ages Sexes and localities formed the basis of work . Diagnosis of suspected infested animals with skin Scabies was made by laboratory means. 109 Cases Showed infestation with Psoroptes bovis. Various Pathological Changes of Skin lesions were reported on different Parts of body begining from Papules to Crusts Formations . Clincal Signs were described and reported . Significant Changes ($P<0.05$) in incidence of mange infestation among buffaloes at Various Localities of Assiut and Qena governorates were reported . Maximum infestation percent with the disease was noticed during the winter Season . Conversely , the percent of infestation was at minimum during the Summer months . High Significant Variation ($P<0.05$) between males and females was observed among animals at both governorates . Infestation in Calves below 6 months was low (3.81%) , followed by rise with ageing. At 1-2 Years old , infestation was 13.61% . Gradual decline of infestation with ageing was noticed in animals over 6 Years. Doramectin (Dectomax , Pfizer) given to diseased Cases in association with the application of Deltamethrin (Butox, Hoechst) to the surrounding environment gave Satisfactory results .

Introduction

Mites infestation is the most important ectoparasite of veterinary and Public health. It is a highly Contagious zoonotic disease of man and domestic animals [1] The disease has an economic value as it affects health, growth and productivity of animals [2]. The disease Causes unpleasant appearance of infested Cases that interfere to animal Commerce with low price , decrease milk yield and reduction in body weight .

Our Fundamental goal was to declare the Prevalence of the disease in buffaloes living under the ecology of Upper Egypt . Clinical , epidemiological and Strategy control were taken into account .

Animals, Material and Methods

A total of 1659 buffaloes of different ages and Sexes formed the basis of the work . The animals were belonged to Private and governomental animal Stations at Assiut and Qena governorates. Diagnosis of Clinically suspected infested Cases with mites was ascertained by laboratory means of Skin Scrapings [3]. The influence of localities ,Seasons ,Sex , age and Climatic temperature was evaluated .Statistical analysis was Carried out according to [4] .

30 infested animals Showing the typical lesions of mange and harboring Psoroptes bovis mites were treated . The animals were divided into 3 groups each of 10 Cases. The First group received one dose ($200\mu g$ /kg.B.wt.,S.C.) of Doramectin . The Second group received two doses of Doramectin with 2 weeks intervals. The third group received two doses of Doramectin with 2 weeks intervals in adjunct with the application of Deltamethrin (0.1%) as

Spray to the Surrounding environment 2 times with 2 weeks intervals.The efficiency of each regime was evaluated .

Results

Skin lesions of mange were seen on neck , brisket , withers , shoulders , thorax , abdomen, back and hind legs . Intense itching and loss of Condition were noticed in most scabied Cases.The lesions were in the form of reddish touchable Papules . The Papules oozes Serum or blood like fluid which Coagulates forming Crusts and thereafter brownish Scabs .The Chronic Cases showed alopecia ,thickened , dried and fissured skin.

Laboratory examination of the Collected Skin Scrapings revealed that Psoroptes bovis was the only detected mites of the affected Cases . A total of 109 infested Cases represented the incidence of 6.57% in both governorates (table , 1).High infestation (8.67% and 11.4%) was reported in winter (clim.temp.19.5'C and 18.5'C) , while in Summer (clim .temp .34 and 37.5^{o}c) showed the lowest infestation (1.88% and 3.5 %) in animals at Assiut and Qena respectively (table,3).Infestation in Calves below 6 months was low (3.81%) followed by rise with ageing . At 1-2 years old, infestation was 13.61% . Gradual decline of infestation with ageing was noticed in animals over 6 years (table,5).The therapeutic trials revealed the recurrence of infestation in 3 and one Cases (30 % and 10%) on the day 50 post treatment in animals of the First and Second groups respectively. On the other side , recovery rate was 100% without recurrence of infestation in animals of the third group.

Tab. 1

Localities	No. of examined animals	No. of positive cases	% of infection
Assiut Governorate	875	47	5.37
Qena Governorate	784	62	7.90**
Total	1659	109	6.57

** Significant (P<0.01)

Tab. 1: Incidence of mange in water Buffaloes in relation to localities.

Discussion

Variation in incidence of mange in animals at various localities (table,2) may be related to the ecologic and climatic changes . The changes in infestation percentage at various seasons explained the increased activity of mites in cool temperature months rather than hot ones. The significant higher prevalence of P.bovis in male hosts (table,4) may be due to the less resistance of them as compared to female ones . The difference may be due to the stimulatory effect of estrogens on immune responses while androgen may have an opposite effect [6] . Decreased incidence of infestation with ageing may be attributed to a build up of acquired immunity.

Conclusion

Psoroptes bovis (P.matalensis) was the only isolated mite Causes mange among buffaloes in Upper Egypt. Factors such as, ecology,climate ,seasons , sex and ages has great influences

Tab. 2

Places of examined animals	No. of examined animals	No. of positive cases	% of infection
Various villages			
Assiut Governorate	382	14	3.66[a]
Qena Governorate	452	38	8.40[b,a]
Veterinary service clinics			
Assiut Governorate	278	17	6.10[c,a]
Qena Governorate	432	24	5.55[d]
Veterinary hospital of Assiut university	215	16	7.45[a,b,c]

In the last column, value followed by different letter is significant (P<0.05)

Tab. 2: *Incidence of mange in water Buffaloes at various places of the governorates.*

Tab. 3

	No.	Winter	Spring	Summer	Autumn
Assiut cases					
Examined	875	265	132	218	260
Infested	47	22	8	4	13
%	--	8.67[a]	6.00[b]	1.83[c]	5.00[d]
Qena cases					
Examined	784	219	196	170	199
Infested	62	25	13	6	18
%	--	11.4[f]	6.6[g]	3.5[h]	9.00[i]

In each row, value followed by different letter is significant (P<0.05)

Tab. 3: *Incidence of mange in water Buffaloes in relation to seasons.*

Tab. 4

	Assiut			Qena		
	No. of		% of	No. of		% of
	Examined	Positive	infection	Examined	Positive	infection
Males	322	19	5.90**	228	27	11.85**
Females	553	28	5.06	556	35	6.11

*** Significant (P<0.01)*

Tab. 4: Incidence of mange in water Buffaloes in relation to sex.

on the prevalence and activity of the parasite . The ideal regime for controlling mange in animals depends on rapid detection, isolation and treatment of infested animals together with the application of efficient acaricide to animal's environment. Repeated therapy seemed to be important according to the life cycle of the parasite on and off the host.

Tab. 5

	Assiut			Qena		
	No. of		% of	No. of		% of
	Examined	Positive	infection	Examined	Positive	infection
< 6 months	150	4	2.66[a]	130	5	3.81[g]
6-12 months	157	8	6.10[b]	120	10	8.33[h]
1-2 years	135	11	8.14[c]	125	17	13.61[i]
2-4years	141	12	8.65[d]	136	16	11.76[j]
4-6 years	147	10	6.80[e]	128	11	8.59[k]
> 6 years	145	2	1.37[f]	145	3	2.00[l]

In each row, value followed by different letter is significant (P<0.05)

Tab. 5: *Incidence of mange in water Buffaloes in relation to age.*

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Does histomonas meleagridis affect establishment and development of heterakis gallinarum in chicken?

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Summary

Effects of insoluble (I-NSP) and soluble (S-NSP) non-starch polysaccharides on establishment and development of *Heterakis gallinarum* in chicken were investigated in two experiments. Chickens were either left un-treated (Experiment I) or treated against *Histomonas meleagridis* (Experiment II). Treatment with dimetridazole against *H. meleagridis* significantly ($p<0.05$) increased *H. gallinarum* infection rate, total worm burden and female worm length. Infection with *H. gallinarum* significantly impaired body weight development of the chicks ($p<0.0001$). *H. meleagridis* aggravated this effect. Animals infected with both parasites had lower body weights than those only infected with *H. gallinarum* ($p<0.05$). Both NSP supplemented diets resulted in lower ($p<0.0001$) body weights when compared with a Control diet, S-NSP being inferior to I-NSP ($p<0.05$). The results indicate that *H. meleagridis* harms the definitive host as well as *H. gallinarum*. NSPs favour *H. gallinarum* infection, whereas S-NSP seems to intensify Histomonas infection, which then impairs establishment and survival of *H. gallinarum*.

Introduction

Floor- and outdoor-rearing systems bear an increased risk of parasitic infections in poultry [1]. Transmission of *H. meleagridis* among individuals within or between chicken flocks solely depends on the presence of *H. gallinarum* [2]. This worm parasitizes the caeca and transmission of Histomonas is brought about by the ingestion of excreted and embryonated eggs of *H. gallinarum* [2]. Therefore, control of *H. gallinarum*, in spite of its often neglected per se pathogenicity, may also contribute controlling Histomonas. Dietary fibres, varying in intestinal fermentability, have been reported to influence major helminth infections of pigs differently [3]. The present study aims at estimating the effects of insoluble and soluble non-starch polysaccharides, as fibre sources in diet, on *H. gallinarum* infection in chicken being treated or not treated against *H. meleagridis*.

Material and Methods

Design, animals and experimental infections. Two experiments (I, II), arranged as 2x3 factorial designs with experimental *H. gallinarum* infection (not infected/infected) and diet [Control (CON), I-NSP (+ insoluble NSP), S-NSP (+ soluble NSP)] as the main factors, were conducted. The CON diet supplied energy and nutrients according to feeding standards for growers. I-NSP contained additional pea bran meal and S-NSP additional

chicory root meal (amounting to 9% of the feed mixtures respectively) as NSP-sources. One-day-old Lohmann Selected Leghorn chicks (n=184; n=224 for I and II, respectively) were randomly divided into 3 feeding groups being fed ad lib. either CON or I-NSP or S-SNP for 11 weeks. At 3 weeks of age, 50% of the chicks of each feeding group were infected with 200 embryonated *H. gallinarum* eggs (positive for Histomonas). In II, animals were additionally treated with dimetridazole (0.05% w/v) via drinking water from 2 day before infection to day 7 p.i.. Animals were individually weighed weekly and group feed consumption was determined daily.

Necroscopy and worm harvest. Necropsies were performed at 2, 3, and 5 weeks p.i. to determine macro- and microscopical lesions and to detect *H. meleagridis* in liver and caecal sections. The residual animals were slaughtered 8 weeks p.i. After slaughtering, the gastrointestinal tract was removed and worm burden was quantified by the procedure described by Gauly et al. [4]. Worm length was measured by measuring a maximum of randomly selected 10 females per each animal.

Statistics. Infection rate of the experimentally infected animals was analyzed with the Chi-square test. Total worm burden was analyzed with the non-parametric Kruskal-Wallis test whereas female worm length was analyzed with one-way ANOVA to estimate effects of the diets. Pair-wise comparisons between the infected groups within each experiment were performed. Furthermore, each feeding group was compared between the two experiments in order to estimate the effect of dimetridazole treatment on infection rate, total worm burden and female worm length with the above-mentioned statistical tests. Growth performance of the chicks was evaluated for a period of five weeks (p.i.), covering the pre-patent period of the nematode. Data for body weight development were analyzed with a repeated-measures ANOVA using a mixed model that included effect of diets (CON, I-NSP, S-NSP), *H. gallinarum* infection (infected, not infected), experiment (I and II), age of animals after infection (1-5 weeks), individual random effect of each animal and residual error term. All post-hoc comparisons were performed with the Tukey test in cases where a significant effect ($P<0.05$) of any factor was encountered. All the statistical analyses were performed with SAS [5].

Results

Effects of dimetridazole treatment and the diets on H. gallinarum infections. Treatment with dimetridazole (Exp. II) significantly increased ($p<0.05$) *H. gallinarum* infection rate, total number of worms and female worm length in all feeding groups. CON led to lower ($p<0.05$) infection rate than both NSP supplemented diets (tab. 1). Moreover, CON resulted in lowest and S-NSP in highest worm burden ($p<0.05$), and feeding I-NSP instead of S-NSP was associated with longer female worms ($P<0.05$). Without dimetridazole treatment (Exp. I), I-NSP caused higher infection rate than S-NSP ($p<0.05$), and total worm burden was higher with I-NSP instead of S-NSP ($p<0.05$).
While single*H. gallinarum* infection, after treatment with dimetridazole, induced no lesions (Exp. II), untreated animals (Exp. I) showed Histomonas-typical lesions such as cheesy core and necrosis of the caeca and liver, respectively, during the first 3 weeks after infection.

Effects of the diets and infection(s) on host growth performance. In both experiments, infection with *H. gallinarum* significantly impaired body weight development of the chicks ($p<0.0001$), however, this effect was

aggravated in dimetridazole un-treated animals ($p<0.05$). Overall average body weight of infected and un-treated animals was 409 g whereas non-infected controls had an average of 457 g in experiment I. In experiment II, Infected and treated animals had an average of 435 g and non-infected controls had an average of 449 g body weight. Both NSP supplemented diets resulted in lower ($p<0.0001$) body weight development compared with CON, S-NSP being inferior to I-NSP ($P<0.05$). Overall average body weight of animals fed CON, I-NSP and S-NSP were 451 g, 437 g and 425 g, respectively. Significant interactions were not observed neither between effects of diet and infection, nor between effects of diet and experiment ($p>0.05$). A triple interaction effect of diet, infection and experiments was also absent ($p>0.05$).

Discussion

Dimetridazole is known to be highly effective against *H. meleagridis* in chicken [6]. Therefore, it can be assumed that the Histomonas-positive *H. gallinarum* eggs resulted in a dual infection in experiment I, as confirmed by microscopical lesions and detection of *H. meleagridis* in liver and caecal sections. In experiment II, the animals were only infected with *H. gallinarum*. The results of the present study clearly indicate that *H. meleagridis* decreased establishment and development of *H. gallinarum*. It might be hypothesized, that the harmful effect of *H. meleagridis* on *H. gallinarum* stimulates a selection pressure that may result in a smaller but more suitable worm population for transmission of Histomonas.

The type of diet seems to play an important role in the interaction of the two parasites, since feeding S-NSP resulted in lower infection rate and total worm burden than I-NSP in case of dual infection (Exp. I), whereas this diet resulted in highest worm burden when Histomonas was eliminated (Exp. II). This finding may indicate the involvement of the caecal bacterial microflora in the interaction of the two parasites. *H. meleagridis* attains full virulence in the caeca of chicken only in the presence of several bacteria species [2,7]. Springer et al. [7] showed that Heterakis larvae were not able to survive when injected into caeca of gnotobiotic birds, indicating that the role of bacteria is more vital for the survival of Heterakis than of histomonads. S-NSP mainly consisted of inulin, a highly fermentable substance, which has been shown to increase bacteria count in turkeys [8]. Abundance of intestinal bacteria after feeding S-NSP may have favoured the development of Histomonas thus imposing selective pressure on *H. gallinarum* and resulting in the lower worm burden in these animals. On the other hand, S-NSP may have favoured the development of *H. gallinarum* in the absence of histomonads as indicated by the highest worm burden in this group of experiment II.

Although *H. gallinarum* infection impaired body weight development of the animals, *H. meleagridis* may have aggravated this effect. Although S-NSP resulted in lower infection rate and worm burden than I-SNP in experiment I, I-NSP led to higher body weight development than S-NSP diet indicating that S-NSP intensified Histomonas infection which then impaired establishment and development of H.gallinarum, exerting an additional negative effect on host growth performance.

Conclusion

The results indicate that *H. meleagridis* harms the host animal as well as *H. gallinarum*. NSPs favour *H. gallinarum* infection. S-NSP seems to intensify Histomonas infection, which then impairs survival and development of *H. gallinarum*.

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Chapter 18

Poster

Comparison of aerobic composting and anaerobic stabilisation from the parasitological point of view

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Summary

The generally known high tenacity of endoparasitic propagative stages and their resistance to various environmental biotic and abiotic factors led us to study the problems of the survival of parasitic germs during the composting process (in the channels and piles) and anaerobic stabilisation (in the piles) of organic wastes. The different waste types, potentially contaminated with parasitic and other pathogenic germs, were used for the recycling: a by-product from Penicillin production (mycelium), animal excrements, sludge from waste water treatment plant, wastes from beer production and plant substrates. The "artificial contamination of trough and piles" with model non-embryonated *Ascaris suum* eggs and *Eimeria* oocysts served to make sure that there was a sufficient number of positive samples in our observations. In aerobic composting in the compost channels in thermophilic temperature range, high temperatures (up to 70°C) and changes of physical and chemical parameters resulted in total devitalisation of protozoa germs as early as after 24 hours and helminth germs after 48 hours. Total devitalisation of *A. suum* eggs in compost piles was observed within 7 days. Anaerobic stabilisation of organic waste at temperature max. 35 °C has only decreased the viability of *A. suum* eggs (by 21 %). Only aerobic composting in thermophilic range reduces a risk of dissemination, survival and potential spread of endoparasitic germs in the environment, as organic wastes processed by such composting are hygienically sound.

Introduction

Animal husbandry, in relation to humans, is connected with whole range of ecological and medical problems. Animals are one of the most frequent sources of environmental contamination. Manure and other wastes from agriculture often contain high concentration not only of animal but also of human pathogens. Animal excrements frequently contain bacteria of the family Enterobacteriaceae, protozoa and eggs or larvae of enteronematodes most of which are of zoonotic character. It has been common practice for thousands of years to dispose of excrements of infected animals on land. Through these faeces the agents of parasitozoonoses spread into the environment. Therefore a direct contact with infected animal but also contaminated environment or contaminated food chain (water, vegetables, vegetable organic wastes – straw, sawdust) is considered a potential risk factor. On the other hand, animal excrements can supply essential plant nutrients and serve as a soil amendment by adding organic matter. With regard to potential presence of pathogenic micro-organisms in these substrates should be viewed as possible health hazard. One of the suitable methods for recycling organic wastes compatible with the environment is composting [1, 2, 3]. Only relatively few studies investigated the impact of composting conditions on the survival of parasite stages. The study described here evaluated the effect of aerobic composting (in the

channels and piles) and anaerobic stabilisation (in the piles) of different organic wastes on the survival of parasitic agents.

Material and Methods

Investigations were carried out under operating conditions in 4 composting factories (CF1-4) in the Slovak Republic to determine the effect of composting of different organic wastes on the survival of parasitic germs. The different waste types, potentially contaminated with parasitic and other pathogenic germs, were used for the recycling: CF1 - by-product from Penicillin production (mycelium), straw, sawdust; CF2 - poultry excrements, straw; CF3 - waste from beer production, sludge from WWTP, straw, wood chips, sawdust; CF4 (anaerobic stabilisation) - wastes from public areas, parks and playgrounds (dog excrements and other). The organic wastes were "artificially contaminated with unembryonated *A. suum* eggs and *Eimeria* oocysts in order to make sure that there was a sufficient number of positive samples in our observations. Eggs and oocysts were filled into polyurethane carriers, prepared according to Plachý and Juriš [4], at adose of 1000 eggs and 30000 - 50 000 oocysts per carrier. For mechanical protection the carriers were placed into perforated PET bottles (50 ml) before introducing them into the piles or channels. The controls with eggs and oocysts were incubated in distilled water for the same time as the exposure time in the experiments. The viability of exposed parasitic germs was determined by incubation up to the embryonated stage in athermostat at 26 °C for 21 days. After exposure in thetrough, samples for parasitological and physical and chemical examination were collected. The methods used for monitoring physical and chemical properties of organic wastes corresponded to the STN 465735 [5]. The C content was calculated according to the content of OM by the method of Navarro et al. [6], and the C:N ratio was calculated. Three samples were taken and analysed at each of the given sampling intervals. Significance of differences between experimental and control groups were determined using Dunnet's multiple comparison test at the levels of significance 0.05; 0.01; and 0.001 [7].

Results and Discussion

Ascaris suum eggs are amongst the helminth eggs most resistant to environmental factors. Their cellular wall is enveloped with an outer layer formed by acid polysaccharides and proteins, central layer consisting of proteins (25%) and lipids (75%, particularly alpha glycosides). Thus this resistant cell wall protects *A. suum* eggs against effects of chemicals and drying. In general context is ascariasis, caused by the type species *Ascaris lumbricoides* and*Ascaris suum*, of major human and animal health importance in many parts of the world. Coccidia oocysts were chosen for comparison because they can also survive for a long time in the environment thanks to their double layered cell wall and are specific for particular species.

In aerobic composting in the compost channels, high temperatures (up to 70°C) and changes of physical and chemical parameters resulted in total devitalisation of protozoa germs as early as after 24 hours and helminth germs after 48 hours (Tab. 1). Total devitalisation of *A. suum* eggs in compost piles was observed within 7 days (Tab. 2-3). The key factors for a viability of parasite eggs are temperature, humidity, changes in physical and chemical parameters of the substrate and a suitable carbon - nitrogen ratio (C:N - 15-30:1). After 150-days of composting, 63.47 4.15 eggs of *A. suum* (Tab. 4) preserved their viability owning to the effect of anaerobic conditions, low temperature (to 40 °C), low C:N

ratio (to 15:1) and changes in the physico-chemical propreties of the composted organic wastes. Similarly, Buchwalder and Wolmer [8] detected devitalization of all *A. suum* eggs during 3 days of composting. Our results also corresponded with those of Steger et al. [9]. The authors reported that during the thermophilic phase of composting thick-walled helminth eggs and protozoa oocysts or sporocysts are destroyed. In contrast to our results Zhu [2] detected that all roundworm eggs were destroyed after 63 days of composting.

Tab. 1: Damage of *A. suum* eggs and *Eimeria* oocysts during aerobic composting in the channels (CF1)

Exposure (days)	Damaged ($\bar{x}$ %±SD)	
	A. suum eggs	*Eimeria* oocysts
0 (control)	16.02±2.61	9.11±0.75
1	98.74±1.18***	100***
2	100***	100***
3	100***	100***
4	100***	100***
7	100***	100***
8	100***	100***
28	100***	100***

***Significance at the level P<0.001

Tab. 2: Damage of *A. suum* eggs during aerobic composting in piles (CF2)

Exposure (days)	Damaged *A. suum* eggs ($\bar{x}$ %±SD)
0 (control)	16.02 ± 2.61
1	28.16 ± 1.18
2	58.26 ± 4.63**
3	69.11 ± 7.22***
4	98.50 ± 3.65***
7	100***
9	100***
14	100***
17	100***
24	100***

**Significance at the level P<0.01, ***Significance at the level P<0.001

Tab. 3: Damage of *A. suum* eggs during aerobic composting in piles in winter and summer seasons (CF3)

Exposure (days)	Damaged *A. suum* eggs ($\bar{x}$ %±SD)	
	Winter season	*Summer season*
0 (control)	13.80±2.61	12.62±2.23
1	64.13±3.46**	ND
3	ND	16.90±9.75
4	88.30±5.47***	45.33±18.71*
5	92.50±6.52***	84.36±18.32***
6	100***	100***
7	100***	100***
10	ND	100***
11	ND	100***
36	100***	ND
62	ND	100***
104	100***	ND

ND – not detected, *Significance at the level P<0.05, **Significance at the level P<0.01, ***Significance at the level P<0.001

Tab. 4: Survival of *A. suum* eggs during anaerobic stabilisation (CF4)

Stabilisation (days)	Damaged *A. suum* eggs ($\bar{x}$ %±SD)
0 (control)	15.52±1.82
35	19.22±1.42
60	24.09±3.66
110	28.60±1.45*
150	36.53±4.15*

*Significance at the level P<0.05,

Conclusion

1. A total devitalisation of non-embryonated *A. suum* eggs was observed after 48 hours of thermophilic aerobic composting in the compost channels. Protozoa were damaged as early as after 24 hours.
2. A total devitalisation of *A. suum* eggs occurred within 7 days of composting in the compost piles.
3. The anaerobic stabilisation failed to devitalise completely the model parasitic stages.
4. From the hygienic and epidemiologic point of view, wastes treated by aerobic thermophilic composting are safe and suitable for further use as fertiliser for direct application on the soil causing no subsequent contamination of surrounding ecosystem with endoparasite agents.

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Inactivation of *Ascaris suum* eggs in sewage sludge composting process

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Summary

The effect of sewage sludge composting process on the inactivation of eggs of the enteric parasite *A, suum* was investigated. Carriers containing eggs were placed in the material in a container and then in a windrow. The study confirmed a high effectiveness of composting process in elimination of *A. suum* eggs. In the summer cycle, invasive eggs were not detected in the carriers after 7 days. In the autumn cycle, the presence of living eggs in the carriers in the top and middle layers was not observed in the 6th day of the process. In the bottom part of the biomass inactivation proceeded slower -14 days in the container and 33 days in the windrow.

Introduction

Eggs of nematodes of the genus *Ascaris* are used in the study as an indicator of parasitic contamination of the composted material with intestinal parasites [5,8]. A complex structure and highly selective permeability of egg shells make them remarkably resistant to unfavourable environmental conditions [9,10]. Although their development in sludge is unlikely, in good conditions in soil they can retain their invasiveness [4]. One of the methods of biowaste utilization is composting. If the process is conducted incorrectly, the final product may contain both pathogenic bacteria and intestinal parasite eggs. Such a compost poses a hazard for the environment and people. The aim of the study carried out was to estimate the effectiveness of inactivation of *A. suum* eggs during the process of sewage sludge composting.

Material and Methods

The research was conducted in a composting plant of waste from municipal green areas and sewage sludge working in the container technology. Properly fragmented and mixed material was placed in containers, where a stage of intensive composting proceeded for approximately 14 days. Then the biomass was removed from the containers and formed in a windrow. In order to provide a proper aeration, the windrow was turned mechanically every two weeks. The stage of the biomass maturation lasted about 4-6 weeks.

The hygienization level of waste subjected to composting was tested on the basis of the inactivation of the enteric parasite *Ascaris suum* eggs. 1 ml of their suspension was introduced into perlon bags of 28μm pore diameter. Prepared carriers were placed in the composted material in a container at the top, middle and bottom layer of the biomass. During the stage of intensive composting the carriers were removed several times from each layer and subject to analyses. After forming the windrow, part of the carriers was transferred into it from the container, and additional carriers were introduced, containing the eggs of the helminth

A. suum. Every several days the carriers were removed, cut out and placed in sterile Petri dishes. The bags were poured with water and incubated for 30 days at a temperature of 28 °C. The dishes were opened in order to provide oxygen and filled up with water. After the incubation, 300 eggs were observed under the microscope, and the percentage of invasive eggs, containing a live larvae, was calculated. The control was the eggs incubated directly after taking from the fragments of *A. suum* uteruses. The results obtained were subjected to statistical analysis using the program Statistica. Regression lines were drawn and were a basis for the calculation of the theoretic time needed for the inactivation of *A. suum* eggs.

Results and Discussion

A study carried out in two cycles, summer and autumn, showed a high effectiveness of composting process in eliminating *A. suum* eggs. In the summer cycle, the presence of living eggs of the enteric parasite was not detected in the carriers as early as after 7 days of the intensive composting phase in the container. Similarly, in additional carriers placed in the windrow, in the upper layers of the biomass, the eggs were already inactivated after 6 days (Fig.1). Only in the bottom layer of the composted material the process of sanitization proceeded slightly slower. The full elimination of eggs capable of further development was obtained after 21 days of the composting maturing phase in the windrow (Tab.1). Such situation was probably related to the action of the high temperature generated during the process. It is reported in the literature that this is a key factor destroying pathogenic microorganisms and intestinal parasites in composted material [7,11]. The inactivation of *Ascaris* eggs can be obtained after a period that ranges from several hours to several days of action of the temperature above 55 °C [1,3]. During the intensive composting stage, an increase in temperature above 50 °C was observed in the upper parts of the material. It remained for several days and then decreased gradually. After removing the material from the container and making the windrow, another increase in temperature above 50 °C was observed (Fig.3). Such thermal conditions determined a fast inactivation of *A. suum* eggs as early as at the intensive composting in containers.

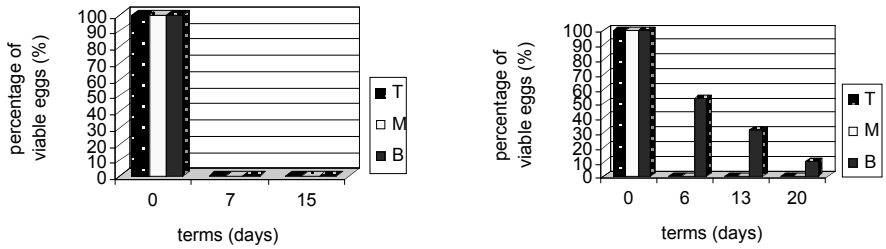


Fig.1: Inactivation of A. suum during composting process in a)the container and b) the windrow in summer.

In the autumn cycle, the elimination of nematode eggs occurred very quickly as well, in spite of not obtaining the appropriate, high temperature values. After 6 days, the presence of eggs that are able to develop afterwards in the carriers in the upper and middle layers were not estimated. In the bottom layer, at the same time, the percentage of living eggs fell to 68% (Fig.2). In additional carriers placed in the windrow, the survival time in the upper and middle layers amounted to 25 and 24 days respectively. The eggs inactivation proceeded slowest in the bottom layer of the biomass - 33 days (Tab.1). A relatively fast process of

dying of *A. suum*eggs, in spite of inconvenient sanitary conditions, indicates the effect of still other factors on them, apart from the high temperature. The presence and activity of microorganisms naturally inhibiting compost, which delay egg development, are essential factors. Aerobic conditions which deteriorate in the course of the composting process, inhibiting the embryonic development of eggs, also of great importance [2,6].

Tab.1: The dynamics of A. suum eggs inactivation in the composted material.

Cycles	Location carriers	Layers of biomass	Regression equations	r^2(%)	Survival of eggs (days)
summer	container	top	-	-	nd (after 7 day)
		middle	-	-	nd (after 7 day)
		bottom	-	-	nd (after 7 day)
	windrow	top	-	-	nd (after 6 day)
		middle	-	-	nd (after 6 day)
		bottom	y= -4.3x+90.71	92.16	21
automn	container	top	-	-	2*
		middle	-	-	2*
		bottom	y= -6.92x+100.07	96.04	14
	windrow	top	y= -4.13x+ 102.14	98.01	25
		middle	y= -4.27x+ 101.62	98.01	24
		bottom	y= -3.36x+ 110.12	90.25	33

nd- no occurrence detected of viable eggs

*- the day of the process when the eggs were last isolated

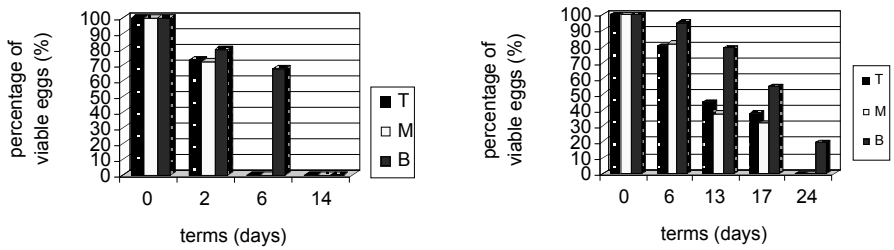


Fig.2: Inactivation of A. suum during composting process in a)the container and b) the windrow in autumn.

Conclusion

The research indicated the high effectiveness of the inactivation of *A. suum* eggs during sewage sludge composting. The simple method applied in the experiments enables the process of compost sanitization to be controlled directly. However, attention should be given to obtaining of the required temperature in the whole biomass in order to secure the environment against contamination.

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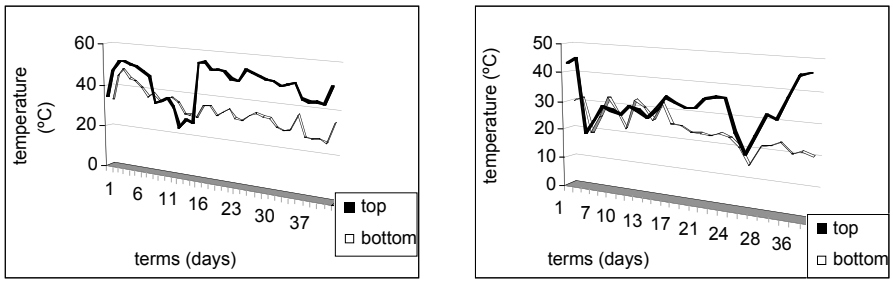


Fig.3: Temperature distribution in the composted biomass in the container and the windrow during a) summer and b) autumn.

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Estimation of genetic parameters for resistance to avian pathogenic *Escherichia coli* (APEC)

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Summary

White leghorn hens were challenged with avian pathogenic *Escherichia coli* (APEC). Survival ability as well as survival time, were used to estimate the genetic components for disease susceptibility caused by APEC. The estimated heritability for survival ability was at 13 percent and for 11 percent survival time. Based on literature studies, candidate genes were chosen for association analysis. A significant association between the Toll-like Receptor 5 and the estimated breeding value for resistance to APEC was observed.

Introduction

Avian pathogenic *Escherichia coli* (APEC) infection results in a significant reduction of performance and liveability of chicken, leading to a distinct economic loss in poultry production. Infection with APEC generally begins as a localized infection of the air sac which in turn may spread to other internal organs resulting in systemic infection. One of the strategies to combat and control this disease is to breed chickens which are more resistant to APEC. The main objective of this research work was to estimate genetic parameters of APEC diseases for development of breeding programmes.

Material and Methods

353 Seventy-week old White leghorn hens (Lohmann) were infected intra-tracheally with a characterized APEC field strain IMT5155 (O2:K1:H5; multilocus sequence type 95) using 10^8 doses corresponding to the established model of infection [1]. The time of mortality occurrence and the number of animals which survived 220 hours of post infection was recorded. Statistical and genetical analysis of recorded mortality data was carried out by the application of a linear logistic model as well as using survival analysis. Compared to the logistic model the analysis of post infection data using survival analysis takes the individual survival time into consideration, whereby the surviving animal is considered as a censored observation. The data was then analyzed with the GLIMMIX macro [2], AsReml software packages [3], and Survival Kit [4] including the effects of tier and hatch. In further analysis based on literature studies, candidate genes NRAMP, iNOS, TLR-4, IL-8 and defence genes with respect to APEC were chosen. These candidate genes were selected on the basis of experimental evidence from mouse studies on uropathogenic *E. coli* which are closely related to APEC and from studies in the chicken in Salmonella infection models. They were used in a gene substitution statistical model for their associations to

the estimated breeding values. The data was analysed using a mixed model including the random effect of sires.

Results

The results of analysis of the logistic model show that there was no significant effect of hatch on survivability. However, there was a highly significant effect of tier on survivability suggesting that the environmental condition have a distinct effect on susceptibility to APEC diseases. It is not clear here which specific environmental effect could be responsible for the increase in mortality. The mortality of artificially infected chicken amounted to 62 percent, demonstrating that there is a high phenotypical variability of APEC inducing consecutive symptoms. The heritability for mortality as an indicator of resistance to APEC was estimated to 0.13 ± 0.07, meaning that 13 per cent of the estimated total variability of susceptibility to the disease is based on additive genetic reasons. The genetically determined susceptibility for individual animals is expressed as breeding values which means in the logistic model, the individual genetic potential for survival ability, after APEC infection. The distribution of estimated breeding values is illustrated in figure 1. Estimated breeding values for survival ability are in the range of 0.29 to 0.45. Despite the low number of challenged animals, the distribution of breeding values shows an extended range for individual genetic potential for susceptibility to APEC.

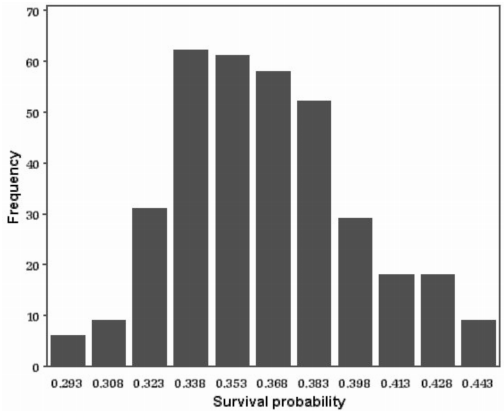


Fig. 1: Distribution of estimated breeding value for survival probability

The result for survival analysis using Cox regression model, including fixed effect of hatch and tier are similar to the logistic model. There was a highly significant effect of tier on hazard rate. The effect of hatch was not significant as detected before in the logistic model. The estimated heritability using the survival analysis is 11 percent and is somewhat lower than estimates in the logistic model. The distribution of estimated breeding values using survival analysis illustrated as relative risk is given in figure 2. In contrast to the nearly normal distribution of breeding values in the logistic model, a great part of animals in the survival analysis shows a similar high relative risk of mortality.

These reflect the breeding value of a part of animals with a high hazard rate or dying very early after an artificial APEC infection. On the other hand, the group of animals that survived the challenge or had a long survival time shows distinctly lower risks of mortality. The variation of estimated breeding values, the individual genetic potential for relative risk, ranged from 0.675 to 1.125.

The result of association analysis presented here (table 1) obviously shows that only the Toll-like Receptor 5 with the fragment

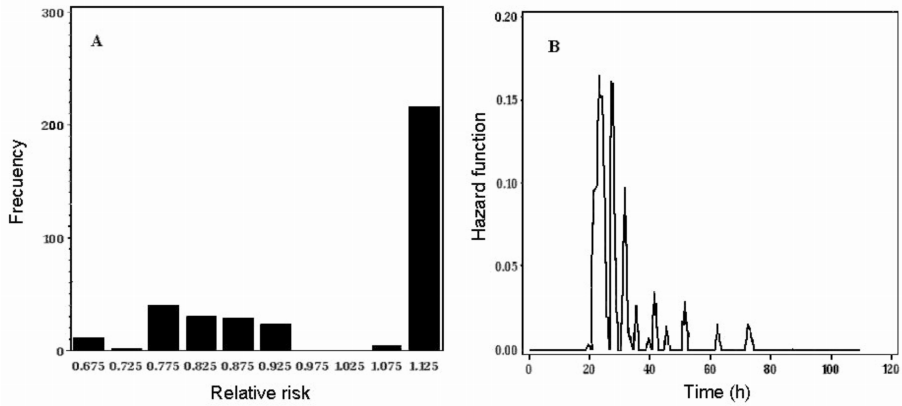


Fig. 2: Distribution of estimated breeding values for relative risk (A) and illustration of hazard function (B)

Tab. 1: Regression coefficient with F-value and significance level for different genes in relation to estimated breeding value

	Logistic regression			Survival analysis		
Gene	Coefficient	F-value	P-value	Coefficient	F-value	P-value
GAL1_1_474	-0.0004	0.02	0.8877	-0.0104	0.21	0.6498
GAL2_567_946	-0.0005	0.12	0.7341	0.0060	0.23	0.6310
TLR5_1834_2325	0.0126	3.09	0.0799	-0.1060	4.03	0.0461
NRAMP_1275_1649	-0.0015	0.23	0.6304	0.0020	0.01	0.9336
NRAMP_274_669	-0.0001	0.10	0.7575	-0.0001	0.00	0.9691
IL2_1_466	-0.0076	0.50	0.4819	0.0750	1.36	0.2465
TLR5_267_706	0.0041	0.37	0.5557	-0.0590	1.34	0.2476
IL2_5527_5920	-0.0076	0.50	0.4808	0.0750	1.36	0.2465

_1834_2325 has a tendentially significant effect (p=0.079) on survival ability as well as a significant effect on relative risk (p=0.046) by artificial infection with APEC. The estimated regression coefficient for survivability exhibited an average gene substitution effect of 1.27 percent and for relative risk -0.106 points.

Discussion

The size of phenotypical variability in survival ability depends on the degree of the severity of infection as well as on the genetically determined resistance of host and virulence factors of the pathogen in interaction with environmental conditions. A major part of the detected variability is due to genetically determined host immune response to the pathogen, which could be expressed as estimated heritability. The estimated heritability using both models is on a low level in general, but in the usual range for fitness traits. Taking into consideration the very high reproductive ability and very short generation interval in chicken, it is feasible to select animals successfully for resistance to APEC disease. However, such a selection strategy requires as a precondition the recurrent challenging of a large number of animals from pedigreed chicks of different lines with APEC, which is not feasible from an animal welfare point of view and also in regard to breeding costs. This makes the relevance of development and application of molecular

genetics tools to improve genetic predisposition of disease obvious. In respect to candidate gene association with susceptibility to APEC there was only a significant association between the Toll-like Receptor 5 and estimated breeding value for resistance to APEC. Taking into consideration the range of variation for breeding values of 15 for living ability, the effect of a 1.27 percent allele substitution, means that 8.5 percent of estimated breeding value is due to associated gene effect. According to the estimated breeding value for relative risk estimated by applying survival analysis, the effect of the associated gene accounts for 23 percent. After verifying the resistance effect using a higher number of animals and a higher polymorphism level, the Toll-like Receptor 5 gene can be used for gene assisted selection in order to improve resistance by selection.

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Part VIII

Human Health and Zoonoses

Chapter 19

Block 4C

Detection and identification of airborne bacteria in a German turkey stable

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Summary

Today's intensive livestock production is often accompanied by high concentrations of airborne microorganisms, their metabolic products and cell constituents at working places. Exposure to those substances may affect the respiratory system leading to health effects. So far, the microbial communities in livestock stable's air have not been studied in detail. For this reason, bioaerosol samples from two turkey stables were investigated by cultivation-based and molecular methods to determine the concentrations of microorganisms and the compositions of bacterial communities. Depending on the used media, colony forming unit concentrations ranged between $8x10^4$ and $8x10^5$ CFU m^{-3} air. The corresponding total cell concentration (DAPI staining), in contrast was two magnitudes higher, showing the clear underestimation of the cultivation based quantification approaches. Isolates were identified by 16S rRNA gene sequence analyses. Sequences of five isolates were found to be closely related to bacterial species *Acinetobacter johnsonii*, *Aerococcus viridans* and *Pantoea agglomerans* which are risk group 2 organisms [1] and known as causative agents for pulmonary diseases. These results show that organisms not only at high concentrations are found, but also species of potential health risk can be detected in livestock stables.

Introduction

Occupational safety has become more important in agriculture with regard to human respiratory tract diseases (e.g. farmers lung, mushroom workers lung). Therefore aerosols are studied in order to determine potential health hazards by the analyses of gases, allergens, and microorganisms. The organic fraction of these aerosols is often composed of non-viable particles, originating from e.g. litter, manure, feed, silage, and feather and viable particulates like fungi and bacteria ("bioaerosols"). Today's large-scale poultry production with densely stocked and enclosed production buildings is often accompanied by high concentrations of airborne microorganisms. Airborne microorganism concentration in poultry stables has been found to reach up to 10^8 cfu m^{-3} air [2, 3] and between 10^7 to 10^{10} cells m^{-3} air, respectively [4]. But not only the total (viable) count but also the composition of the present bacteria has to be examined for risk assessment. So far, the microbial communities in livestock stable's air have not been studied in detail. For this reason, bioaerosol samples from two turkey stables were investigated by cultivation-based and molecular methods to determine the microbial concentrations and the composition of bacterial communities.

Material and Methods

Bioaerosol samples were taken in a conventional farmed turkey fattening facility (2 x 7,000 animals) in Germany. The complex

consists of two equally constructed barns. Turkeys were housed separately by male (n=6526) and female (n=3,500) birds and were 7 weeks old at the time of aerosol sampling.

Bioaerosol sampling was conducted according to VDI 4252 part 2 and [5, 6] in the middle of the barns. Cells from polycarbonate filters were detached into isotonic NaCl using a Stomacher 80 (Biomaster, Seward, UK), impinger liquids were directly used for further analyses. For isolation and quantification of culturable bacteria, Tryptone Soy Agar (TSA), MacConkey-Agar (McC) and Actinomycete Isolation Agar (AIM) were used. Bioaerosol sample liquids were serially diluted and aliquots of 0.1 ml plated onto media plates. Visible colonies were counted after an incubation time of 3 days (McC), 7 days (TSA), and 14 days (AIM) at 37 °C, respectively.

From these plates, isolates were obtained and purified. After DNA extraction according to [7], 16S rRNA genes of the obtained isolates were amplified and sequenced.

The 16S rRNA gene amplicons from DNA directly extracted from three different bioaerosol samples, were used to generate one *E. coli* clone library per sample (using the pGEM** T vector system kit from Promega, USA). For analyses, from each clone library 60 clones were randomly picked. Gene inserts of three times 60 randomly picked clones were sequenced (900bp) and phylogenetically assigned.

Quantification of total cell count (TCC) was performed by DAPI fluorescence staining according to [8] followed by microscopic analysis.

Results

Quantification of airborne bacteria. Depending on the applied culture media, concentrations of abundant cultivable bacteria varied between 0.8 and 8 x 10^5 CFU m^{-3} air. The corresponding TCCs were found to be up to two magnitudes higher. Concentrations of airborne bacteria (CFU and TCC) sampled by the impinger device were always below the corresponding concentrations obtained by filtration sampling.

Community analyses. Sequencing of the 16S rRNA genes of 28 isolates showed highest sequence similarities to *Nocardiopsis* sp. (n=4), *Bacillus* sp. (n=4), and *Brevibacterium stationis* (n=3). Five isolates were found to be next related to species of potential health risk: *Aerococcus viridans* (n=1), *Acinetobacter johnsonii* (n=2), *Pantoea agglomerans* (n=1) and *Shigella flexneri* (n=1).

Most abundant sequences detected by cloning analyses showed highest similarity to 16S rRNA gene sequences to species of the genera *Aerococcus*, *Lactobacillus*, and *Megamonas*. The dominance of these genera within the clone libraries was found for both sampling locations independently from the applied sampling method. In addition, in all samples high proportions of sequences not assignable to yet cultured bacteria were found. However, these showed similarities to poultry intestinal microbiota gene sequences obtained by molecular investigations. *Salmonella* spp. were not found, neither by cultivation nor by molecular methods.

Discussion

Up to now, standardized methods for quantitative as well as qualitative exposition assessment rely on cultivation based methods. In our study, both cultivation and cultivation-independent approaches were applied to investigate concentrations and compositions of airborne bacteria in poultry stable air. Concentrations of airborne microorganisms in investigated stables' air are comparable to those found in earlier investigations [9-11]. Cultivation-independent TCC determination by DAPI-staining yielded concentrations two

magnitudes higher compared to cultivation-based concentrations. This can be due to dead bacteria, viable but non-cultivable organisms (VBNC) [12] or overall non-cultivable bacteria showing the clear limitation of cultivation-based methods for quantification of airborne bacteria.

Independent of the analytical approach, community analyses revealed species phylogenetically related to those of potential health risk (*Aerococcus viridans*, *Acinetobacter johnsonii*, *Pantoea agglomerans* and *Shigella flexneri*). The predominant genera in the bioaerosol samples differed depending on the applied method. This discrepancy can be attributed to the different selectivity of both methodological approaches. Both, the cultivation –based techniques and the molecular techniques are limited either by the applied selective cultivation conditions or by the coverage of DNA extraction and amplification selectivity.

Cloning analyses showed a high consistency of dominating genera independent of sampling location and sampling procedure. Because molecular methods detect both, living and dead bacteria, this could be an advantage in comparison with the cultivation-based methods, particularly regarding a possible allergenic potential of dead organisms.

The high proportion of gene sequences highly similar to poultry intestinal microbiota gene sequences obtained by molecular investigations, indicate the release of poultry faecal bacteria into the air. Inhalation of those cells may offer an additional health risk for employees.

Conclusion

Results of the molecular approach clearly show its applicability for qualitative exposure measurements. With both, cultivation-based and molecular methods, species of potential health risk (*Acinetobacter johnsonii*, *Aerococcus viridans*, *Pantoea agglomerans* and *Shigella flexneri*) were identified. Many clone sequences could not be assigned to yet cultured bacteria, but were most similar to poultry intestinal microorganisms. This is an indication for the release of poultry faecal bacteria into the air. These results underline the necessity of an adequate breathing protection (cf. TRBA 230) during work in poultry stables.

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Detection of airborne bacteria in a German duck hatchery

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Summary

Today's large-scale poultry production is often accompanied by high concentrations of airborne microorganisms at working places. However the microbial communities in those bioaerosols are rarely characterised. Therefore in this study we investigated the bacterial diversity in bioaerosols from a duck hatchery by both cultivation based and molecular methods. Depending on used media, concentrations of airborne culturable bacteria varied between 6 x 10^1 and to 7 x 10^6 cfu per m^3. The corresponding total cell count of DAPI stained microorganisms was 2 x 10^7 cells per m^3. Analyses of bacterial isolates and 16S rRNA gene clone libraries revealed a quite low bacterial diversity in hatchery's air. More than 50% of isolates are phylogenetically next related to bacterial species of the risk group 2. *Acinetobacter*, *Enterococcus* and *Staphylococcus* are the predominant genera at the investigated workplace. The unexpected high concentration of isolates next related to species with potential health risk, require further detailed investigations for these bacterial species.

Introduction

Exposures to bioaerosols at agricultural workplaces can cause a wide range of respiratory disorders. In a preliminary study in a duck hatchery it has been assumed that breathing the with dust and microbes highly polluted air result in a decline in workers lung function during their working day. The microbial diversity in those bioaerosols in general is rarely characterised. Therefore in this study we investigated bioaerosol samples from a duck hatchery by both cultivation based and molecular methods to get a first dataset about the abundant bacteria.

Material and Methods

According to VDI 4252-Blatt_2 bioaerosol samples (n=6, each 300 liter air) from a duck hatchery were collected on gelatine filters. Samples were taken during packaging of approximatley 80,000 ducklings. Cultivation based quantification was performed according to VDI 4253 Blatt_3 using six different agar media Tryptone-soy-agar (TSA), Actinomycete-isolation-agar, MacConkey-agar, Middlebrock-agar, Glycerol-arginine-agar and Oatmeal-agar. Bacterial isolates were grouped according to similar colony morphology and Gram-staining. After DNA extraction (GenElute^{TM} Plant Genomic DNA Miniprep Kit, Sigma), the 16S rRNA genes were amplified, sequenced and phylogenetically assigned. For molecular approaches like total cell counting and gene library generation three bioaerosol samples were collected additionally on polycarbonate filters as described above (in 2008, in parallel to cultivation approach) and for long time approach by personal sampling equipment on polycarbonat filters collecting the inhalable dust fraction (in 2007).

Cells were detached from the filters into 10 ml cell free isotonic NaCl solution using a Stomacher 80 (Biomaster, Seward, UK). Cells out of 5 ml were fixed by the addition of 50μl formaldehyde (37%) and quantified after DAPI staining method as described by Porter and Feig [1]. Cells in remaining 5 ml were concentrated by centrifugation and used for direct DNA extraction. Amplicons of 16S rRNA gene specific PCR were employed to generate one *E. coli* clone library per sample (using the pGEM®T vector system kit from Promega, USA). For analyses from each clone library 64 clones were randomly picked. Plasmid inserts were subsequently sequences and phylogenetically assigned.

Results

Depending on the selectivity of the used media, the concentrations of cultivable bacteria were at least one up to six dimensions lower than those observed by fluorescence microscopic quantification (2 x 10^7 cells m^{-3}).

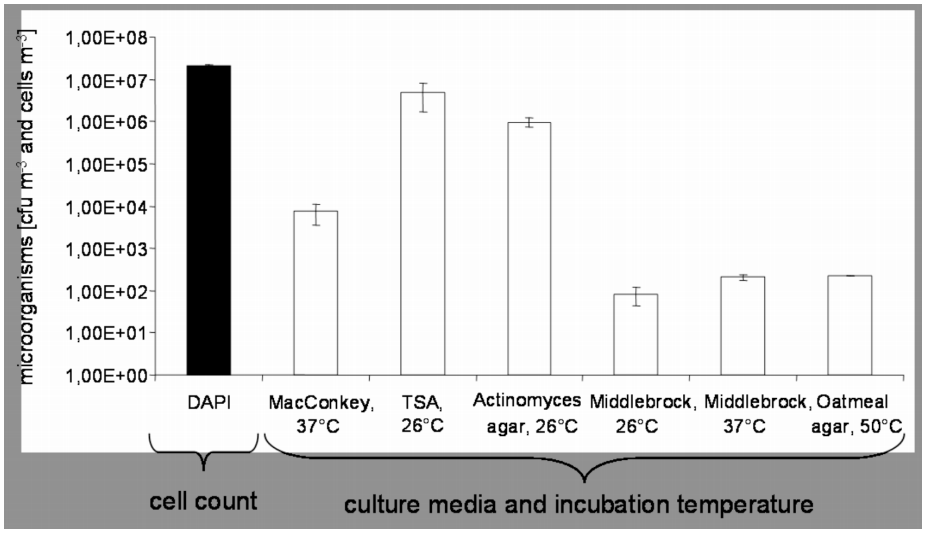


Fig. 1: Total cell count of DAPI stained microorganisms per m^3 in comparison to concentrations of cultivable bacteria (cfu per m^3) from parallel collected bioaerosols, depending on selected media and incubation temperature, both taken in duck hatchery. Values are means of triplicates ±SD.

Analyses of 16S rRNA genes from 41 obtained isolates show a low bacterial diversity in hatchery's bioaerosols. The isolates were identified as members of 13 different genera in the phyla Actinobacteria (11%, 2 isolates), Firmicutes (47%, 9 isolates), Bacteroidetes (5%, 1 isolate) and Proteobacteria (37%, 7 isolates). Most abundant isolates are next related to *Acinetobacter baumannii* (1 x 10^6 cfu per m^3), *Staphylococcus aureus* (5 x 10^5 cfu per m^3), *St. delphini* (3 x 10^5 cfu per m^3), and *Enterococcus faecium* (2 x 10^5 cfu per m^3). Further, bacterial isolates whose next related species (based on 16S rRNA gene sequence) are classified to biological agents of risk group 2 are: *Mycobacterium fortuitum*, *Stenotrophomonas maltophilia*, *Shigella* sp., *Pusillimonas noertemannii*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia* sp..

Analyses of 16S rRNA gene clone libraries from stationary bioaerosol sampling showed

similarly a low bacterial diversity in hatchery's bioaerosols. Most abundant OTU's of clone library were next related to 16S rRNA gene sequences of the genera: *Acinetobacter*, *Enterococcus* and *Staphylococcus*. Most 16S rRNA PCR products (56 %) from cloning results could assigned to *Staphylococcus aureus*.

Discussion

In comparison to ambient air (e.g. in dwellings, offices and outdoor) the detected concentrations of culturable airborne bacteria in the investigated hatchery (10^6 cfu bacteria per m^{3}3 air on TSA agar) are elevated. However, depending on the used quantification method there are clear differences in detected concentrations (Fig. 1). Depending on the selectivity of used media, the concentrations examined by cultivation were one to six magnitudes lower than those observed directly by fluorescence microscopy (Fig. 1). These results clearly show the limitation of cultivation based methods for quantitative exposure measurements if only one selective media is used.

Analyses of 16S rRNA-genes from clone libraries as well as from isolates show a very low bacterial diversity in hatchery's bioaerosols. This result indicates a low number of niches for bacterial growth in the hatchery, presumably due to the intensive cleaning activity.

Both approaches revealed that the majority of present bacteria are next related to species of the risk group 2. As well, both methods indicates *Acinetobacter*, *Enterococcus* and *Staphylococcus* as the predominant genera at this working place. These consistance indicates that the majority of airborne bacteria in the investigated hatchery air are augmentable. Concentration of some risk group 2 isolates extened to 10^5 - 10^6 cfu per m^3 air. Therefore under certain circumstances, a negative health effect for employees can not be excluded.

Conclusion

Due to the high concentrations of risk group 2 bacteria, which are described in association to different respiratory disorders, we recommend an adequate breathing protection for workers in duck hatcheries.

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Detection of airborne bacteria in a German duck stable

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Summary

Bioaerosols in stables' air are interesting in two different aspects. These bioaerosols can affect workers' respiratory system and surrounding environments can be inoculated with facility specific microorganisms via air transport. Despite the acquaintance until now no detailed investigations exist on the microbial communities in stables' air. Therefore in this study the bacterial diversity in bioaerosol samples of duck stables were investigated by cultivation based and molecular methods. Depending on the used media the concentrations of cultivable bacteria varied between 200 cfu m^{-3} on MacConkey-agar and 2 x 10^5 cfu m^{-3} on TSA agar. The corresponding total cell count concentration of DAPI stained cells, in contrast was 1 x 10^8 cells m^{-3} showing the clear underestimation using cultivation based approaches.

Phylogenetic 16S rRNA gene analyses of 115 bacterial isolates revealed a high bacterial diversity. Isolates could be assigned into 29 different bacterial genera of the phyla Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria. Next related type strains of several isolates have been classified to biological agents of the risk group 2 and some are known as causatives of negative pulmonary health effects. Further isolates represent new bacterial species.

Molecular cloning analyses were used the first time in combination with a personal bioaerosol sampling device revealed a quite constant exposition scenario for employees during egg sampling activity. These results clearly show the applicability of molecular methods for a reproducible exposure assesment. Most abundant sequences in clone libraries are next related to 16S rRNA genes of members of the genera: *Staphylococcus*, *Streptococcus* and the unexpected genus *Jeotgalicoccus*.

Introduction

Poultry meat is increasingly produced and consumed in European countries during the last decades. In reverse, due to an increasing animal production industry the number of farms for animal production significantly decreased. These changes in animal processing often is accompanied by new working situation for employees. Permanent duties e.g. at the same location are often normal case. Exposures to bioaerosols at those working places can cause a wide range of respiratory disorders. Early case reports e.g. in poultry processing describe hypersensitivity pneumonitis and epidemiological studies later revealed the presence of extensive chest symptoms and changes in respiratory function [1, 2]. However, the microbial diversity in livestock stables' air is so far rarely characterised. In this study we investigated the bacterial communities in bioaerosol samples of duck stables by both cultivation and molecular based methods.

Material and Methods

According to VDI-4252 p.2, bioaerosols were collected in two duck stables each accommodated 2500 animals. For isolation of abundant cultivable bacteria 6 different solid agar media were used (Tryptone-soy-agar (TSA), Actinomycete- isolation-agar, MacConkey-

agar, Middlebrock-agar, Glycerol-arginine-agar and Oatmeal-agar). Bacterial isolates were grouped according to similar colony morphology and Gram-staining. After DNA extraction (GenElute^{TM} Plant Genomic DNA Miniprep Kit, Sigma), the 16S rRNA genes were amplified, sequenced and phylogenetically assigned.

For molecular approaches and total cell counting three bioaerosol samples were collected by personal sampling equipment collecting the inhalable dust fraction on polycarbonat filters (3,5 cm). Cells were detached from the filters into 10 ml cell free isotonic NaCl solution using a Stomacher 80 (Biomaster, Seward, UK). Cells out of 5 ml were fixed by the addition of 50μl formaldehyde (37%) and quantified after DAPI staining [3]. Cells in remaining 5 ml were concentrated by centrifugation and used for direct DNA extraction. Amplicons of 16S rRNA gene specific PCR were employed to generate one *E. coli* clone library per sample (pGEM®T vector system kit, Promega, USA). For analyses from each clone library 64 clones were randomly picked. Plasmid inserts were subsequently sequenced and phylogenetically assigned.

Results

Depending on the selectivity of the used media, the concentrations of cultivable bacteria were at least one up to six dimensions lower than those observed by fluorescence microscopic quantification (1 x 10^8 cells m^{-3}).

In total, 213 bacterial isolates were obtained. After arrangement in 115 different groups the 16S rRNA gene sequence of one representative isolate of each group was identified. The isolated species were identified as members of 29 different genera in the phyla Actinobacteria (84%, 96 isolates), Firmicutes (11%, 13 isolates), Bacteroidetes (1%, 1 isolate) and Proteobacteria (4%, 5 isolates). Among them, isolates closely related (>99.2% 16S rRNA similarity) to *Cellulosimicrobium funkei*, *Corynebacterium falsenii*, *C. xerosis*, *Mycobacterium arupense* and *Staphylococcus epidermidis* were determined at concentrations to the extent of 10^4 cfu m^{-3}. Most bacterial isolates (n=40) contained 16S rRNA – genes which are next related to species of the genus *Streptomyces*. Several isolates represent new phylogenetic taxa.

Independent cloning analyses (n=3) of 16S rRNA genes from personal bioaerosol sampling showed i) high reproducible results and ii) a high bacterial diversity in duck stable's bioaerosol. OTUs of 3 x 64 randomly analysed clones revealed sequences associated to 8 genera of the phylum Actinobacteria, 13 genera of the phylum Firmicutes, 2 genera of the phylum Bacteroidetes and 5 genera of the phylum Proteobacteria. Most abundant sequences (each >15%) were found are next related to *Staphylococcus* spp., *Streptococcus* spp., and *Jeotgalicoccus* spp.. Some detected sequences (19%) are next related to until now uncultured bacteria.

Discussion

Currently the quantification of bacterial exposure at working places is carried out by cultivation-dependent approaches. "Total" exposition levels here are examined on "low-selective" culture media e.g. on TSA. However, by culture based methods only microorganisms which survive the sampling stress are potentially detectable. Microorganisms with more specific cultivation requirements remain undetected as well as dead microorganisms which may, however, also provoke allergic or inflammation reactions. Results from direct microscopic counts of bioaerosols from investigated duck stable bioaerosol samples showed that the microbial exposition is substantially underestimated by cultivation based approaches. Especially taking into account a high sampling

stress, a long sampling time is counterproductive in connection to a cultivation dependent analyses. However, for a meaningful exposure measurement at working places a personal sampling, ideally across the entire working day, is indispensable. In this study molecular cloning analyses were used the first time in combination with a personal bioaerosol sampling device. Analyses revealed a quite constant exposition scenario for different employees during egg sampling activity. These results clearly show the applicability of molecular methods for qualitative and reproducible exposure assesment.

Generally 16S rRNA-gene analyses, from bacterial isolates as well as from clone libraries revealed a high diversity and several new phylogenetical taxa in investigated duck stable's bioaerosols. Five isolates are phylogenetically close related to bacterial type strains of the risk group 2 [5]. Concerning these isolates with concentration of extend to 10^4 cfu per m^3 air under certain circumstances, a negative health effect can be supposed. Particularly with regard to the unexpected high concentrations of *Mycobacterium* spp., which are known as causative of different pulmonary health effects [4] in future we have to investigate these isolates in more detail.

First investigations of duck stables' bioaerosols revealed that beside *Staphylococcus* spp. and *Streptococcus* spp., 15% of 16S rRNA-genes in clone libraries were next related to *Jeotgalicoccus psychrophilus* and *Jeotgalicoccus halotolerans*. These results indicate that the general assumption, that bacterial populations in animal stables are most often dominated by species of the genera *Streptococcus* and *Staphylococcus* should be reconsidered.

Conclusion

Due to the high concentration of risk group 2 bacteria, which are described in association to different respiratory disorders, we recommend an adequate breathing protection for farm workers in duck stables. However, we call on microbiologists to investigate stables' bioaerosol as an interesting microbiological environment containing several new species with unknown medical and ecological importance.

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Farmers exposure to ammonia in new systems of laying hens

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Summary

After banning conventional cages to keep laying hens other system must be introduced and evaluated. In stables with floor keeping, aviary and large furnished cages all relevant measures were investigated. Occupational concern is given by ammonia and dust inside the building, wereby ammonia is in the focus of this paper only. Large differences between the systems were found. Seasonal variations could be observed.
Ammonia concentration ranged at all between 1 ppm and 60 ppm, especially influenced by the manure management.

Introduction

Due to the european ban of conventional cages for layers alternative systems are pushed and new systems are developed. These systems must follow the intentions of the society concerning high animal welfare. Nevertheless for evaluation of different systems requirements of protection of work and environment should not be neglected. In this paper measurements are presented only which are focussed to the exposure of farmers against ammonia in different new types of layer husbandry.

Material and Methods

Four stables, three on commercial farms and one experimental stable as reference, are investigated in a still running study. Main parameter is the keeping system itself.
Concentration of airborne particles and ammonia were measured in an aviary with integrated litter space (1), a floor keeping system with integrated litter space (2), a floor keeping system with outdoor access (3) and a furnished cage system (4). The particular data for the different systems are given in table 1.

Table 1: Data of the investigated systems

keeping system	stocking	litter	manure management
1	900	sand/wood shavings	manure belt, weekly
2	8000	horselitter/wood shavings	storage inside
3	3000	without	storage inside
4	1500	without	manure belt, weekly

All stables are force ventilated and artificially lighted with special programs for day and night. From the sytems with storage inside the stables the manure was removed together with the birds after the laying period.
As an additional hygienic effect the stable with the furnished cages was swept daily.
The birds were from the line Lohmann SLS and Lohmann brown.
Concentrations of ammonia in the stables are measured with a multi gas monitor Innova 1302 an opto acoustic device once each month as a hourly spot at noon time to indicate the influence of the keeping system. Measuring at the same time in the course of a day should ensure comparability between the different systems. By an additionally 24h monitoring the spots are classified with respect to the average of a total day and the dark and bright time periods. A 24h monitoring is added for each stable once in a quarter of a year to have the opportunity to indicate how the spot measurements reflect the course of a day and a possible influence of the season. Measurements are carried out at a centre location in the stables in an height of 1.5 m above the ground floor, which was found previously to be representative to describe long term effects in the stable. Figure 1 shows typical courses of a day for ammonia in the floor keeping system with outdoor access.

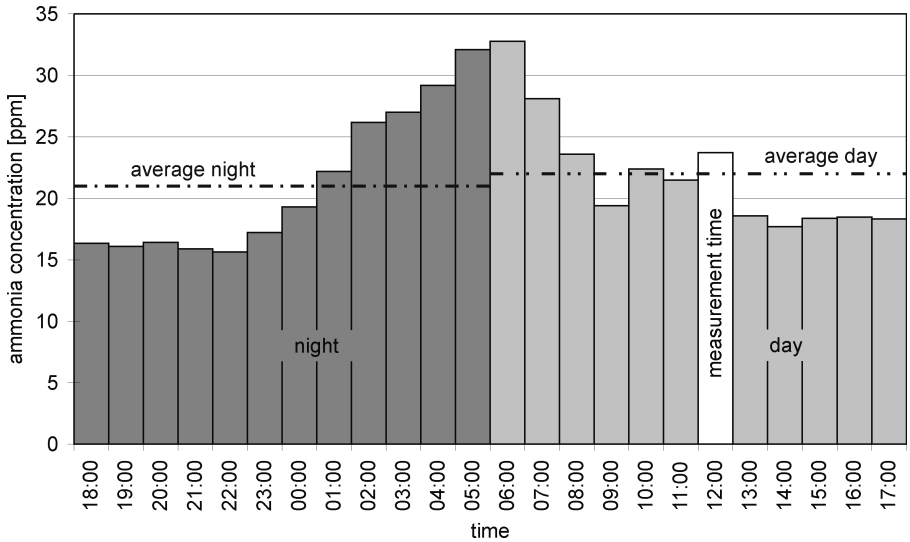


Figure 1: Representative measurement time in a daily course of ammonia in the floor keeping system with outdoor access

Each column represents an average of 1 hour. For ammonia day and night time averages a quite similar and are fitted by the spot measurement at noon rather well with a little overestimation. With view to the air quality during farmers working time in the stable these results are sufficient enough for estimations and comparison.

Results

In the following the results are presented for the single stables basing on 24h monitoring and the spot measurements. The diurnal profiles of figure 2 show levels of concentration in

the range between 5 ppm and 60 ppm. Ammonia concentration showed more or less smooth profiles for all systems. In the furnished cage system significant lower values were observed.

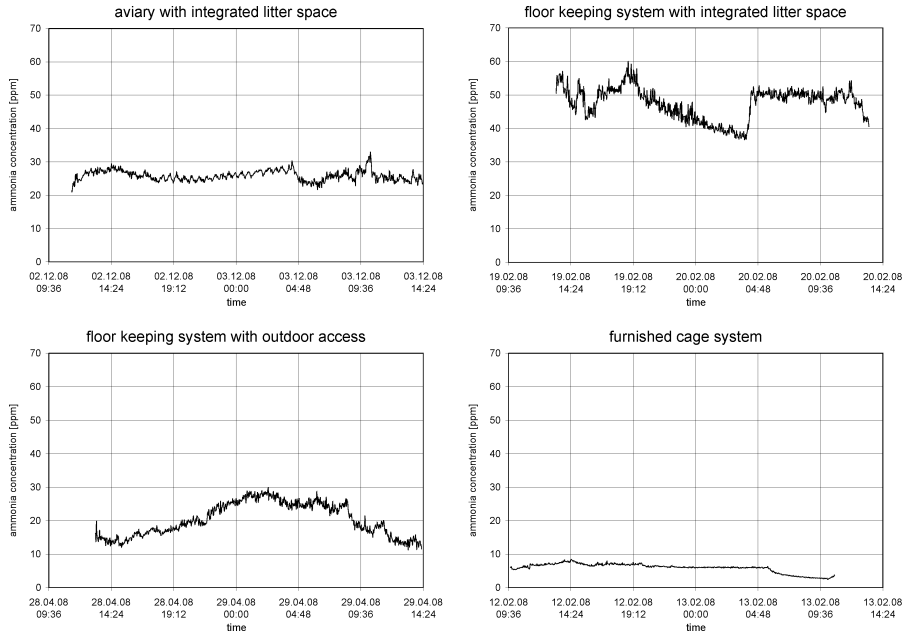


Figure 2: Typical course of ammonia concentration during a day in the four alternative housing systems

A seasonal dependency was found shown in figure 3 in a box and whiskers plot for example of the floor keeping system with outdoor access.

Discussion

The concentration levels in the different keeping systems will be compared and evaluated with respect to given or wanted occupational limit values. That means 20 ppm should not exceed for ammonia [1, 2]. For some systems the limit value of 20 ppm is kept for other exceeded. One explanation can be given by the manure management storage inside for the period of laying, the ventilation strategy and at last the size of the stock. Especially in the stable with the floor keeping system with integrated litter space the values increased dramatically. The best values showed the large furnished cage system. It is the only stable which kept the 20 ppm limit all over the time of observation.

One reason might be the system but another reason is to see in the stable management with a sophistically high hygienic demand. This stable is part of a research facility in contrast to the other stables which are operated commercially.

Mainly in winter the limit value was exceeded and the variations were wider than in summer.

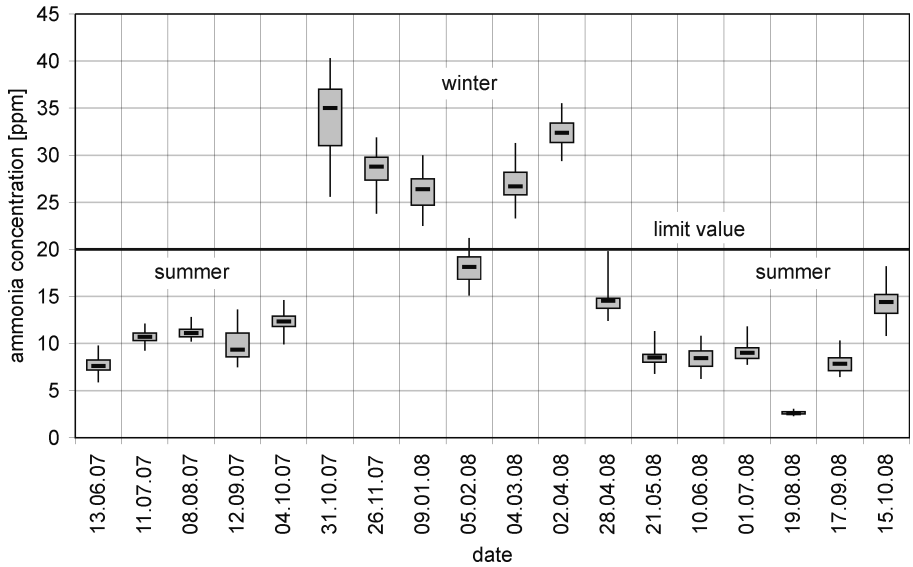


Figure 3: Box and whiskers plot of the ammonia concentration in the floor keeping system with outdoor access with view to seasonal deviations

Conclusion

New systems for keeping laying hens are developed and on the market.
Ammonia concentration depends on the system itself, ventilation strategy but especially on the manure management.
More investigations are necessary to validate this results.

Acknowledgement

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Occupational exposure to airborne bacteria in four alternative housing systems for laying hens

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Summary

Airborne bacteria were measured regularly during the course of 24 month using impingement (AGI 30) in four different housing systems: a floor keeping system with integrated litter space, an aviary with integrated litter space, a floor keeping system with wintergarden and outdoor-access (free range) and a small colony system (german 'Kleingruppenhaltung').
The concentrations of mesophilic bacteria ranged from 1.9 x 10^4 ('Kleingruppenhaltung') to 6.7 x 10^6 (floor keeping system) CFU/ m. Staphylococci showed a very similar range from 1.3 x 10^4 ('Kleingruppenhaltung') to 6.1 x 10^6 (floor keeping system) CFU/ m, but the share of staphylococci of total bacteria differed between the housing types. The quantitative distribution of haemolysing streptococci, enterococci and actinomycetes among the four systems followed the same order but in much lower concentrations.
The results indicate an urgent need to improve the air quality at the work place of farm workers e.g. by developing low emission farm systems, better ventilation devices or by using breathing masks in order to prevent negative health effects.

Introduction

Airborne micro-organisms in and from animal husbandry are associated with multiple health effects in humans and animals. Comparatively high concentrations can be found in the poultry keeping units, especially in broiler barns [3]. With the move to alternative housing systems for laying hens in the EU (1999/74/EC), in order to improve animal welfare, new systems have been introduced which allow the hens more free movement than in the former battery cages. However, little is known about the air quality in these systems and the possible health effects of air pollutants on animals and people working in these atmospheres. Therefore a study was carried out on the concentrations of airborne bacteria in four different alternative keeping systems for laying hens over 24 month.

Material and Methods

The collection of bacteria was conducted by impingement (All-Glass-Impinger type AGI 30). This method is known to show the highest yield of micro-organisms in stables under regular conditions [1, 2]. For each measurement there were six samples collected in three turns with two impingers running parallel. The impingers were charged with phosphate buffer (after Soerensen) to avoid a change in pH-value due to a drag-in of ammonia. Collection of air samples took place in 150 cm height in the centre of the barn and middle of the daily maintenance patrol. The four exemplary keeping systems for layers were a floor keeping system with integrated litter space

and manure pit, an aviary with integrated litter space and manure belt, a floor keeping system with manure pit, wintergarden and outdoor-access (free range) and a small group system (german 'Kleingruppenhaltung') with manure belt. During sampling impingers were kept in isolated and temperated aluminium tubes to allow for constant sampling conditions over the year. After sampling the impingers were stored and transported to the laboratory at 4 °C. Estimation of the concentrations of airborne bacteria was done by aerobic plate count method (APC) on various culture media. Aliquots of the samples were incubated on Blood Base Agar (Oxoid, Wesel, Germany) at 36 °C for 24 hours, on Mannitol Salt Agar (Oxoid, Wesel, Germany) at 36 °C for 24 hours, on Azide- Blood-Agar (Oxoid, Wesel, Germany) at 36 °C in a 5% CO_2 atmosphere for 48 hours, on Enterococcosel-Agar (Becton Dickinson, Heidelberg, Germany) at 36 °C for 48 hours and on Aktinomyceten-Agar at 25 °C and 50 °C for 72 hours. After incubation colony forming units (CFU) were counted for the concentrations of total culturable, aerobic and mesophilic bacteria, staphylococci, hemolysing streptococci, enterococci and actinomycetes. Mean values of CFU per m were calculated for every month (n=36) to get the mean concentrations of airborne bacteria during the course of one year.

Results and Discussion

Figure 1 shows the burden of airborne aerobic, mesophilic bacteria during the course of the year. There is a clear difference in the concentrations between the examined alternative keeping systems. The highest concentrations of total mesophilic bacteria can be found for the floor keeping system, followed by the aviary, the floor keeping system with outdoor access and the 'Kleingruppenhaltung'. Very similar values appear in figure 2 for the staphylococci. The airborne staphylococci have a main share of total bacteria but the share differs in its height between the keeping systems (figure 3). While staphylococci seem to make up to nearly 100 % of the total bacteria in the floor keeping system and also have a very high share in the aviary and in the 'Kleingruppenhaltung' the share of staphylococci in the system with outdoor access system is much lower presumably due to an increased exchange with bacteria from ambient air and a resulting higher variance in bacteria. An inferior share of this spectrum consists of bacteria from the actinomycetes group that can only be found regularly in the air of the free range system, even if the concentrations remained low altogether (figure 4).

The concentrations of airborne enterococci follow the course of the total bacteria and staphylococci but in much lower values (figure 5). This holds also true for hemolysing streptococci (figure 6) where there were no clear differences between the housing types though. Highest burden of airborne hemolysing streptococci was found in the air of the aviary system. Especially for the concentrations of staphylococci and total bacteria there is an evident seasonal course with higher values in the winter months.

The seasonal course of the bacteria concentrations is presumably due to the ventilation. In winter month at low temperatures the ventilation is reduced to avoid heat losses. Apart from the weather conditions and the season the integrated litter space seems to have a noticeable effect on the burden of airborne bacteria. Dust borne bacteria (p.e. of faeces origin or from the animals' skin) can easily be dispersed into the air when the animals show their normal behaviour (such as scratching, pecking, flying and dust bathing) which is supported by the integrated litter space and the allowance of three dimensional movement. Additional influence on the concentrations of bacteria in the air of laying hen housings can be assumed for the waste management. In housing systems with manure pit, in contrast to the systems equipped with a manure belt, the manure is usually stored until the end of the laying period and contributes - with increasing charge - to the concentrations of airborne bacteria.

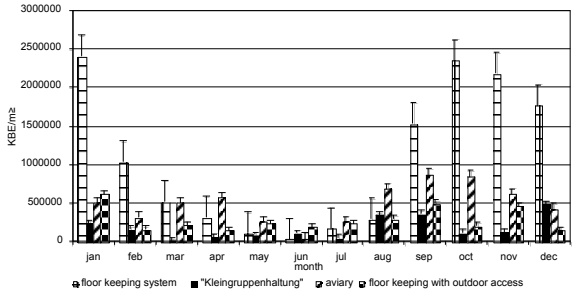


Figure 1: The mean concentrations of airborne total culturable aerobic mesophilic bacteria (n=36) in the air of four different housing systems for laying hens during the course of one year

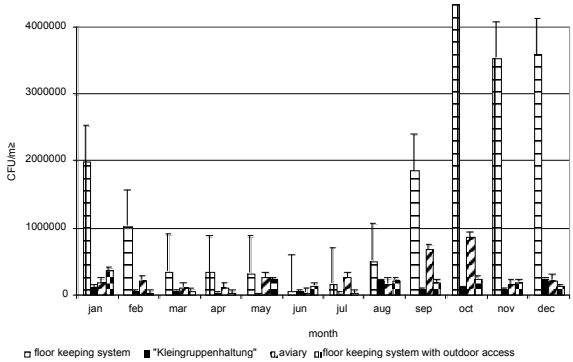


Figure 2: The mean concentrations of airborne staphylococci (n=36) in the air of four different housing systems for laying hens during the course of one year

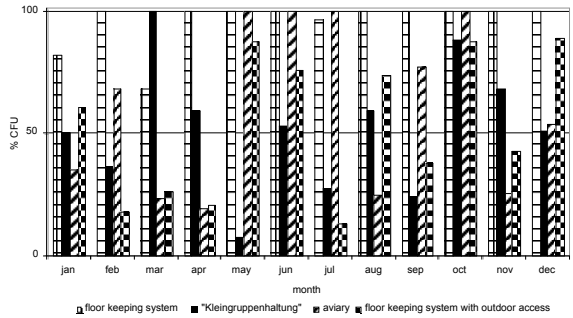


Figure 3: Share of staphylococci of the total airborne bacteria in four different housing systems for laying hens during the course of one year

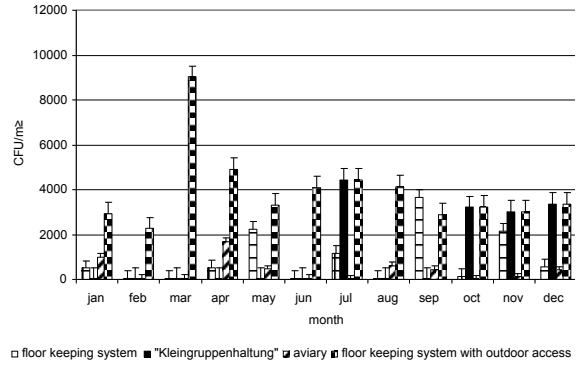


Figure 4: The mean concentrations of airborne actinomycetes (n=36) in the air of four different housing systems for laying hens during the course of one year

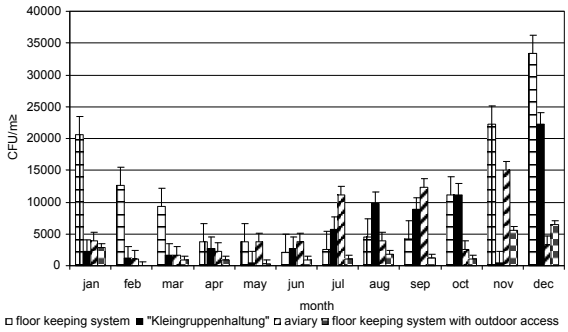


Figure 5: The mean concentrations of airborne enterococci (n=36) in the air of four different housing systems for laying hens during the course of one year

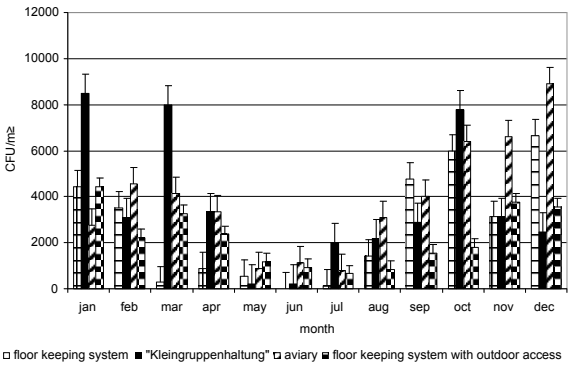


Figure 6: The mean concentrations of airborne hemolysing streptococci (n=36) in the air of four different housing systems for laying hens during the course of one year

Conclusion

Bacteria concentrations in the air of alternative housing systems for laying hens exceed the natural concentrations in ambient air by a factor of up to 10 000. There is a strong influence of housing type and season on bacterial counts.
The results indicate an urgent need to improve the air quality at the work place of farm workers e.g. by developing low emission farm systems, better ventilation devices or by using breathing masks in order to prevent negative health effects.

Acknowledgement

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Chapter 20

Block 5C

Inactivation kinetics of avian influenza virus, feline calicivirus and ECHO virus in fermented raw sausages: Implications for foodborne virus transmission

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Summary

Viruses are an important cause of acute gastroenteritis in humans worldwide. In several cases outbreaks and sporadic infections are caused by contaminated foodstuff. However little is known about survival time and inactivation kinetics of viruses in different food products. Referring to bacterial pathogens raw sausages are known as a risk product. A study with feline calicivirus (FCV) as a surrogat for norovirus, human enterovirus (ECHO virus) and avian influenza (AIV) virus was conducted to obtain data on viral persistance and inactivation kinetics in raw fermented sausages. During processing sausages were experimentally spiked with each virus and then underwent common conditions of storage and ripening. Inactivation of AIV could be observed within a short time. ECHO virus and FCV decreased in viral titer during ripening but no complete loss of infectivity was observed by day 56 in long fermented raw sausage. Influence of temperature on viral inactivation was observed for all viruses used in this study.

Introduction

Viral transmission due to the consumption of contaminated foods is increasingly recognized as a significant public health concern. From an epidemiological point of view, the most important viral foodborne pathogens are mainly human noroviruses, hepatitis A viruses and rotaviruses with regard to the number of outbreaks and number of people affected in the Western world. It is known that particularly raw products such as lettuce, fruits or shellfish can pose a possible health risk for consumers. But also animal products like raw meat or dairy products may be involved in foodborne-viral transmission.

Apart from studies with hepatitis A virus or poliovirus little is known about survival time and inactivation kinetics of viruses in different food products.

Due to the present data situation consumption of raw meat products might pose a risk for virus transmission.

The objective of this study was to obtain data about survival time and inactivation kinetics of viruses in short and long fermented raw sausages. Therefore low pathogenic AIV (subtypes H3N8 and H5N6), ECHO virus as a surrogat for the genus Enterovirus and FCV as a surrogate for norovirus were used for investigations.

Material and Methods

A strain of FCV (KS 20), ECHO vrius 1 (Farouk) and two isolates of AIV (H3N8 A/duck/Ukraine/1/63 and H5N6 A/duck/Potsdam/2216/84) were used for this study. Virus titration and propagation of FCV was done in a line of CRFK cells, for ECHO virus a line of BGM cells was used. AIV was propagated and titrated in 9 to 11 day-old chicken eggs. Raw sausages were produced according to common terms and conditions. During processing the sausage meat was spiked experimentally with each virus. Conditions for storage and ripening differed in temperature (Tab. 1). Samples for virological and physico-chemical testing were regularly taken.

Tab. 1: Temperature of ripening for short fermented raw sausages

day	temperature of ripening for ECHO and FCV ('C)		day	temperature of ripening for AIV ('C)	
0-3	22	22	0 – 14 / 18	22	7
4-28	22	7		22	7

Tab. 2: Temperature of ripening for long fermented raw sausages

day	temperature of ripening for ECHO and FCV ('C)
0-3	23/22
4-56	18

Results

In short fermented raw sausage stored at 22 °C no infectious virus was detectable for AIV by day 3 (H3N8) or day 7 (H5N6). At 7 °C both isolates showed decrease in invectivity but were still detectable by day 14. No culturable virus could be detected on day 17 (H5N6). Mean infectious doses observed for AIV during storage were reduced significantly (Mann-Whitney test $p<0.05$) in comparison to initial recovery, for both temperatures respectively.

More stability in infectivity was observed for FCV and ECHO virus. In short fermented raw sausages a decrease in viral titer was detected for both viruses. Decrease of infectivity was positively correlated with time of storage and followed linear regression with $r^2 = 0.73$ for ECHO and $r^2 = 0.65$ for FCV (for 22 °C both). Decrease was more obvious for 22 °C than 7 °C. Statistical significant differences (Student t-test $p<0.05$) in viral titers were observed between temperatures at day 21 and 28 for both viruses. By the end of storage FCV and ECHO showed maintenance of infectivity. In long fermented raw sausages infectious FCV and ECHO viruses were still detectable by day 56. Decrease in viral titer was observed in similarity to short fermented sausages. Linear regression of virus titer verus time was $r^2 = 0.86$ for ECHO virus and $r^2 = 0.89$ for FCV. From day 7 on mean viral titers observed during storage were reduced significantly (Student t-test $p<0.05$) in comparison to initial recovered titer.

Observed values for pH as well for a_w (activity of water) met the characteristics of raw fermented sausage products. Lactid acid bacteria up to 8 log cfu / g were of characteristical high amounts.

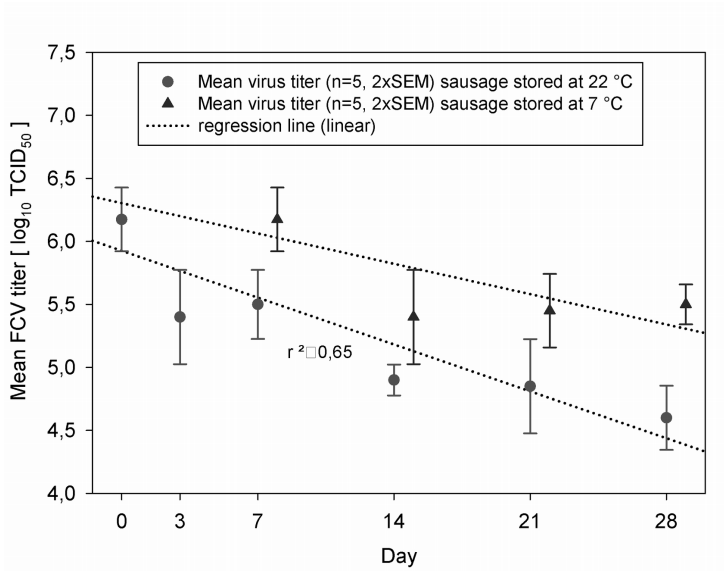


Fig. 1: Linear regression plot for persistance of FCV in long fermented raw sausage, similar results were observed for ECHO virus

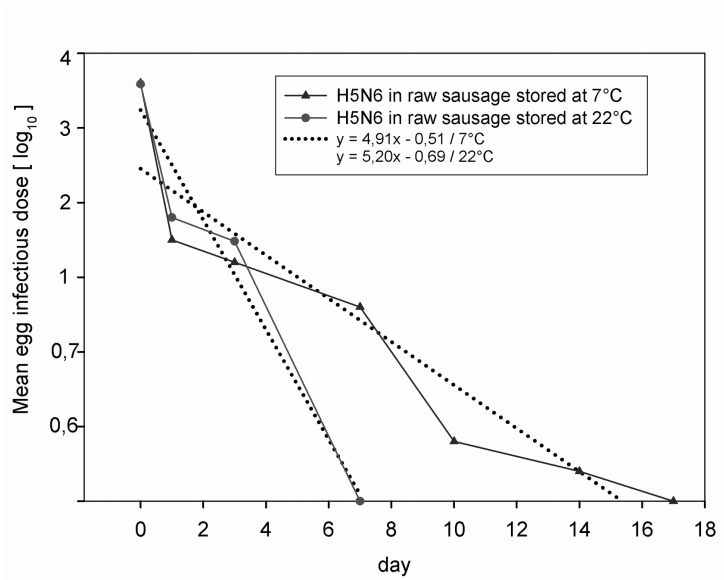


Fig.2: Linear regression plot for persistence of H5N6 in short fermented raw

Tab. 3: Range of pH values in raw fermented sausages

Fermented raw sausage	Range of pH value in raw sausages			
	ECHO	FCV	H3N8	H5N6
short	5.6 - 4.8	5.5 – 4.9	5.6 – 5.1	5.5 – 5.1
long	5.7 – 4.9	5.5 – 5.0		-

Discussion

Belonging to the *orthomyxoviridae* family avian influenza viruses are considered to be sensitive to acid pH values. According to studies of Stallknecht et al. [4] and Brown et al. [1] strong interaction between temperature, pH and salinity on virus survival could be demonstrated. In our findings both AIV strains showed low stability in raw fermented sausage – conditions like low pH and low salinity levels led to inactivation in dependance on temperature and time of storage. Human enteroviruses are kown for exceptional viral resistance in harsh environments [ ]. In raw fermented sausage they showed slowly decline in infectivity. Conditions in raw fermented sausage were not sufficient for effective viral inactivation. This results correspond to earlier findings of Kantor and Potter [ ]. With FCV an other environmentally stable virus was investigated. In accordance to ECHO virus FCV also showed prolonged persistance in raw fermented sausages. High persistence of FCV was also observed by Mattison et al.[2] by inoculation of ham. Influence of temperature on viral persistance was observed for all viruses used in this study. Although ECHO virus and FCV showed much more stability than AIV in raw fermented sausage, temperature of 22' C enhanced viral inactivation. By now little is known about the mechanims ambient temperatures affect viral particles and which target structures of viral capsid or genome are involved.

Conclusion

It could be demonstrated that FCV and ECHO virus can persist for prolonged time in short and long fermented sausage. These findings show that for norovirus or enterovirus the risk of an virus infection due to the consumption of raw fermented sausages has to be taken into consideration when viruses are introduced into the food chain.The infection with avian influenza virus by consumption of fermented raw sausage has to be considered as a risk referring to short fermented sausages riped within a few days under cooling conditions only. For other sorts of raw fermented sausage an infection is rather unlikely.

Acknowledgement

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Vibrio parahaemolyticus AND *Vibrio vulnificus* DETECTED IN SLOVENIAN MUSSELS

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Summary

Consumption of raw bivalve molluscs may cause outbreaks of food poisoning due to *Vibrio parahaemolyticus* and *Vibrio vulnificus* strains.
Blue mussels (*Mytilus galloprovincialis*) is one of the most commonly harvested and consumed shellfish in Slovenia. The purpose of our study was to investigate the prevalence of *V.parahaemolyticus* in blue mussels from Slovenian coast. The results of the three years (2006 – 2008) study showed low percentage of *V. parahaemolyticus* and a slightly higher percentage of *V. vulnificus* in blue mussels.

Introduction

Vibrio strains are commonly found in marine environments, including inland waterways and estuaries. They may be often isolated from molluscs.
First detected in Japan in 1950s, *V.parahaemolyticus* is today one of the most important foodborne pathogen in the world. Consumption of raw or undercooked seafood is often associated with gastroenteritis, caused by *V.parahaemolyticus*.
Only some of the strains have enteropathogenic potential, associated with the production of thermostable direct hemolysin (TDH), encoded by the *tdh* gene and the thermostable direct hemolysin-related hemolysin (TRH), encoded by the *trh* gene. The relationship between strains, isolated from estuarine environments, and those, isolated from food and human clinical samples is poorly understood. Less than 1% of food or environmental strains express the *tdh* gene, while more than 90 % of clinical isolates do. In the environment, the frequency of *V. parahaemolyticus* strains expressing the *trh* genes appear to be very low. The species specific genes are *toxR* and *tl* (thermolabile hemolysin gene), among them only *toxR* is associated with pathogenicity. The occurrence of *V. parahaemolyticus* strains is supposed to be correlated to water temperatures; higher densities of total and pathogenic strains were observed in waters with higher temperatures [1].
The reference method ISO/TS 21872-1 for detection *V.parahaemolyticus* includes the two step enrichment medium, followed by the isolation on agar.
Vibrio vulnificus is distinguished from other members of genus *Vibrio* in its ability to ferment lactose. Its presence in estuarine environment is not due to contamination. Under certain conditions it has the ability to cause serious and often fatal infections. Consumption of raw or undercooked shellfish and wound infections, acquired through contact with shellfish or marine waters, where *V. vulnificus* is present, can reflect to invasive septicemia.
V. vulnificus was first isolated in 1964 in USA, at that time identified as a virulent strain of *V. parahaemolyticus*. The total viable *V. vulnificus* cells presence is influenced with the ambient temperature. At temperatures below

10^oC, the number often falls to nearly undetectable level. It is hypothesized that in this conditions the bacteria enters into viable but nonculturable state (VBNC). Filter-feeding molluscs concentrate bacteria in the gut and other tissues [2].

Material and Methods

Sampling. One hundred and sixty eight shellfish samples, consisting of 15 mussels (*Mytilus galloprovincialis*) were sampled for laboratory analysis. Samples were collected between May 2006 and December 2008 at Slovenian coast. They were transported in portable coolers to laboratory and analyzed immediately after arrival.

Sample preparation. Mussels were washed under potable water to remove sand and algae, and opened with sterilized scalpels. Dead and damaged shellfish were discarded. Digestive glands were removed from the rest of the body and collected in sterile glass Potter-elvehjem. Glands were homogenized and used for inoculation on media as described.

Isolation and biochemical identification. The gland suspension was inoculated onto alkaline saline peptone water (ASPW) and the bacteria were isolated on thiosulphate citrate bile sucrose (TCBS) agar. Blue-green colonies, approximately 2 mm in diameter, defined as *V. parahaemolyticus* were isolated on saline nutrient agar (SNA) for further oxydase test. The oxydase positive strains were identified using API 20E biochemical galleries (bioMérieux). Cell suspension of the defined cultures was prepared in 2 % NaCl.
V. vulnificus suspicious colonies isolated from TCBS agar were identified using API 20E biochemical galleries (bioMérieux).

DNA extraction from the enrichment broth. 0,1 ml of enrichment broth, taken from just below the broth's surface, was boiled in a water bath for 10 minutes, put immediately on ice for 5 minutes and mixed by inversion. Suspension was centrifuged at 12000 g for 15 minutes. The obtained supernatant was transferred in new tubes, heated at 65^oC for 5 minutes and vortexed. Released DNA was stored at -20^oC until use.

DNA extraction from pure colonies. Bacterial suspension was made by taking 10 l loop full of cells and 100 l of molecular grade water. Cell suspension was lysed by boiling for 10 minutes, tubes were removed immediately, put on ice for 5 minutes and mixed by inversion. Centrifugation followed for 15 minutes at 12000 g. Supernatant was heated at 65^oC for 5 minutes and vortexed. Released DNA was stored at -20^oC until use.

Molecular detection of *V. parahaemolyticus***.** PCR reactions were performed in a thermal cycler with a reaction mixture containing 10xPCR buffer, 1,5 mM MgCl2, 1 mM dNTP, 1 U Platinum®Taq DNA Polymerase and oligonucleotide primers for *toxR* (Kim et al. (1999)), *tl, trh* and *tdh* (Bej et al. (1999)), respectively. The final reaction volume was 50μl. All products were checked by electrophoresis on 1,6 % agarose gels stained with ethidium bromide and illuminated on UV transilluminator. Strains without amplification of DNA specific for *toxR* and *tl* genes were considered negative for *V. parahaemolyticus* and were not subjected to further analysis for *tdh* and *trh* genes.

Results

*V. parahaemolyticus***.** In the year 2006, sixty-two samples were analysed for *V. parahaemolyticus*. In one sample (1,6 %) *V. parahaemolyticus* was detected by direct plating. After the enrichment *V. parahaemolyticus* was detected by PCR in 4 (6,4 %) samples. The PCR results were positive only for *toxR* and *tl* genes (Table 1).

In 2007, out of 32 tested samples 4 (12,5 %) were found *toxR* and *tl* positive and one of them showed also trh positive sequence.
In 2008, 16 out 74 tested samples (21,6 %) showed *toxR* positive sequence from enrichment broth, but *V. parahaemolyticus* was not isolated.

Tab. 1: Number of detected *V. parahaemolyticus* strains by PCR method

Year	*Nr. of samples*	*toxR, tl positive (culture)*	*toxR, tl positive (enrichment)*	*trh positive*	*tdh positive*
2006	62	1	4	0	0
2007	32	0	4	1	0
2008	**74**	**0**	**16**	**0**	**0**

All samples were diluted till 10^7 and inoculated on TCBS agar. At that time mainly lactose positive and some lactose negative colonies grew up. None of them were confirmed as *V. parahaemolyticus*.

*V. vulnificus***.** From July to October in the years 2006 – 2008 *V. vulnificus* was isolated by plating directly on agar in average concentration of 10^4cfu per gram of shellfish (Table 2). In that period of the year water temperature is in the range of 18 - 25°C.

In 2006, 3,2 % (2/62) of samples were positive on *V. vulnificus*. Similar percentage (3,1% (1/32)) of positive samples were defined in 2007, while the number of positive samples grew up on 10,8 % (8/74) in 2008.

Tab. 2: Concentration of *V. vulnificus* in shellfish samples during July and October

Year	Month	Water temperature (‘C)	V. vulnificus cfu/g
2006	October	23	10^3, 10^5
2007	October	19	10^5
2008	July August October	25 22 18	$3x10^5$,10^4, 10^3 10^5, 10^5 10^4

Discussion

A three year investigation of shellfish bacterial contamination indicated the presence of *Vibrio* strains in blue mussels from the Slovenian coast.

Laboratory testing showed that the conventional bacteriological methods had serious limitations for *V. parahaemolyticus* detection in shellfish samples [3]. Due to the overgrowth of the other natural bacterial flora, present in coastal marine environment, and due to the

problems, associated with the limited cultivability of *Vibrio* strains under adverse conditions the use of culture plate media was not efficient enough for the detection of these bacteria in shellfish samples. As well, the isolation of *V. parahaemolyticus* after enrichment broth was not successful.

One of possible explanation is, that digestive gland accumulated a high number of different bacteria that overgrow *V.parahaemolyticus* after enrichment. Especially in the summer, a great number of lactose positive bacteria were observed. To surpass these problems, the molecular methods were introduced for the detection of *V. parahaemolyticus*.

The results indicated that the PCR used for the detection of *V. parahaemolyticus* directly in the enrichment broths was more effective than the plate culture method with TCBS agar.

Direct plating method was more successful for *V. vulnificus* detection in blue mussels during the summer months when these bacteria exist in viable state and are possible to be cultured on specific media plates.

Conclusion

Vibrio parahaemolyticus and *Vibrio vulnificus* were detected in blue mussels harvested at Slovenian coast. *Vibrio vulnificus* was isolated during the summer months more often than *Vibrio parahaemolyticus*. For the detection of *Vibrio parahaemolyticus* molecular methods were found more realiable than conventional bacterial methods. These bacteria were detected in the enrichment broth by the PCR amplified the *toxR* and *tl* gene sequences.

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The influence of storage conditions on animal feed quality with reference to toxigenic fungal contamination and their mycotoxins detection in serum, tissues and milk samples from selected areas of South Africa

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Summary

The major objectives of this study were to analyze and assess the hygiene quality of animals feed in reference to toxigenic fungi and mycotoxins and their possible effects on animal health, production and products quality such as milk, tissues and serum obtained from selected rural areas, commercial farms and shelves of South Africa. Fungal cultures and Polymerase Chain Reaction were used for fungal isolation. Thin layer chromatography, Anion-exchange cartridges, immuno-affinity columns and high pressure and liquid chromatography were used for mycotoxin extraction and analysis. *Aspergillus, Fusarium* and *Penicillium*species were the most prevalent fungal contaminants found in all feed samples. Mycotoxins were detected in higher levels in rural animal feeds than commercial feed with aflatoxins (188,8 $\pm$270,3μg/kg), fumonisins (584,4 $\pm$1034μg/kg), ochratoxinA (0.13 $\pm$9.8μg/kg), deoxynivalenol (0.2 $\pm$15μg/kg) and zearalenone (0.001 $\pm$11μg/kg) being the major mycotoxins found. The same mycotoxins were found in pigs serum and tissues with high incidence in samples from rural areas as compared to commercial samples. Similarly, aflatoxin M_1(100%), ochratoxinA (16%) and fumonisin B_1 (03%) were detected at higher frequency and levels in milk samples from rural animals than those from commercial farm animals.. Animal feed hygiene and storage conditions highly influence the quality of both feed and animal products with possible high health risks for animal and human consumers and particularly in rural areas.

Introduction

Mycotoxins are secondary metabolites of fungi, which may contaminate animal feed and human food at all stages of the food chain. Their global occurrence is considered as an important risk factor for human and animal health, as up to 25% of the world crop production may be contaminated with various mycotoxins [2,4]. Mycotoxins have significant economic impact in terms of productivity and in addition nutritive value of the infected cereals and forages, which always is affected. These mycotoxins can be excreted in animal products such as milk, meat and in animal biological products such as serum, urine and faeces. In animals, mycotoxins have negative effects on the production, reproductive rate, growth efficiency and immunological defence, as well as often provoking various forms of damage in the major organs such as heart, liver and kidney. Animal feeds in commercial farms are stored in sterile environment with consequent

maintenance of quality. The feeds in rural area however are kept under poor storage conditions with adverse effect by humidity, bacteria, fungi and mycotoxins on the quality. Animals fed such rural feeds do not thrive as much as the commercial farm animals.



Figure1: Illustration of poor storage conditions of animal feed in rural areas

Material and Methods

Fungal culturing, isolation, identification and confirmation from animal feed samples were done by direct and indirect plating (serial dilution) as described by [6,8]. Microscopic and polymerase chain reactions (PCR) methods were used for fungal confirmation. Mycotoxins extraction as well as quantification were done using thin layer chromatographic (TLC), high performance liquid chromatographic (HPLC). Immunoaffinity column (VICAM) and HPLC methods were used for aflatoxin M_1, fumonisin and ochratoxin A analysis in milk samples. Mycotoxins extraction from plasma and tissue were done using the methods of [1,5]with some modifications.

Results and Discussion

Fungal analysis in both rural and commercial animal feeds revealed contamination by *Aspergillus niveus, A. niger, A. candidus, A. penicellioides, A. clavatusand, A. flavus, A. flaviceps, A. fumigatus, Fusarium verticillioides, F. proliferatum, F. graminerium, Penicillium crustosum, Penicillium verrucosum*, and *Mucor spp.* In addition, high levels of mycotoxins were detected in feed from rural areas as compared to commercial animal feed (Table 1) with aflatoxins (188,8-270,3μg/kg), fumonisins (584,4-1034μg/kg), ochratoxinA (0.13-9.8μg/kg), deoxynivalenol (0.2-15μg/kg) and zearalenone (0.001-11μg/kg) being the major mycotoxins found. These results confirm that obtained by [2,6,9]. The same mycotoxins were found in milk samples with high incidence in samples from rural areas as compared to commercial samples (Table2). The results show that animal feed hygiene influenced by poor storage conditions in rural area adversely affects the quality of these feeds with resultant decrease in livestock production.

These maize crops are kept on the field until they are completely dry, no proper storage facilities exist and harvested crops are kept on the roof, in the kitchen or on the ground conditions which highly influence the quality of both animal feed and animal milk quality in relation to fungi and mycotoxins with possible high health risks for animal and human consumers. These results [1,3,10] who also detected the same mycotoxins in milk samples.

The presence of aflatoxins, fumonisins and ochratoxinA in tissues and plasma (Table 3) in this work, correlates with the findings of [1,5,7,11]. The low contents of these mycotoxins in blood and tissues might be as a result of the rapid metabolism of mycotoxins by animal system and difficulty to extract them from animal tissues [1,5].

Table 1: Levels of mycotoxins (μg/kg) in rural and commercial animal feeds as determined using immunoaffinity column (VICAM) and HPLC.

Sampling Area		Aflatoxins (µg/kg)	Deoxynivalenol (µg/kg)	Zearalenone (µg/kg)	Fumonisins (µg/kg)	OchratoxinA (µg/kg)
Rural	Incidence	135 (90%)	49 (32.2)	78 (52%)	149 (99.3%)	18 (12%)
n=150	Ranges	188-270	0.2-15	0.001-11	584, 4±1034	0.13±9.8
Means		6.54	7.99	1.36	735.00	5.32
Commercial	Incidence	36 (80%)	16 (32%)	25 (50%)	41 (82%)	21 (42%)
n=50	Ranges	0.13- 40	0.2-6.2	0.001-4.5	0.28- 7.0	0.001-11
Means		6.17	5.38	1.44	7.85	4.9

Table 2: Mycotoxins analysis in milk as determined using immunoaffinity column (VICAM) and confirmed on High Pressure Liquid Chromatography (HPLC) ('nd' = not detected)

Area		Aflatoxin M_1 (µg/kg)	Fumonisins (µg/kg)	ochratoxinA (µg/kg)
Rural n=90	Incidence%	90 (100%)	03 (03%)	16 (17.7%)
Ranges (Means)		0.21-59(6.54)	0.05-0.11(0.09)	0.001-3.9(1.36)
Commercial n=80	Incidence %	36 (45%)	nd	09 (11.3%)
Ranges (Means)		0.13- 40 (6.17	-	0.001-4.5 (1.44)

Table 3: Mycotoxin contents of pig tissues and plasma as determined on HPLC.

		Tissues			Plasma		
Sampling		**Aflatoxins**	**Fumonisins**	**ochratoxinA**	**Aflatoxins**	**Fumonisins**	**ochratoxinA**
Area		**(µg/kg)**	**(µg/kg)**	**(µg/kg)**	**(µg/kg)**	**(µg/kg)**	**(µg/kg)**
Rural	Incidence	16 (32%)	03(6%)	09 (84%)	21 (42%)	03 (6%)	05 (10%)
n=50	Ranges	0.115.3	0.5-6.9	0.021-1.2	0.006-11.5	0.13±9.8	0.05-6.35
Means		6.54	3.54	1.02	5.3	3.6	2.56
Commercial	Incidence	09 (15%)	01(02%)	11 (22%)	23 (46%)	nd	0.6 (12%)
n=50	Ranges	0.3- 4.63	0.06	0.001-4.5	0.003-6.9	-	0.56- 5.6
Means		6.17	0.6	1.44	5.85	-	3.25

Conclusion

Mycotoxin problems tend to be a serious issue particularly to rural areas in comparison to commercial farms. It is assumed that this is due to large amounts of contamination occurring in both field and storage structures, and poor management of the feed and feeding programmes. The presence of toxigenic fungal such as *Aspergillus, Fusarium* and *Penicillium* species and of fumonisins, aflatoxins, zearalenone, Deoxynivalenol and ochratoxin A in animal feed, milk, tissues and serum of animals show how much storage condition can influence the feed hygiene and expose animal feed to toxigenic fungi and mycotoxins with health consequences on both animals and consumers of animal products.

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Investigations in order to establish a concept for using dioxin exposed floodplains by beef cattle under food safety aspects

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Summary

Grassland near the river Elbe (in the Northern part of Germany) is known for increased dioxin levels due to floodings with water containing sediments which are highly contaminated with dioxins (longstanding pollution from former locations of industry). Grazing ruminants use those areas traditionally. Beef cows are fed with grass/grass silage from these contaminated areas and produce calves that are fattened. For minimizing the dioxin load in the cattle carcasses, recommendations for farmers should be given concerning the duration of the suckling period (dioxin contaminated milk/green fodder) and the following fattening period (based on dioxin uncontaminated feed).

In this study, several groups of cattle were investigated. The animals differed in age, feeding and housing conditions and in the duration and intensity of dioxin exposure. To determine the prenatal dioxin load, muscle and liver tissues of newborn calves were tested. Furthermore, on the one hand, a group of calves that were continuously kept on potentially contaminated grassland for 8 weeks or 6 months with the cows and, on the other hand, a group that was transferred after these periods of time and then fattened with uncontaminated feed were investigated. Additionally, old beef cows which were continuously exposed were tested. Furthermore, the dioxin concentrations of feed and rumen content were measured. The dioxin concentrations were analyzed by HRGS coupled with HRMS.

The dioxin concentrations (WHO PCDD/F-TEQ) in the tissues of suckling calves varied between 2.8 and 9.7 pg/g fat in the muscle and between 10.3 and 94.3 pg/g fat in the liver tissue. After a 6 month period with uncontaminated feed, the dioxin concentrations in the analyzed tissues decreased markedly (muscle: 0.4–4.1 pg/g fat; liver: 2.6–35.8 pg/g fat).

Liver tissue of old beef cows exceeded the maximum level (6 ng WHO PCDD/F-TEQ/g fat) in all cases, whereas half of the muscle samples showed dioxin contents below the maximum level (3 ng WHO PCDD/F-TEQ/g fat).

Dioxin exposed grassland could be used for beef cows to produce calves but not for fattening the reared calves. A final fattening with uncontaminated feed appears to be a great chance to reduce the body burden of these animals and thus to minimize human exposure.

Introduction

The chemical substances belonging to the classes of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are being summarised under the term "dioxins". Due to their toxic potential and high environmental persistence, they deserve continuous attention and observation in order to avoid of their entry into the food chain [2]. More than 90 % of the human dioxin exposure can be traced back to food of animal origin

(milk, meat, fish, eggs).
As a result of regular floodings with water containing dioxin-contaminated sediments, increased dioxin loads in the soil and vegetation of the floodplains at the river Elbe (Germany) are to be considered [4,5]. This region served as a model for the present study which is centred on the examination of the dioxin contamination of cattle grazing at the riversides.
The present investigations focused on the effects of feeding and housing conditions on potential dioxin contaminations. The variation of dioxin loads in muscle and liver tissue of cattle in dependence on their mode and duration of exposure was a matter of particular interest. In this respect, differences in production conditions (use of potentially contaminated feed) as well as differences regarding age and body weight were required. The effects of translocation at different times and subsequent fattening with "uncontaminated" feed on the dioxin load of muscular and liver tissue were investigated.

Animals, Material and Methods

Within the scope of this study in the floodplains of the river Elbe, 20 cattle were allotted into several groups differing in age and housing conditions. *Newborn or stillborn calves* (n = 4) served for assessing the prenatal contamination load, i.e. before milk ingestion. *Calves* were sampled after weaning for examining the effects of the suckling duration on the dioxin load in tissues of calves. These suckling calves were raised on exposed grassland up to an age of 8 weeks (n = 3) and 6 months (n = 3), respectively, and analysed for dioxin concentrations in muscle and liver tissues. Furthermore, 6 calves were relocated at the age of 8 weeks and 6 months, respectively, and thenceforward fattened with feed not contaminated with dioxins for 6 further months. In comparison to that, a group of *cows* (> 7 years, n = 4) was comparatively tested. The muscle tissue is of particular importance for human nutrition, the liver is known for a potent dioxin accumulation.
Moreover, corresponding *feed samples* (n = 7) and *rumen contents* (from calves aged 6 months, n = 3) and the "uncontaminated" hay were analysed concerning their dioxin burden. All samples were analysed with high resolution gas chromatography (HRGC) in combination with high resolution mass spectrometry (HRMS).

Results

None of the feed samples turned out to be clearly contaminated (0.15–0.38 ng WHO PCDD/F-TEQ/kg, based on 88 % dm). The analysed rumen contents of the exposed 6 months old calves showed a higher contamination than the tested feed (1.00–2.10 ng WHO PCDD/F-TEQ/kg, based on 88 % dm).
Newborn and stillborn calves, respectively, of beef cattle from an exposed area, which were fed with potentially contaminated feed during gestation, already showed increased dioxin levels in muscular (1.2–5.5 pg WHO PCDD/F-TEQ/g fat) and liver tissue (4.7–7.3 pg WHO PCDD/F-TEQ/g fat).
The dioxin load in samples of muscle and liver of early weaned calves (8th week), originating from exposed grassland, exceeded the maximum level by far. The highest values were found in calves weaned after a 6 month suckling period from the exposed area (table 1).
The dioxin contents in the muscular tissue of old cows varied between 1.9–6.3 pg WHO PCDD/F-TEQ/g fat. PCDD/F concentrations in the livers of cows were clearly higher (6.4–41.9 pg WHO PCDD/F-TEQ/g fat).
The muscular and liver tissue of animals weaned at an early age and subsequently fattened with uncontaminated feed for a period of six months showed tolerable dioxin levels, without any exception. In the muscular tissue, contents of up to 1.1 pg WHO PCDD/F-TEQ/g fat were measured, whereas the liver

tissue reached values of up to 5.9 pg WHO PCDD/F-TEQ/g fat.
Animals after a longer suckling period (and subsequently fattened with uncontaminated feed) showed dioxin levels of up to 4.1 pg WHO PCDD/F-TEQ/g fat in the muscular tissue, but still reached values of up to 35.8 pg WHO PCDD/F-TEQ/g fat in the liver tissue (table 1).

Tab. 1: Dioxin contents in young beef cattle after different exposures to dioxin

dioxin content [pg WHO-PCDD/F-TEQ/g fat]	**muscle** (max. level: 3.0)	**liver** (max. level: 6.0)
suckling period of 8 weeks (exposed)		
- at the end of 8 weeks (contaminated milk/fodder)	2.8 - 9.7	10.3 - 48.6
- after 6 further months of fattening (uncontaminated feed)	0.4 - 1.1	3.0 - 5.9
suckling period of 6 months (exposed)		
- at the end of 6 months[1] (contaminated milk/fodder)	4.0 - 7.9	14.1 - 94.3
- after 6 further months of fattening (uncontaminated feed)	0.9 - 4.1	2.6 - 35.8

[1] Dioxin concentrations in rumen content (i.e. in the ingested feed): 1.0 - 2.1 ng PCDD/F-TEQ/kg dm

Discussion

An appropriate sampling of plants from the grassland is a major difficulty and of particular importance; meanwhile rumen contents seem to be a better indicator for the dioxin intake by grazing animals than the feed [3]. The most significant cause of variations regarding the dioxin burden in feed is the composition of the matrix next to the soil, which means the contamination of roughage during grazing and/or mechanical harvesting. In the soil and the matrix next to the soil, dioxin concentrations of 4.1 ng up to 1,531 ng WHO-PCDD/F-TEQ/kg dm and 1.49 ng up to 147 ng WHO-PCDD/F-TEQ/kg dm, respectively, were measured [2]. Considering these high values, the question arises whether it is necessary and possible to use such areas for growing forage crops. However, the floodplains are used for flood control and therefore, vegetation encroachment or a silvicultural use should be avoided. The cultivation of high rising fodder plants like corn could be an opportunity without the risk of a higher dioxin burden because dioxins represent an exterior contamination that is basically determined by contamination with soil or the matrix next to the soil [3].
The results of these studies definitely proved the possibility of a reduction of the dioxin load in beef cattle which is high when the cattle ingest PCDD/F [1]. This can be achieved by systematic modifications in the management when exposed pasture areas are used.
All calves weaned at an early age and then subsequently fattened with uncontaminated feed for six months showed contamination levels below to the maximum level both in the muscular and in the liver tissue. Thus, weaning as soon as possible (approximately eight weeks) and a following fattening period with uncontaminated feed turned out to be highly effective. Some animals weaned at an age > 6 months showed dioxin loads above the maximum level even after a subsequent fattening period of six months (in the muscular tissue one and in the liver tissue one out of three samples each). The livers in particular still showed significantly higher dioxin levels.
A relocated production of beef provides a

concept complying with the requirements of flood control, reasonable interests of involved farms and demands of food safety.

Conclusion

The tissues of cattle fed green fodder/silage from areas known for a high dioxin load can reach and exceed the dioxin maximum levels. Dioxin exposed grassland could be used for beef cows in order to produce calves but not for fattening beef cattle. A period of fattening with uncontaminated feed appears to be a great chance for reducing the body burden of these animals and thus minimizing human exposure. The exposed grassland should only serve for supplying the reproducing animals. The calves should only receive mother's milk for a very short period of time. The following fattening should take place at another location, based on uncontaminated feed. After a repeated use of the old cows, these animals must not be utilized as food. The economic benefit of those animals is limited to the production of offspring and potential subsidies that are given for keeping the floodplains clear of vegetation encroachment.

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Quality Fresh Milk Standards and Bacterial Content to Some Dairy Cattle Farms in Kosova

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Summary

The main goal of this research was to study the influence of bacterial content (CFU/ml) on the quality of fresh milk in some cattle dairy farms being based on the existing standards for milk quality in Kosovo. Research results derived from 3021 individual samples of milk figured from the program of milk recording in the period August 2007 – July 2008. Influence of cow breed, month and farm on bacterial content was analyzed being based on the existing legislation for the year 2007, 2008 and 2009. A very strong influence on bacterial content of fresh milk ($P < 0.001$) was shown by farm and month of the year effect. A slight significant effect on milk quality was shown from the breed of cow ($P < 0.0478$).The results gained show that if milk quality produced proceeds being the same as currently, in 2008 and 2009, to the category of non hygienic milk (CFU/ml $<$ 300.000 and CFU/ml $<$ 100. 000) would belong about 26.72% and 47.48% respectively. While to the standard of the extra quality milk (CFU/ml $<$ 100.000, CFU/ml $<$ 80.000 and CFU/ml $<$ 50.000) would belong about 52.44% for 2007, 47.25% for 2008 and 37.18% for 2009. According to the results shown, we could say that bacterial content on fresh milk in these farms is an urgent problem that asks for an immediate solution in order to increase the profit of the farms and ensured consumers health in Kosova.

Introduction

Kosovo milk industry is lastly faced to new consumer's demands asking for lower costs and higher milk quality according to the approved standards in the country. Even though this industry is developing faster toward the commercialized and organized farms, still the milk quality produced on diary farms needs a continuous care to face competition of open trade, increase farm profit and produce fresh high quality milk. Milk is food product synthesized by special cells of mammal gland, is almost sterile and secreted on udder alveolus [6]. The micro-organisms in the raw milk derive from various sources of contamination, such as the udder from non healthy cows, from the surface of the teat, the air and from milk contacted surfaces of the milking storage and transport equipments [1]). Milk in the udder of a healthy cow is more or less free from Bacteria. By passing the strichkanal, the milk is contaminated with 100 – 1000 CFU/ml or 100 – 10000 CFU/ ml [3,5]. High number of bacteria has a negative impact on raw milk quality and milk products.

Material and Methods

Data registration, manipulation and sample analysis: In this study were included 13 commercial cattle dairy farms all around Kosovo. The analyzes of bacterial content derive from fresh milk samples using the standard method – A4 according to International Committee for

Animal Registration – ICAR, for the period August 2002 – February 2008. The amount of 40 – 50 ml of milk makes up the milk sample made up from both samples of morning and evening that were mixed and put together on a sterile bottle. AZIDOL was used as a conservancy for sample preservation. Bacterial content analyzes were made using "BactoScan-Foss" equipment. The results gained were compared according to the fresh milk quality standards in Kosovo. Statistical analyzes were performed using JMP- starter packet a business unit of SAS [4]. To test the difference between means of different variables the Duncan test was used [7].

Results

The milk samples per farm, breed and month, their least squares means, standard error, standard deviation and variance analyses were shown in Tab. 1.

Results gained show that regarding bacteria content on fresh milk for both variables (farm and month) the variations were highly significant differences ($P < 0.0001$), while for cow breed the differences were at the border of significant effect ($P < 0.0478$). Farm V was seen to produce the poorest quality milk, followed by farm VI, and then by XIII, with a bacteria content of 2115.61, 558.29 and 449.28 respectively. Extra quality milk was produced on farms IV, IX and XII with a bacteria presence of 20.93, 24.61 and 59.36 respectively. Cows of Crosses (1124.33), Black Holstein (561.75) and Simmental (458.34) tend to be more exposed to milk contaminated with bacteria compared to Montbeliard (161.97) and Brown Swiss (286.80). During winter season's months (December, January and February) bacteria contamination was higher (908.32, 672.73 respectively 739.58), compare to fall season (September=292.83, October=202.39 and November=517.08). Figure 1, depicts differences share of milk categories according to milk standards. From the overall total of milk produced in this period, the largest percentage belongs to class extra milk, with its higher results in October (58.33%). Generally, 1st, 2nd and 3rd classes of milk quality standard represent 3.39% until 19.50% of milk with small variations between months and between these milk categories during the research period. A large quantity of milk, based on standards belongs to the so called poor hygienic milk. Results from Fig. 1 also show that there were big differences between months regarding milk contamination with bacteria, and this variation range from 12.28% until 32.08% or more. If we consider that milk production continues to be of the same quality based on standards foreseen for 2007, 2008 and 2009 (Fig. 2), there is a negative slope regarding milk quality from the extra class to por hygienic milk. On 2007, extra quality milk represented 52.44%, in 2008, 26.72% while based in 2009 criteria only 37.18% of the same milk is qualified to extra class. On the contrary the non-hygienic milk ratio is increased by 47.48%, in 2009, 47.13% in 2008, while in 2007 there were no criteria to range milk as poor hygienic quality milk. There are large differences between those who produced extra quality milk during all the period of study and the standards for 2007, 2008, and 2009 respectively, especially to those farms whose milk is considered poor hygienic for 2009 standards, which is a ratio of 92.96%(i.e. farm V).

Discussion

This results lead to the conclusion that bacterial content in fresh milk in our diary farms is very high, compared to existing standards. This is an issue to be worried about for all interested parties linked to milk industry chain, considering that 26.72% (CFU/ml < 200.000) in 2008 and 47.48% (CFU/ml < 100.000) in

Tab. 1: Least squares means (LSM), standard error (SE), standard deviation (STD DEV) and analyses of variance (Pr>F) for effect of farm, breed and month on bacterial content in milk (CFU/ml).

Effect	Bacterial content in farm (CFU/mil – 000)		
	N	LSM±SE	STD DEV
Effect of farm*			
Farm I	47	283.75 ± 150.53$^{b\,c\,d}$	276.50
Farm II	99	105.25 ± 103.72^{d}	78.73
Farm III	43	282.54 ± 157.37$^{b\,c\,d}$	323.66
Farm IV	28	20.93 ± 95.02$^{c\,d}$	14.82
Farm V	270	2115.61 ± 62.80^{a}	2177.72
Farm VI	43	558.29 ± 157.37^{b}	398.83
Farm VII	140	59.36 ± 87.22^{d}	136.97
Farm VIII	104	165.17 ± 101.19$^{c\,d}$	408.96
Farm IX	71	24.61 ± 122.47^{d}	7.14
Farm X	54	127.44 ± 140.43$^{c\,d}$	208.25
Farm XI	223	146.22 ± 69.11^{d}	281.10
Farm XII	82	61.18 ± 113.96^{d}	75.84
Farm XIII	87	449.28 ± 110.64$^{b\,c}$	590.13
Effect of breed			
Black Holstein	368	561.75 ± 66.84^{c}	1177.68
Brown Swiss	215	286.80 ± 87.45^{d}	836.15
Crosses	93	1124.33 ± 132.96^{a}	1764.50
Montbeliard	75	161.97 ± 148.06^{d}	276.31
Simmental	220	458.34 ± 86.45$^{c\,d}$	1311.46
Effect of month			
August	159	874.78 ± 101.44^{a}	1770.41
September	203	292.83 ± 89.78$^{c\,d}$	559.53
October	228	202.39 ± 84.71^{d}	521.07
November	191	517.08 ± 92.55$^{b\,c}$	1101.93
December	200	908.32 ± 90.45^{a}	1879.23
January	177	672.73 ± 96.14$^{a\,b}$	1507.43
February	133	739.58 ± 110.91$^{a\,b}$	1056.18
Analyses of variance	*df*	Pr>F	
Effect of farm	12	0.0001	
Effect of breed	5	0.0478	
Effect of month	6	0.0001	

*Farm names are presented by Roma letters.

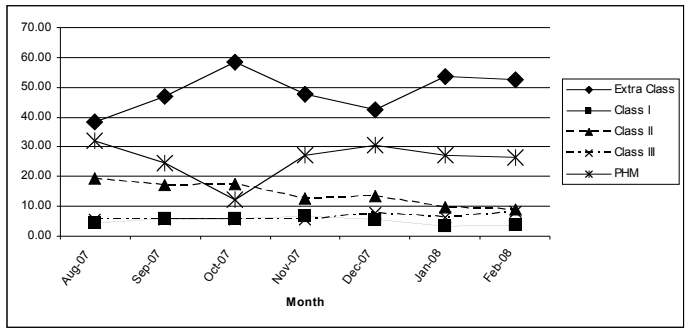


Fig. 1: Bacterial content (CFU/ml) in milk; according to hygienic standards for milk quality; for the period August 2007 – February 2008.

2009 do not fulfil the standards for fresh quality milk. As in the study finding of Bytyqi et al. [2], there were huge differences between farms, included in the study, followed by managerial divergences, which in many cases are not linked only to expensive infrastructure (closed milking system etc.) but they derive directly from negligence and improper management. It is noticeable that there is a negative correlation between cattle breeds with high production (i.e. HF and HK) and bacteria in milk. There is a concern that if the dairy commercial farms continue to produce low quality milk, some of these farms will not be able to sell their milk legally (to dairies, milk processors, etc) and thus they will be obliged to sell their milk illegally (green market, direct consumers, smaller milk processors, etc).

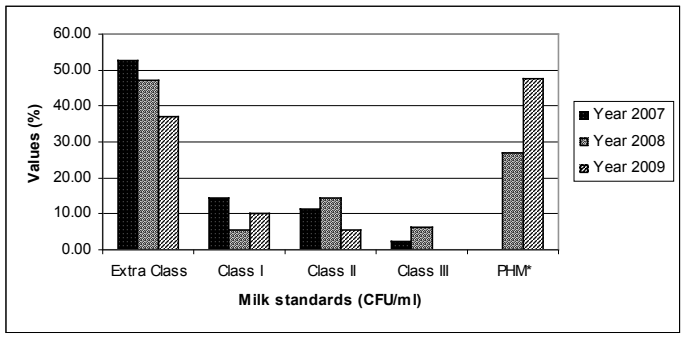


Fig. 2: Bacterial number (CFU/ml) in milk; according to hygienic standards for milk; as predated for the years 2007, 2008, 2009

Conclusion

The fresh milk quality produced in Kosovo, according to existing standards, is still low and it needs an immediate improvement. Many expressed divergences, especially those dealing with farm management, could be improved by applying more advanced practices and with a small intervention in infrastructure (i.e milking equipment and adequate preservation of milk, overall hygiene in farm, etc). The data from our research include just the half of possibilities of milk contamination with bacteria. However, our study opens the possibility of a direct access in avoiding the possible ways of milk contamination and as such it increases directly the customers trust and the farmer profitability.

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Chapter 21

Block 6C

Research on the occurrence of Methicillin-resistant Staphylococcus aureus in domestic pigs and wild boars in Germany

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Summary

This paper describes three studies on the occurrence of MRSA in conventional and organic pig herds and in wild boars to draw preliminary conclusions on the influence of husbandry systems, animal density and antimicrobial treatment.

Introduction

The occurrence of Staphylcoccus aureus strains that are resistant against Methicillin (MRSA: Methicillin-resistant Staphylococcus aureus) has been known since the 60's of the past century [1]. The identification and monitoring of MRSA was initially limited to hospital infections. Over the following decades, however, MRSA strains were more and more often found in the regular population [9], which resulted in the differentiation between hospital acquired MRSA (haMRSA) and community acquired MRSA (caMRSA) [7]. The fact that animals obviously are one of the reservoirs for caMRSA was highlighted for the first time by the detection of MRSA as causative agent of mastitis in a cow herd in Belgium [4]. In the following years, there was an increasing number of reports about the sporadic occurrence of MRSA in other domestic animals: in horses [2], and in dogs and cats [5]. Since about two years, there are reports from The Netherlands [3] about an obviously high frequency of the occurrence of a special clonal MRSA line, the Multilocus Sequence Typing (MLST) type ST398, in pig herds. Soon afterwards, MEEMKEN et al. [6] reported about the occurrence of MRSA in German pig herds. All pig isolates both in The Netherlands and in Germany were assigned to the MLST type ST398. Although the reports from several countries about MRSA in pigs show that ST398 is almost the only clonal MRSA line to be found in porcine populations, it is not justified to assume that this is a clonal line that is exclusively adapted to pigs, since e.g. the data of the National Reference Centre for Staphylococci at the Robert Koch - Institute in Germany show that ST398 has been isolated from other mammal species including humans, both as latent colonizer and as cause of severe infections [11]. These facts clearly demonstrate that the MRSA complex has a zoonotic component, which is in the case of ST398 closely linked to food animal populations: apart from pigs, there are also reports on calves and cows [10] and poultry [8]. Therefore, the term livestock associated MRSA (laMRSA) has been newly established. The following investigations are a contribution to the knowledge on the occurrence of MRSA in different pig populations and husbandry systems.

Material and Methods

Our investigations on the occurrence of MRSA in different pig populations with different husbandry systems were carried out in three studies:

1) MRSA as nasal colonizer of domestic pigs from conventional husbandry system. Therefore we tested 687 pigs from 347 different farms and age groups, which were sent to the Field Station for Epidemiology for clarification of herd health problems by necropsies and laboratory diagnostics. Before dissecting the pigs, nasal swabs were taken, sent to the Nation Reference Centre for Staphylococcus of the Robert Koch-Institute, Germany, and cultured on MRSA-selective chromagar plates. From all MRSA isolates the MLST-type was determined.

2) MRSA in dust samples taken in 6 organic pig husbandry systems and in 7 conventional husbandry systems. Conform to the European Food Standard Agencys approach of the survey on the prevalence of Methicillin-resistant Staphylococcus aureus in herds of breeding pigs (SANCO/3100/2007 – Rev.2), we collected 5 dust samples (500 cm^2 each) per herd, pooled, enriched and cultured them in our laboratory.

3) MRSA as nasal colonizer of wild boars hunted in Germany. From 75 hunted wild boars nasal swabs were taken and cultured on selective MRSA-plates.

Results

As for a) Out of the 678 tested pigs from conventional husbandry systems, 85 had a nasal colonization with MRSA. All MRSA isolates turned out as to belong to the livestock-associated MRSA type ST398. At the individual animal level, a frequency of positive animals of 13%, and at the herd level, a frequency of positive herds of 18% (62 positive herds) was found. Table 1 demonstrates the results according to the different age groups.

Tab. 1: MRSA positive pigs according to the age groups

Age group (weight class)	**No. of pigs (%)**	**MRSA ST398 positive (%)**
Suckling pigs (1,4 kg – 8 kg)	197 (29%)	20 (10%)
Weaning pigs (> 8 kg – 25 kg)	252 (37%)	44 (17%)
Grow-finishers (> 25 kg – 40 kg)	88 (13%)	12 (14%)
Finishers (> 40 kg – 110 kg)	102 (15%)	9 (9%)
Productive sows (> 110 – 291 kg)	39 (6%)	0 (0%)
Total No. of animals	**678**	**85 (13%)**

In our study group the panel of weaners and grow-finishers had the highest frequency of positive results with about 15%. Suckling pigs and finishers had about 10% positive MRSA results. And in none of the tested 39 breeding sows we could find a nasal colonization with MRSA, but this may be associated to the small number of tested sows. As shown in Tab. 2 the rate of positive herds was steadily increasing with the number of submitted pigs per herd. Only 7% of the herds with one submitted pig were positive, 20% of the herds with 2 pigs, 25% of the herds with 3 pigs, and even 50% of the herds with 4 submitted pigs were positive.

As for b) The current results of the dust samples of organic pig farms show a considerably lower prevalence of MRSA compared to the samples of tested conventional herds (Tab. 3).

As for c) All 75 tested nasal swabs from hunted wild boars were MRSA negative. In 10 cases methicillin-sensitive Staphylococcus aureus (MSSA) was detected.

Tab. 2: Rate of MRSA positive tested herds according to the number of tested pigs

No. of tested pigs per herd	No. of herds	No. of positive tested herds (%)
1 tested pig	151	11 (7%)
2 tested pigs	118	24 (20%)
3 tested pigs	47	12 (25%)
4 tested pigs	20	10 (50%)
> 4 tested pigs	11	6 (55%)
Total No. of tested herds	**347**	**63 (18%)**

Tab. 3: Results of testing dust samples from organic vs. conventional husbandry systems

	No. of tested herds	MRSA positive herds (%)
"organic" dust samples	6	1 (16%)
"conventional" dust samples	7	7 (100%)

Discussion

Whereas our results on the MRSA frequency in pigs (nasal colonization) at the individual pig level seem to be in accordance with most of similar studies in other regions [3, 10, 11], our measured herd prevalence of 18% from pigs raised in conventional husbandry system seems to be highly underestimated (cf. Table 2). Like in our study, only few animals per herd were tested. However, from our results it is clearly be concluded that the more pigs per herd we tested, the more MRSA positive herds (one positive pig and more) could be identified. The true herd prevalence, therefore, must be reckoned with as to be far above 50%.

In our studies, the MRSA load in conventional husbandry systems (in the nasal cavity and in dust samples) turned out to be higher than in organic husbandry systems and even much higher in the population of wild boars. The latter cannot be attributed to a natural non-susceptibility of wild boar for Staphylococcus aureus, since we found in ten wild boar specimen MSSA. Possible reasons for the higher MRSA detection rate in conventional husbandry systems than in organic herds could be the higher animal density in conventional herds (i.e. smaller distances between the animals, better transmission conditions), the commonly more frequent use of antibiotics, and the more frequent contacts (circulation of MRSA between herds) with other conventional herds through animal movements than in organic pig herds.

Conclusion

The differences in the MRSA frequency found in our studies do not justify a direct link between the occurrence of MRSA and the use of antibiotics at a specific farm (an indirect link is more than obvious [10]), since the spread of MRSA between conventional herds and the lack of this spread to organic pigs and to wild boar can explain the differences as well. More detailed research into the risk factors of MRRSA is needed.

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Intra-herd Prevalence and Colonisation Dynamics of Methicillin-resistant Staphylococcus aureus (MRSA) ST398 in Pig Herds

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Summary

The study presents first data on the intra-herd prevalence and colonisation dynamics of Methicillin-resistant Staphylococcus aureus (MRSA) in two independent pig herds located in the Northwest of Germany. Swabs taken from the anterior nasal cavity of sows and piglets, from the udder of the sows and from the direct environment of the pigs were tested microbiologically for MRSA. The piglets were identified individually and then swabbed again every 3 weeks from birth to sale at the age of 63 days (farm A), and at slaughter respectively (farm B).
The intra-herd prevalence differed between the two farms. In addition, both farms differed concerning the point in time of the first detection of MRSA in piglets: While MRSA was detected in piglets on farm A within three days after farrowing, positive results in piglets did not occur before three weeks of life. An MRSA-positive result of the sow increased the number of MRSA-positive piglets on days 1-3. The results of this study support the suggestion, that an early colonisation of the piglets with MSSA has a protective effect. Over time, sows and piglets changed their microbiological status of colonisation. Samples from the surroundings of the pigs revealed that the obviously proper cleaning and disinfection on one of the study farm had a reducing effect on the MRSA occurrence in the farrowing pens and in the flat deck.

Introduction

An increasing number of studies in several countries describe the occurrence of livestock-associated MRSA ST398 (laMRSA) in pig populations at a regional level. Publications from The Netherlands (1), Canada (2), Belgium (3) and Germany (4,5) report a regional frequency of farms with MRSA findings up to 70%. The detection rate on the farm seems to increase with the number of samples (5). VAN DUIJKEREN et al. (6) showed transmission of laMRSA from multiplier to finisher farms. And BROENS et al. (7) describes a strong association between positive farms and positive suppliers. According to a Belgian study (3), multiple source farms seem to have a higher in-herd prevalence of positive animals (94%) versus farrow-to-finish farms (56%).
However, there are only few data illustrating the in-herd patterns of colonisation during the pigs' lifetime: ZWAMBAG et al. (8) followed up the piglets of 10 sows. The authors found piglets of MRSA-positive sows to have a higher risk being positive for MRSA at day 28 than those of MRSA-negative.
Our study presents information about the prevalence and the colonisation dynamics of MRSA in pigs from birth to the end of the nursery in one herd and from birth to slaughter in another herd. Moreover it illustrates the impact of cleaning and disinfection on the occurrence of MRSA.

Animals, Material and Methods

Data collection took place between November 2008 and May 2009 on two pig farms in the Northwest of Germany. Farm A is a sow herd with 80 sows. Piglets are sold after leaving the flat deck. Farm B is a farrow-to-finish system with 170 sows. On both farms piglets are weaned after a suckling period of approximately 24 days. Pharyngeal swabs (between day 1 and 3) and later nasal swabs (on days 21, 42, and 63) from 100 piglets (2 piglets each from 50 sows: farm A 30 sows, farm B 20 sows) were collected. The interval of sample collection in piglets was three weeks beginning within three days after farrowing. The sows of both farms were tested for MRSA by taking nasal swabs. On farm A swabs from the udder after entering the farrowing crate were also taken. Additionally the animals' environment in the farrowing crate and in the flat deck (also farm A) was tested. For this purpose, swabs were taken before and after cleaning and disinfection the crates and pens. All samples were tested microbiologically for MRSA.

Results

Farm A: Nasal swabs of 18 sows (62%) were tested positive for MRSA immediately after farrowing. The swabs of the udder were positive in only two sows (5%).
A total of 45 piglets (78%) were tested positive for MRSA at least once during the longitudinal study. Most swabs were tested positive on day 21 and on day 63. Table 1 demonstrates the results of the piglets over all sampling dates of the trial.

Tab. 1: Microbiological results of the nasal swabs of 58 piglets at 4 different points in time on farm A

	MRSA	MSSA	MRSA + MSSA	S. aureus-neg.
day 1-3	9	10	0	39
day 21	28	15	0	15
day 42	15	19	4	24
day 63	30	19	18	9

Piglets from MRSA-positive sows were more often MRSA-positive (7/9) at the first sampling than piglets from MRSA-negative sows (2/9). However, an MRSA-positive result of the sow had no influence on the number of MRSA-positive piglets on days 21, 42, 63. The total number of positive piglets over the study period was equal with regard to positive and negative sows (42/41).
The piglets that were MRSA-positive on the day of the first sampling (n=9), were positive on day 21 to a significantly higher percentage (67%) compared to those piglet that were colonised with MSSA at the first sampling (30%) or when they were free from S. aureus (19%). Piglets that were free from S. aureus at the first sampling carried later on most frequently MRSA.
In dust samples from the farrowing crates MRSA were detected in 29 of 34 samples (85%) before cleaning and disinfection swabbing a surface area of 500 cm^2. After cleaning and disinfection the results of all dust samples were negative. The situation on the flat deck was similar: 14 out of 20 pens (70%) were MRSA-positive before cleaning and disinfection. After cleaning and disinfection there were no positive findings any more.
Farm B: No MRSA-positive sows were identified and no positive piglet was found on the first sampling date. The first positive results in the tested piglets occurred on day 21 of the survey (3/39). A repeated testing of the sows that gave birth to the then positive piglets delivered still negative results. Table 2 shows the microbiological results of the nasal swabs of 39 piglets on farm B at 6 sampling dates.

Tab. 2: Microbiological results of the nasal swabs of 39 piglets at 4 different points in time on farm B

	MRSA	MSSA	MRSA+MSSA	S. aureus-neg.
day 1-3	0	4	0	35
day 21	3	5	0	31
day 42	10	1	0	28
day 63	3	24	0	12
day 84	2	24	1	12
day 105	8	4	0	26

Nine out of the 10 piglets that were tested positive for MRSA on day 42 were found in one barn including the 3 piglets found positive on day 21. Only two of them also carried MRSA on day 63. The status of the others turned to MSSA, which was found to be the most predominant coloniser on days 63 and 84. However, most of the animals were free from S. aureus on day 105. In contrast to this, 7 out of 8 piglets being positive for MRSA on day 105 had never been MRSA-positive on one of the sampling days before.

Discussion

Our investigation in two independent pig farms revealed that the in-herd MRSA prevalence varies remarkably (farm A 78%; farm B 47%). On farm B even no positive sow was detected. This might explain the altogether lower finding rate on farm B, which underlines the importance of the sow-piglet transmission route of MRSA for the in-herd prevalence, although the strongest influence on the in-herd prevalence of this route was detectable on the first days after farrowing. Furthermore, our results support the assumption that an early colonisation with MSSA might have a protective effect due to a phenomenon similar to a competitive exclusion between MSSA and MRSA. To be free from S. aureus early in life seems to lead to more MRSA findings at older ages.

The MRSA-findings in three suckling piglets on day 21 on farm B, and the fact that the sows remained all the same negative, show that piglets get also colonised from other sources than from their mother. This finding supports the statement of ZWAMBAG et al. (8), that the piglets get infected not only by the sow-piglet transmission route. Almost 70% of the MRSA-findings in piglets on farm B occurred after moving the weaned piglets to the flat deck or the finisher-unit. This fact indicates that mixing of piglets even from the same source seems to be a risk factor for a higher in-herd prevalence of MRSA.

The practiced procedure of cleaning and disinfection on farm A was very successful in reducing the occurrence of S. aureus (MSSA and MRSA) in the crate and pen environment of the pigs.

Conclusion

The in-herd prevalence and colonisation dynamics of MRSA and MSSA vary remarkably between farms. The MRSA colonisation status of pigs can change during the animals' lifetime. The MRSA-status of the sow has a strong impact on the MRSA-status of the newborn piglet, and a weaker impact on the colonisation at later points in time during the animals' lifetime.

The findings on farm A show, that an early colonisation with MSSA (between day 1 to 3) might reduce the risk of an MRSA-colonisation at older ages.

In all-in-all-out systems, proper cleaning and disinfection seems to be able to reduce the MRSA frequency in the environment (walls, crates, pens) of pigs, even if positive animals had been in the barn during the previous production cycle.

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Methicillin-resistant Staphylococcus aureus (MRSA) in Veterinarians and Meat Inspectors

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Summary

In several studies, veterinarians and meat inspectors were tested for their nasal colonisation with Methicillin-resistant Staphylococcus aureus (MRSA). First, selected veterinarians visiting exclusively pig farms were tested, 36% turned out to be positive for MRSA ST398, the personnel of the Field Station for Epidemiology of the University of Veterinary Medicine Hannover (pig necropsies and pig farm visits) was even in 38% of the cases positive (also ST398). Second, meat inspectors in two large-scale pig slaughterhouses were tested and found positive with MRSA ST398 in 14% of the cases. Finally, 688 veterinarians of all disciplines were tested for their nasal colonisation with MRSA at a veterinary congress in November 2009. In total, 9% of all tested veterinarians were positive, out of the veterinarians working with companion animals, 8% were positive. However, 21% of those veterinarians that mainly work with pigs turned out to be positive. These frequencies of the MRSA colonisation in veterinarians are significantly higher than the estimated 1–2% MRSA prevalence in the regular population. The German Human Health Authority has therefore decided to add veterinarians and meat inspectors exposed to pigs and/or pig carcasses, like pig farmers, to the traditional groups of people at risk.

Introduction

During the last two years data on Methicillin-resistant *Staphylococcus (S.) aureus* isolated from different farm animal species in various countries have been published [1,2,3,4]. Most MRSA isolates from farm animals were from the Multilocus Sequence Type (MLST) ST398. This type was also found to colonize farmers, veterinarians and slaughterhouse staff, usually without any clinical implications [5,3,6]. However, ST398 has also been reported to be involved in some rare cases of clinical diseases of variable severity in humans [7,8]. These facts clearly demonstrate that the MRSA complex (for a long period of time seen as a problem of human medicine and hospital hygiene only) has a zoonotic component, which is in the case of strains of the clonal line ST398 closely linked to food animal populations. Apart from pigs, there are also reports on MRSA in cows [9], calves [10] and poultry [11]. Therefore, the term "livestock-associated MRSA" (laMRSA) has been established, which adds to the technical terms "hospital-acquired MRSA" (haMRSA) and "community-acquired MRSA" (caMRSA) [12] in human medicine. Furthermore, MRSA ST398 was isolated from meat and meat products of a number of food animal species collected at retail level [13]. The paper reports about several studies into the nasal colonisation of veterinarians and meat inspectors with different degrees of exposure to pigs.

Material and Methods

Nasal swabs from the following professional groups were taken:
- veterinarians fully specialised in pigs,
- research personnel involved in pig pathology and clinics,
- veterinarians with various specialisations (mixed species and companion animal practitioners), and
- meat inspectors in pig slaughterhouses.

All samples were cultured (without pre-enrichment) on selective MRSA plates (Chromagar plates, Becton-Dickinson, Heidelberg, Germany) and simultaneously on Columbia blood agar plates (Heipha, Eppelheim, Germany). Most of the MRSA isolates were tested for their MLST-type or their typing is still pending.

All tested persons filled in a questionnaire (major focus on: antibiotic treatments prior to sampling, and regular contacts to people in hospitals and/or elderly homes).

Results

All these groups of persons occupationally exposed to animals (live animals or carcasses) showed an MRSA colonisation frequency between 8% and 38% (see Table 1). All subtyped MRSA-isolates of the pig-exposed persons turned out to be only strains of the clonal line MLST389, i.e. laMRSA. The subtyping results of all other MRSA findings (mixed species and companion practitioners) are still pending.

Analysing the questionnaires, no obvious association between a nasal colonisation with MRSA in the sampled persons and their previous antibiotic treatments or a frequent contact to persons in hospitals and elderly homes could be identified.

Tab. 1: MRSA results in persons with different degrees of exposure to pigs

Profession: degree of exposure	**No. of tested persons**	**MRSA positive (n)**	**MLST types**
Veterinarian: full-time pig practitioners	28	36% (10)	ST398
Research personnel: pig pathology and clinics	8	38% (3)	ST398
Meat inspector: pig slaughterhouses	50	14% (7)	ST398
Veterinarian: mixed species practitioner	164	21% (35)	pending
Veterinarian: companion animal practitioner	380	8% (29)	pending

Discussion

The frequency of the nasal colonisation with MRSA in veterinarians with close and frequent contacts to pigs (full-time pig herd health management and pig research/diagnostics), as well as the frequency of the nasal colonisation of meat inspectors in pig slaughterhouses are definitively higher than the frequency in other groups of veterinarians (mixed species and companion animal practitioners). These

frequencies, even the relatively low frequency of nasal MRSA colonisation in the companion animal practitioners are far above the estimated MRSA prevalence in the general population of Germany (estimation of the Robert Koch-Institute, Germany). The fact that all MRSA-positive pig-exposed persons carry only the livestock-associated MRSA ST389 proves the zoonotic link between pigs and exposed persons. The assumption of the circulation of laMRSA between pigs and occupationally pig-exposed persons is underlined by the fact that in the presented studies the frequency of known risk factors (e.g. antibiotic treatment and contact to persons in hospitals and elderly homes) for an MRSA colonisation of humans was evenly distributed between laMRSA-positive and laMRSA-negative persons.

Conclusion

Although there has been so far no acute increase of the findings of MRSA ST398 in human clinical cases, it is advisable to closely monitor the occurrence and epidemiology of MRSA ST398, since pigs must be regarded as an important reservoir of MRSA. Humans occupationally exposed to pigs should be educated about the potential risk for themselves and about their potential carrying MRSA into a hospital. The Robert Koch-Institute (the German Human Health Risk Assessment Authority) has therefore decided to assign veterinarians and meat inspectors exposed to pigs and/or pig carcasses, like pig farmers, to a new group of people at risk.

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Hygiene interventions to reduce spread of MRSA in animal production

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Introduction

Staphylococcus aureus including Meticillin-resistant *S. aureus* (MRSA) is frequently present on the skin, in the nose or in the mouth of human hosts without causing illness [6]. However, in some instances, *S. aureus* can cause disease when it enters wounds or damaged skin, and can cause abscesses, pneumonia, meningitis, endocarditis and septicaemia particularly in immune suppressed patients who are difficult to treat. Recently a specific clone (CC398) appears to be emerging which has been found in nearly all production animals, particularly in pigs, across Europe that colonises also farmers and veterinarians, and concerns rise that it may enter hospitals and the food chain [2]. Clear abatement strategies are not yet in place in animal production. This paper will describe control options on farms and between farms suitable to avoid or reduce transmission of these micro-organisms.

Animals, Material and Methods

Staphylococcus is a genus of Gram-positive bacteria that are coccoid (spherical) and approximately 1 micrometer in diameter, have thick peptidoglycan cell walls, have a low G + C content, and grow in clusters similar to grapes (staphyl = grape). They are commensals (normal bacterial flora) of mammals, but can also survive in a variety of environments and survive desiccation (drying). There are over 30 species of staphylococci, the most pathogenic species for humans is *S. aureus* which can be differentiated from other staphylococcal species colonising humans (e.g. *S. epidermidis, S. haemolyticus, S. hominis, S. saprophyticus, S. capitis*) in the diagnostic laboratory by a positive coagulase reaction. *S. aureus* occurs in the human nose, and can also be found in the throat, auxilla, rectum, perineum or gastrointestinal tract. It is important to recognise different states of interaction between *S. aureus* (including meticillin resistant *S. aureus*, MRSA) and its environment. These can be defined as infections in both animals and humans where growth of the bacterium occurs together with overt or covert pathological changes indicating the presence of disease. Carriage or colonisation also occurs in both humans and animal where *S. aureus* (including MRSA) multiplies in the nares, throat or other superficial sites but without causing disease. Contamination occurs in humans, animals, food, the environment etc where *S. aureus* (including MRSA) is present due to exposure from another site (i.e and infected or colonised host or the environment such as dust). Animals or humans can be contaminated at external surfaces, and there is no multiplication of *S. aureus* and no clinical symptoms [2].

Results

S. aureus is transmitted from human to human, animals to human and vice versa and through other viable or inanimate vectors. Further viable vectors, which transmit the bacterium, are e.g. flies or ticks [9]. These vectors are defined as *mechanical vectors* if the transmission occurs by means of their body parts without a multiplication of the infectious agent. Inanimate vectors could be medical devices, contaminated material or objects, food, milk and dust. The latter is known as a vehicle for the airborne transmission of *S. aureus* and may play a role in the spread of infections. Transmission and spreading in general is supported through the tenacity outside the host and through the adaptability of this bacterium.
The transmission routes of methicillin-resistant *S. aureus* (MRSA) are probably similar to those of methicillin-sensitive *S. aureus* (MSSA) but there may be differences in the efficiency of host colonization due to genetic diversity. The following table 1 shows examples of vector related transmissions of MRSA strains from the literature.

Table 1. MRSA transmission by known or suspected vectors

source	**vector**	**host**	**reference**
faces of patients	hospital personnel medical instruments	patients	Akane Kuramoto-Chikamatsu et al., 2007
unknown	health care workers patients	patients health care workers	Blok et al., 2003
unknown	lactating mothers infants	infants lactating mothers	Kawada et al., 2008
wrestling team	infected or colonized wrestler	team members	Lindenmayer et al., 1998
unknown	parents or children	family members	Huijsdens et al., 2006
unknown	football players	team members	Kazakova et al., 2005
presumably human	horses	humans with animal contact	Weese et al., 2005
unknown	staff of a veterinary teaching hospital	horses	Seguin et al., 1999
unknown	Pig	humans with animal contact	Meemken et al., 2008
unknown	Cows or farm worker	farm worker or cows	Juhasz-Kaszanytzky et al., 2007
unknown	domestic pets humans	humans pets	Weese et al., 2006
nares of a dog	Dog	human couple	Manian, 2003
unknown	nurse	dog	Duijkeren, 2004
Health-care worker	Food	patients	Kluytmans et al., 1995
food preparer	Food	humans	Jones et al., 2002

The origin of MRSA strains, which colonized or infected different vectors, is often not traceable. Skin to skin contact is in all likelihood the main route of transmission between humans, humans to animals, animals to human and between animals, however contaminated materials, surfaces, food or dust could play also

an important role in transmitting the agent. It is obvious that there are specific settings acting as reservoirs for HA MRSA and CA MRSA. For example hot spots for HA MRSA strains are hospitals or nursing homes. Circumstances such as overcrowding and understaffing or high colonization pressure in such facilities may increase the transmission risk whereas MRSA control programs and the compliance of strict hygiene roles could decrease the transmission risk.

In contrast CA MRSA are transmitted within population groups such as families, sport teams, prisoners, injection drug users or men who have sex with men [4]. Crowding, frequent skin-to-skin contact, compromised skin, contaminated surfaces and shared items and lack of cleanliness are assumed to contribute to the transmission of MRSA inside of this groups. The increasing cases in CA MRSA presents also a problem in MRSA control in hospitals since it is established that transmission of CA MRSA in hospital lead to infections.

Recent studies revealed that farm animals and small animals could be also reservoirs of MRSA strains. The origins of the strains are unknown, however investigations indicates an international spread of specific clones (Wulf and Voss 2008). The transmission of MRSA from animals to humans is evident and people with direct contact to the animals such as pet owner, farmers, stockman, veterinarians and the staff at slaughterhouses showed a higher prevalence of the agent as less or unexposed people. In this context it is of particular concern that an inpatient with pneumonia was infected with a sequence type (ST398) that is spread under European pigs. A ST398 strain was also responsible for a human endocarditis [3] and this indicates that MRSA in pigs may pose a public health risk to human contact. Further studies in particular the investigation of transmission routes of MRSA between animals and humans and vice versa are necessary to assess the risk of the zoonotic potential of this agent.

Conclusion

In spite of the presently still limited understanding of the transmission routes it is even more important to apply and constantly improve bio-security measures in order to protect herds against the entry of MRSA which are likely to be equally applicable to control other infectious agents. Such general measures on farm level can help to reduce the entrance and the spread of infectious agents both within and between herds and can be described as follows:

- Restriction of traffic of cars and trucks and in particular people transporting in and bringing out animals, feed stuff and equipment as well as slurry or manure.
- Avoid contact with animals. Provide protective equipment, including clothing, gloves and breathing masks when required.
- New animals have to undergo veterinary inspection, testing and a quarantine time before entering the herd.
- Observe strict hygienic practices when milking and with milk tank facilities.
- Store fallen animals in separate and safe containers or places until removed as soon as possible by specialised companies.
- Restrict contact with rodents, insects and wild birds on the premises in order to avoid transfer of infectious agents and MRSA from farm to farm and between herds in a farm. Prevent cats and dogs of the farm to have access to the livestock.
- Make sure that regular checks of the health status of personnel with regard to MRSA and other zoonotic diseases are carried out.
- Apply regularly strict cleaning and disinfecting regimes to the different housing systems.
- In order to avoid airborne transmission of MRSA from existing farms to new farm buildings the right location for the farm should be selected with sufficient distance to the next farm or residential dwelling ("safe distances" to neighbouring farms and residential areas). For existing farms technical devices such as biofilters or bioscrubbers (VDI guidelines) can be used in order

to reduce or to eliminate bacterial emissions from farms [7]. However, experiences with these techniques are still missing for MRSA control.
- There is an urgent need for more research in this field for a better understanding of the transmission pathways and hence the development of prevention and control strategies.

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Salmonella - A MODERN EUROPEAN PERSPECTIVE

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SUMMARY

Salmonella is a worldwide public health issue and the European Union (EU) has showed clear initiatives to take on the challenge of reducing *Salmonella* in food animal production. The European Union countries have a very diverse *Salmonella* situation, ranging from some countries having negligible levels in the food animal production to other countries where *Salmonella* is highly prevalent. The dominant serotypes of *Salmonella* vary between regions and countries. Most serotypes do not cause clinical disease in animals, but are more of public health concern.
The EU has been implementing an enhanced monitoring and control of *Salmonella* in food animal production. Producers are now sharing the responsibility for the safety of food production. Egg and egg products are considered the major *Salmonella* risk food source for humans, and the *Salmonella* control programmes have initially been targeted to the poultry production. Meanwhile, there is legislation and plans to implement *Salmonella* reduction measures in pig production. These EU plans are however pending a cost benefit assessment. The methods of reduction of *Salmonella* will vary dependent on food animal production species, the agricultural infrastructure, prevalence in the animal population, resources and focus of the industry concerned. All member states have *Salmonella* control programmes in poultry, starting with the breeding birds, and some member states have initiated control programmes in pigs and cattle based on antibody titres to *Salmonella*.
This paper will briefly present the *Salmonella* scenario in Europe, the *Salmonella* legislative measures, the control programmes, and some of the challenges of reversing the *Salmonella* trend in food animal production.

INTRODUCTION

Salmonella **monitoring in the European Union.** EU Directive 2003/99/EC [1] on the monitoring of zoonoses and zoonotic agents improved the old data collection system for zoonotic agents, including *Salmonella*. Two EU institutions, The European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), collate data, publishing summaries, and advise on harmonized procedures for sampling and testing.[1] EFSA also evaluates risks associated with *Salmonella* in food production animals, and the optimal control programmes. [15, 16]

OVERVIEW

In 2007, approximately 152,000 human salmonellosis cases were reported in the European Union and 39.5% of all foodborne outbreaks were *Salmonella* associated. [17, 18] Cases of salmonellosis are not always diagnostically confirmed and reported, diagnostic procedures, methods and reporting vary between nations, making international comparisons very difficult, and the true number of *Salmonella* cases is likely very much higher. A study

of human *Salmonella* antibodies has estimated that there were 325 *Salmonella* infected humans per microbiological culture-confirmed case.[22] However, the reported incidence of reported salmonellosis continues to decrease in the European Union with a statistically significant trend over the last four years.[19] In the EU, among the foodborne cases of human salmonellosis, eggs and egg products are still the most frequently implicated sources. Meat is also an important source of foodborne salmonellosis, with poultry and pork implicated more often than beef and lamb. In the EU, *Salmonella*Enteritidis and Typhimurium are the serovars most frequently associated with human illness. A EU wide study in 2007 of meat-based products indicated that *Salmonella*was found in 5.5% of fresh poultry and 1.1% of pig meat samples.[18] It should be noted that microbiological culture of final products is an insensitive method, and *Salmonella* may therefore be present in much higher levels.

An EFSA study in 2004-2005, conducted on commercial large-scale egg-laying hen farms in the EU, found a range of *Salmonella* levels in layer flocks between 0% and 79% and 20% of all farms were positive for *S.* Enteritidis and/or *S.* Typhimurium.[11] Another EFSA study in 2005 to 2006 indicated that 23% of broiler flocks were *Salmonella* positive and the levels varied from 0 to 68% between countries. [12] The most common serovars in broilers in decreasing order were *S.* Enteritidis, *S.* Infantis, *S.* Mbandaka, *S.* Typhimurium and *S.* Hadar.

A survey of *Salmonella* in turkey production estimated that all serotypes were estimated to present in 30.7% of turkey flocks reared for human consumption and in 13.6% of turkey flocks kept for breeding purposes. [14] *S.* Enteritidis and *S.* Typhimurium were detected in 3.8% of flocks reared for human consumption and in 1.7% of breeding flocks. The five most frequently isolated *Salmonella* serovars from fattening turkey flocks in decreasing order were: *S.* Bredeney, *S.* Hadar, *S.* Derby, *S.* Saintpaul and *S.* Kottbus.

In a survey conducted in the EU in slaughter houses in 2006-2007, an estimated 10.3% of all pigs were *Salmonella*-positive. The *Salmonella* prevalence in these slaughter pigs varied from 0% to 29% between countries. The five most frequently isolated serovars from carcasses were respectively in decreasing order *S.* Typhimurium, *S.* Derby, *S.* Infantis, *S.* Bredeney and *S.* Brandenburg. [13]

European Union Legislation regarding *Salmonella* in food animal production. The first European Union legislation for controlling several zoonotic diseases came in 1992 with Council Directive 92/117/EEC. Data was collected in all EU countries on zoonotic diseases in production animals, and control programmes very initiated in breeding poultry for *Salmonella* Enteritidis and *Salmonella* Typhimurium. An evaluation of the control programmes after a decade indicated that the control of the breeding poultry did not result in any decrease in *Salmonella* in the production birds, and the EU decided to extend the control measures to other production categories of poultry, and also lay down foundation for introducing controls in other production animals, such as turkeys and pigs.

EU Regulation 2160/2003/EC [2] now obliges farmers and member state authorities to work together to reduce *Salmonella* , especially serotypes of public health significance, such as *S.* Enteritidis and *S.* Typhimurium. This regulation sets the framework for species-specific control plans. There are general legislation of food safety that legislates that *Salmonella* should not be found in food. The EU Food Hygiene Regulation 852/2004/EC, on the hygiene of food stuffs [3] states that the responsibility for the production of safe food lies with the food business operator and this includes the primary animal producer up through the food chain to the consumer; a farm-to-fork approach. Regulation 2073/2005/EC on microbiological criteria for foodstuffs [5] has rules on the absence of *Salmonella* in certain food products. The criteria apply during the whole shelf life and products must be withdrawn from the market if they do not comply.

There are also specific legislation that describes control programmes. Strategies and

reduction goals vary between countries depending how much *Salmonella* there is in the production animals. The European Union has three regulations for reducing and controlling the prevalence of *Salmonella* in breeding poultry 1003/2005/EC [4], broilers 1168/2006/EC [7] and layers 1177/2006/EC [6]. Targets for reduction of the prevalence of *Salmonella* in herds of slaughter and breeding pigs will be established at the end of 2009 and will apply to all *Salmonella* serotypes with public health significance supported by a cost-benefit analysis.[2]

Control and surveillance programs. Sampling programmes can strongly influence the estimated levels of contamination in herds and countries. In the case a control programme is legislated on EU level, sampling programme and bacteriological culture method has been established. Countries, industries and farmers may chose to use alternative sampling programmes. It is important to assess the sampling programme and methodology used or to be used. [9] Several countries have initiated surveys and control programmes based on monitoring antibodies to *Salmonella* in the herds using ELISA (enzyme linked immunosorbent assays). The herds are thereafter categorized based upon the percentage of animals that are showing high ELISA results. The relationship between herd serology and bacterial shedding is complex, but it can still be a useful tool for herd-level classification. [8,9] Reducing *Salmonella* in production animals requires multiple time and resource consuming interventions. Biosecurity, animal purchase, animal flow, feed, management, nutrition, herd immunity are all very important factors, and the length of this paper prevents detailed descriptions of all control points. Vaccination has shown to be successful in reducing *Salmonella* levels in poultry and pigs, and is used in countries where *Salmonella* is prevalent. [9,10] However, *Salmonella* vaccinations interfere with antibody-based surveillance of the flocks. Organic acids in the feed [23]and in the litter [21] have also been shown to successfully reduce *Salmonella* levels in birds and but not consistently shown in pigs [20]. A wide variety of other antimicrobial interventions have been tested in the live animal production and in the post-harvest procedures with varying results. The importance of validating individual study findings in further studies and in field trials should be emphasized, to avoid adopting procedures that are of questionable value.

Conclusion

The future of *Salmonella* control. The challenge to reduce *Salmonella* in food animal production will in many production systems entail huge time and financial investments. Many of the existing animal husbandry systems are not easy to sanitize, or prevent *Salmonella* from entering and spreading. The food animal industry needs to work together to minimize Salmonella in all pre- and post-harvest production steps. All available tools (such as vaccinations, feed supplements, litter treatments, immune-enhancing drugs, disinfection systems, etc) that have the ability to reduce *Salmonella* should be employed to achieve European Union and national goals.

Further resources. A website www.KnowSalmonella.com on *Salmonella* in food animal production has been developed by Alltech Inc. Links to legislation and EFSA reports are also found on this site All European Union legislations can be retrieved on http://eur-lex.europa.eu and all EFSA publications can be retrieved on http://www.efsa.europa.eu.

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The Role of Nutritional Solutions in a Salmonella Control Program

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Summary

Salmonellae are responsible for a number of transmissible and non-transmissible pig and poultry diseases. Public health concern about the spread of this pathogen through the food chain to humans is currently of major importance and increasingly the scientific community is concerned about the spread of antimicrobial resistance determinants within *Salmonellae*. As none of the on farm salmonella control strategies have been successful on their own, control must be addressed through an integrated approach, taking into account animals, housing, management, cleaning, disinfection as well as the feed. The role the feed plays is two-fold. First, the feed can serve as a vector bringing Salmonella into a production unit. Second, the diet has a great impact on the GI environment as well as on the animal's immune defence. Feed additives such as short and medium chain fatty acids, essential oils, probiotics, prebiotics and certain specific carbodydrates such as mannan-based compounds have demonstrate anti-salmonella properties when added to the feed and their use should be considered as part of an integrated control approach.

Introduction

Salmonellosis is generally accepted to be one of the most important zoonoses transmitted by meat and eggs in the developed world. The impact of salmonellosis on human health results in significant economic losses [1]. In the European Union 5'609 food-borne outbreaks were reported by member states in 2007 [2]. While total EU Salmonella contamination levels have decreased the last few years, antimicrobial resistance of the Salmonella isolates is still increasing. *Typhimurium* serotypes in particular is causing concern, as about 40% of all *Salmonella typhimurium* strains isolated in 2006 were resistant to 4 or more antimicrobials. The magnitude and the complexity of the problem make integrated control programs very important.

There is little doubt that surveillance programs that timely detect salmonella contaminations in the entire food chain (feed, living animals, slaughterhouses, retail sector and restaurants) together with sanitary measures are essential to limit the occurrence of human *Salmonella* infections. Traditionally the industry has been relying too strongly on the final consumer to cook the product properly to eliminate the pathogen. However, greater awareness on the issue as well as new governmental regulation such as the EU regulation on salmonella monitoring at the slaughter house and financial penalties on producers with high levels of Salmonella is forcing farms to reduce systematic action. As none of the on farm salmonella control strategies have been successful on their own, control must be considered in terms of an integrated approach. First of all, good farming and hygienic practices need to be implemented, in order to avoid introduction of Salmonella on the farm or to reduce infection pressure when Salmonella is present. Hygienic measures should take into account animals, housing, management, cleaning, disinfection as well as the feed. The role the feed plays in a control program is two-fold. First, the feed is a potential vector to introduce Salmonella into the farm. Proper control programs must

therefore been in place at any feed manufacturing site as well as on the farm to assure the absence of Salmonella in the diet at the moment when it is consumed by the animal. This must starts with the control of the incoming ingredients. Pelleting at adequate temperatures has been shown very effective in decontaminating feed. If thermal treatment is not integrated in the production line, acid addition or where allowed the application of formaldehyde or their combinations can be used as an alternative decontaminating measure. Feed has to be stored and transported to the feeder with properly cleaned and well maintained installations. Second, besides being a potential vector, the diet has a great impact on the GI environment thus influencing the microbial composition in the GI tract and in the barn as well as on the animal's immune defence and its ability to fight infections. For many years the prophylactic use of antibiotics has been playing a key role in controlling infectious diseases. Since their prophylactic use has been banned in a number of nutritional programs alternative additives are used as part of integrated control programs. There is a large number of commercial products available which can be used via feed or drinking water to reduce salmonella. However, the effects are not well documented for all of them. Decisions on product use should manly be made based on *in vivo* data coming from peer reviewed publications and data from large scale field surveys.

Results

Nutritional strategies to control Salmonella. Nutritional effects can either be achieved through general diet formulation or the addition of additives. Through diet formulation the availability of fermentable substrate and the buffering capacity can be influenced. However, as nutrient requirements have to be respected at all time, the possibilities to affect the conditions in the digestive tract are limited. However, a number of feed additives have gained commercial acceptance in helping to reduce salmonella:

CE-cultures: Nurmi and Rantala [3] showed that newly hatched chicks can be protected from subsequent salmonella infections by accelerating the establishment of a complex, protective microflora. The most efficient way to achieve good protection is the application of a complex CE-culture from a pathogen free mature bird. Much attempt has been made to limit the number of strains in such cultures in order to develop better defined products. However in most cases this was accompanied by a loss in efficacy.

Probiotics – *Lactobacillus sp., Lactococcus sp., Bifidobacteria sp., Pediococcus sp., Enterococcus sp., Saccharomyces sp.* Numerous research studies have investigated the addition of live lactic acid bacteria or yeast to the diet to act in the manner of competitive exclusion. While seeding cultures are only administered once, probiotics are feed continuously to achieve a shift in the microflora.

Prebiotics – fructooligosaccharides and inulin. Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth of a limited number of bacterial species and thus work to improve host health [4]. However, some of the data with FOS is inconsistent for control of Salmonella. Research conducted in rats demonstrated an increased colonisation of Salmonella in caecal contents and enhanced translocation of Salmonella [5].

Short chain fatty acids – formic, acetic, propionic and butyric acids all demonstrate anti-salmonella properties when added to the feed at sufficient concentrations. These acids are often coated or encapsulated to protect them until they reach the lower intestine where they are effective.

Medium chain fatty acids – caproic, caprylic, capric acids. These fatty acids have been shown to reduce the numbers of Salmonella *in vivo* [6]. It was proposed that they reduce the invasive capacity of Salmonella by decreasing the expression of genes specifically involved in invasion.

Essential oils – a range of essential oils have

been shown to have bacteriostatic or bacteriocidal properties against *Salmonella*. It is critical to apply them at the dose in the field at which the anti-salmonella effects have been observed in research.

A number of carbodydrates (based on glucose, mannose, galactose, fucose) have been shown to have anti-infective properties. Mannose and its polymers are the most commonly used products as feed additives. The initial interest in using mannan based carbohydrates as a feed additive originated from work done in the late 1980s looking at the ability of mannose to inhibit salmonella infection in broilers. Mannose based sugars can bind to type-1 fimbriae of Salmonella and thereby block adhesion to epithelia cells and mucus, thus reducing their chance to colonize the digestive tract. Similar findings have been demonstrated with mannan oligosaccharide (Bio-Mos, Alltech Inc.) at significantly lower concentrations than that required for purified mannose [7].

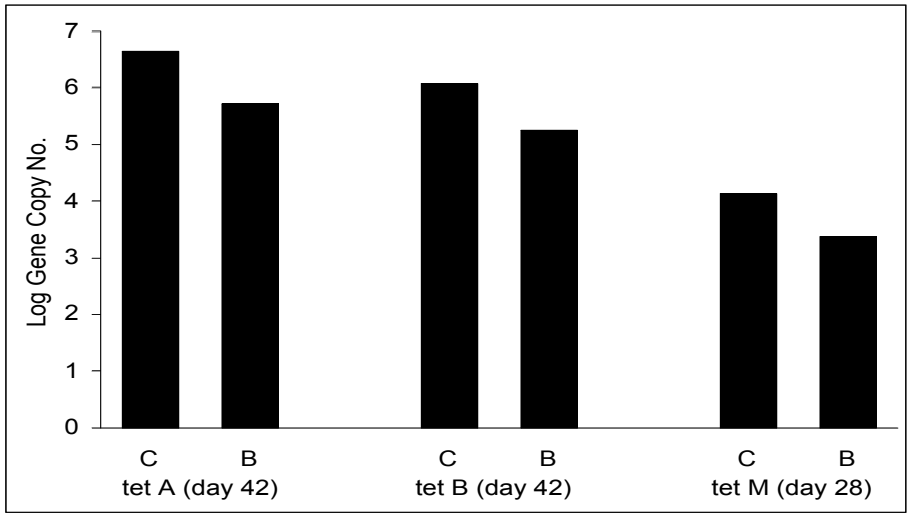


Figure 1. Effects of control (C) and Bio-Mos$^{(R)}$, Alltech Inc (B) on levels of tetracycline resistance genes tet A, tet B and tet M in caecal contents of turkeys (tet A, P<0.050; and tet B, P=0.037) and 28 (tet M, P=0.026). (adapted from [8])

Nutritional strategies to reduce antibiotic resistant Salmonella. Beside efficiently controlling salmonella levels in the production system there is also a great need and interest to reduce antibiotic resistance in Salmonella. Novel data on a mannan based product (Bio-Mos, Alltech Inc.) have demonstrated its ability to reduce the prevalence of antibiotic resistant bacteria in turkey (Figure 1)[8]. It was proposed that the reduction is achieved by affecting plasmid transfer rates, which could be due to inactivation of specific receptors on the bacterial cell wall, probably the same receptors that are involved in bacterial adhesion to gut mucosal cells. Follow up work is needed to better understand the effect in the entire food chain.

Conclusion

Salmonella control must be addressed through an integrated approach, taking into account animals, housing, management, cleaning, disinfection as well as the feed. Feed additives with proven anti-salmonella effects should be part of this integrated approach.

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Chapter 22

Block 7C

Evaluation of risk factors for the occurrence of Salmonella in pig farms in Lower Saxony, Germany

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Summary

In this study farms with a low frequency of animals with antibodies against Salmonella are compared to farms with a high frequency of animals that are serologically positive to evaluate the relevance of presumed risk factors and/or to identify so far unknown risk factors. A multitude of data representing the characteristics of the study farms was recorded in a standardised way by means of a validated questionnaire. To substantiate the results of the survey and to verify the salmonella-specific relevance of the potential risk factors, faeces and environmental samples were gathered and analyzed by means of real-time PCR.

The results confirm that at a regional level, not applicable for single farms, significantly more faeces and environmental samples from farms with pig herds with a high frequency of serologically positive animals were salmonella-positive than those from with a low frequency. At farm level, however, it is obvious that every salmonella-positive farm has its own very farm-specific set of reasons for the Salmonella infection of the animals, for the circulation of Salmonella within the herd, and for the perpetuation of Salmonella from production cycle to production cycle.

The results prove the justified, but so far poorly evidenced assumption that farms with a low frequency of serologically positive animals, even those farms with no serologically positive animals, are not necessarily salmonella-free. The results also show that there is a set of quite general farm and management factors (hygiene level, rodent control, bird access, mixing of animals etc.) that increase the risk for pig herds of being salmonella-positive, but every salmonella-positive herd needs to be specifically analysed for its farm-specific factors that are "responsible" for the introduction into the herd and the circulation on the actual farm in question.

Introduction

Salmonella spp. is, according the latest EU Zoonosis Report [1] still the most common cause of outbreaks of food borne diseases in humans in the European Union (EU). The importance of this pathogen has been known for long, therefore, in 2003 a new EU-Zoonosis-Legislation was passed to monitor, control, and prevent the introduction of zoonotic pathogens including Salmonella into the food-chain via infected food animals including slaughter pigs and contaminated pig carcasses. In accordance with the principle of the "farm-to-fork" approach, the focus of the legislation was on controlling the problem of contaminated food reaching the table of the consumer at all stages of the food chain, including the pig farms themselves. Germany passed a national law in 2007 in accordance with the EU-Legislation which demands from all pig farm owners producing slaughter pigs to have their herds risk categorized by having a certain percentage of animals tested at slaughter serologically for antibodies against Salmonella. The result is a categorisation of herds (run since 2004) into Category I (= "low risk herds" with less than 20% of their tested pigs being antibody positive), Category II (= "medium risk herds" with

between 20% and 40% of the tested pigs being antibody positive), and Category III (= "high risk herds" with more than 40% pigs being antibody positive). The owners of herds in Category III are requested to find the source of the Salmonella infection and to take measures such as improving their hygiene-management to constantly minimise the bacteria.

Many risk factors for Salmonella infections have already been identified and discussed since the mid 90's in the scientific literature. However, all these risk factors have been evaluated by means of relatively small sets (10 to 20) of farms.

The goal of this study was to find out by epidemiologically analysing two large sets of farms what differences in the farm characteristics and management quality, if any, are between farms of high and low serological prevalence in order to establish guidelines against Salmonella-infections.

Material and Methods

By April 10, 2009 77 farms in Category III and 34 farms in Category I have been examined via a questionnaire and altogether 2000 environmental and faeces samples have been collected for the PCR testing. The questionnaire deals with the following areas that potentially have an impact on the occurrence and frequency of Salmonella on a farm and in a pig herd, respectively: farm type, herd size, animal movement, hygiene standard, housing conditions, feeding and management procedures of every study farm.

Fifteen to 20 samples were taken per farm. The kinds of samples taken were standardized as much as possible, though some allowances must be made, since farms may have specific characteristics that are not to be found on each study farm, but may be important for identifying Salmonella sources.

Examples for the most frequently kinds of samples taken are: Faeces collected from pens, partitions between pens, hallways, tools for driving animals, pig toys, drinking nipples, feeders and troughs, boots, fans, rodent droppings and flies, loading ramp surfaces, scales, transporters, ante rooms, dust from cables and pipes, etc.

All samples were enriched in buffered peptone water at 37 °C for 18 hours. Subsequently, each enrichment broth was tested by the standardized real-time PCR protocol. The PCR was carried out with the TaqMan$^{(R)}$ Salmonella Detection Kit according to the manufactures instructions. Reactions were performed on a real-time PCR System 7500 and the results were analysed with SDS software package V. 1.4 (all Applied Biosystems; USA).

Results

The results of the still ongoing study show that contrary to popular belief farms with low serological prevalence are not salmonella-free (Tab. 1).

The environmental samples can be divided into two major types depending on their location of collection: the direct animal environment and the indirect animal environment. The direct animal environment can be further divided into housing components (pen walls) and equipment within the pen (drinking nipples, toys such as chains, and feeding equipment). Frequent samples that were positive in this category are: partitions between the pens, drinking nipples and the pig toys.

Out of the indirect animal environment, a high percentage of positive samples were from the hallways outside the pens, the ante rooms and the animal scales.

Tab. 1: PCR results of the samples per sample type and serological Salmonella category

Sample	Sum	positive total		Cat. I	positive Cat. I		Cat. III	positive Cat. III	
		n	%	total (n)	n	%	total (n)	n	%
Faeces from pen floors	354	86	24%	52	7	13%	302	79	26%
Hallways outside pens	185	67	36%	23	4	17%	163	63	39%
Driving boards	167	43	26%	39	7	18%	128	36	28%
Feeders and Troughs	153	47	31%	33	8	24%	120	39	33%
Central hallway in barn	134	46	34%	35	7	20%	98	39	40%
Pig Toys	123	33	27%	45	10	22%	78	23	28%
Pen walls	120	41	34%	30	8	27%	90	33	37%
Drinking nipples	105	35	33%	37	9	24%	68	26	38%
Ventilation (fans)	96	20	21%	34	4	12%	62	16	26%
Pipes	92	23	25%	28	6	21%	64	17	27%
Ante rooms	70	20	29%	16	3	19%	54	17	31%
Boots/Shoes of Farmer	68	20	29%	27	6	22%	41	14	34%
Walls	67	8	12%	25	2	8%	42	6	14%
Ceilings	47	12	26%	8	0	0%	39	12	31%
Animal scales	44	14	32%	13	2	15%	31	12	39%
Gas heaters	40	10	25%	12	4	33%	28	6	21%
Loading ramps	30	6	20%	6	2	33%	24	4	17%
Lamps	17	6	35%	2	0	0%	15	6	40%
Transporters	15	5	33%	1	0	0%	14	5	36%
Faeces from rodents	13	6	46%	1	1	100%	12	5	42%
Flies	9	1	11%	4	0	0%	6	1	17%
Feed/Water storage	8	0	0%	1	0	0%	7	0	0%
Feeding pipe interiors	8	1	13%	3	1	33%	5	0	0%
Other	35	6	17%	5	1	20%	29	5	17%
Total:	**2000**	**556**	**28%**	**480**	**92**	**19%**	**1522**	**464**	**30%**

Discussion

As Salmonella are ubiquitous bacteria which can be found everywhere and have a high tenacity in the environment, it is not truly surprising that even the study farms with the low serological prevalence are not salmonella-free. However, in the majority of the sample types, the samples from the farms with Category III herds were considerably more often salmonella-positive than the samples of the same sample type from farms with Category I herds. Further research is needed to understand why it is that in some cases of Category I herds an amount of Salmonella in environmental samples that is almost as high as in many Category III, does apparently not lead to an increase of pigs with Salmonella antibodies.

The relatively high amount of positive samples in the direct animal environment does not come as a surprise, because these direct environmental samples are as a rule contaminated with pig faeces. In the case of the toys in the pens, however, it must be assumed that saliva plays the most important role - tonsils of pigs shedding Salmonella via faeces are mostly salmonella-positive as well [2, 3]. It is kind of a dilemma that the toys intended to improve the well being of the animals, are unfortunately a risk factor for spreading Salmonella within the herd. The number of positive samples from the indirect animal environment, such as the hallways outside the pens, the ante rooms and the animal scales can possibly be explained with the fact that since in these areas pigs are not at all or at least not constantly present, the necessity for regular cleaning and disinfection is not present in the minds of the farmers. A critical point is apparently the ante rooms, which are supposed to represent a barrier so that no bacteria and other infectious agents can enter

the barn. With almost 30% of the ante rooms tested positive for Salmonella, however, they appear, if not properly managed, to be more of a distributing point for the bacteria than an active barrier against them.

Conclusion

Final conclusions must await the review of all data. However, current data and analyses from the presented study suggests that while the assessment of risk factors may give a clue to the root of the Salmonella problem of a given farm, there is no single and ultimate "recipe" for preventing and/or diminishing Salmonella at herd level. Each farm must be seen as an individual entity and specifically examined to identify the sources of the introduction and the circulation of Salmonella, which then in turn is the basis for developing a farm-specific control programme. Finally, one point might be made which applies to all farms, regardless of the Salmonella-Category: it appears that much more must be done to inform the farmers of the necessity of a comprehensive biosecurity and hygiene concept, which goes beyond the regular cleaning and disinfection of the animal holding compartments, to include all areas and equipment of the barn starting at the entrance itself to reduce the indirect spread of salmonella within the farm environment.

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Strategies and methods for the investigation of *Salmonella* in pig farms

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Summary

Due to European legislation, the member States are obliged to reduce and take actions against the prevalence of Salmonella and other foodborne zoonotic agents. Subsequently in most member countries various laws and regulations are put into force. Since 24.03.2007 the relevant regulation concerning Salmonella in pig in Germany, is the Schweine-Salmonellen-Verordnung (SchwSalmV). Hereby a fixed number in blood sample of animal tests are taken in certain time intervals and examined for antibodies against Salmonella. Bacteriological-epidemiological investigations are only necessary in farms classified as category III. According to our results it is necessary to monitor farms of all categories routinely by bacteriological -examinations at critical control points.

Introduction

With the European Directive 2003/99/EC on zoonoses and zoonotic agents and Regulation (EC) No 2160/2003 on the control of Salmonella and other foodborne zoonotic agents, a legal framework for reducing Salmonella prevalence in pig herds was created by the European Union and thus the basis for the establishment of national control programs for Salmonella and other zoonotic had been established. On 24.03.2007 the regulation was implemented in Germany for decreasing the Salmonella prevalence in slaughter pigs (Schweine-Salmonellen-Verordnung, SchwSalmV). The regulation stipulates that owners of final fattening farms have to take blood samples in each separated part of the animal house in order for examination on antibody against Salmonella. The method to be used is the national standard DIN Norm 17025/2005. Samples have to be taken according to the following random check key (Tab. 1).

Tab.1: Random check key from SchwSalmV

Number of the prospective to the slaughter delivered pigs per year	Number of the examined pigs
Less than 26	All pigs
Less than 45	26
45 to 100	38
101 to 200	47
More than 200	60

The appreciation of the results takes place as describe in Tab. 2.

If the final fattening farms of category III, the following measures must be implemented

Tab. 2: Appreciation of the results from SchwSalmV

Salmonella antibody status of the operation or the operation section	Category	Percentage positive results of samples (%)
Low status	I	0 to 20
Medium statuss	II	More than 20 to 40
High status	III	More than 40

under the supervision of the veterinarian contracted by the farmer.
1. To determine the source of Salmonella introduced into the herd by bacteriological and epidemiological examinations
2. Cleaning and disinfection of all parts of the animal house which could be contaminated as well as Rodent pest control.

Since retrospective classification based on blood samples collected during slaughtering comes to late for effective preventive measures, the development of a strategy for effective Salmonella prevention on farm level was the aim of this investigation.

Material and Methods

In the monthly intervals and 8 repetitions 1576 samples were taken from 4 pig attitude enterprises and were microbiologically examined for Salmonella with different standardized methods. The aim of this investigations were to find out what the most effective of sampling method and the best sampling site will be in order to optimise the self monitoring strategy of the farmer. The sampling included samples collected from the surroundings including faeces, litter and feed from different locations on the farm, dust, liquid manure, water samples from the water supply and from the distribution system in the animal house as well as flies and mice. Also rectal swabs of selected animals and swab samples from the surroundings of the pigs as for example drink water, trough or feeding system and surfaces such as walls or animal-toys were taken.
The best Salmonella detection method found out in the experiments may be also applied for purposes of monitoring se can be applied in both self-control and for further investigations in the food chain for the contamination of Salmonella.

To investigate for Salmonella, the following five methods were applied and compared
- Method I: DIN EN ISO 6579 Horizontal method for detection of salmonella
- Method II: DIN EN ISO 6579/A1 The isolation of Salmonella spp. with the modified method of Rappaport-Vassiliadis (MSRV)
- Method III: Modified method according to FD CEN / TR 15215-3 process of liquid enrichment in buffered Peptone with novobiocin followed by Rappaport Vassiliadis, qualitative detection of Salmonella
- Method IV : ttr-based PCR (Real time PCR) modifies according to Malorny et al. (2007): the DNA Extraction takes place from Rappaport Vassiliadis broth after the pre-enrichment in buffered Peptone water without Novobiocin
- Method V: ttr-based PCR modified according to Malorny et al. (2007): DNA Extraction made from Rappaport Vassiliadis broth after pre-enrichment in buffered Peptone water with Novobiocin

Results

With the comparison of the positive results with the proofed method which are the most clearly positive results, 333 samples, with the method V were achieved. 261, 76, 47, and 43 positive samples were determined according to the method IV, method III, method II, and method I, respectively (Fig. 1)

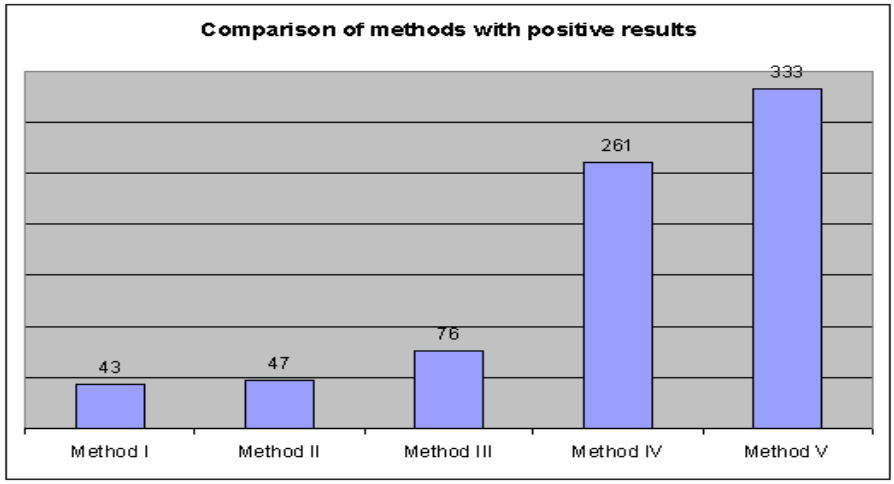


Fig. 1: Comparison of methods with positive results

The comparison of different Sample site with cultural positive results shows that the most positive results with method III provided feed from different locations on the farm, collected samples from the surroundings including faeces, dust, and manure. (Fig. 2)
In the comparison of PCR positive results from different Sampling site, it indicated that the most clearly positive results provided from method V were , feed from different locations on the farm, collected samples from the surroundings including faeces, rectal swab, dust and water from supply. (Fig. 3)

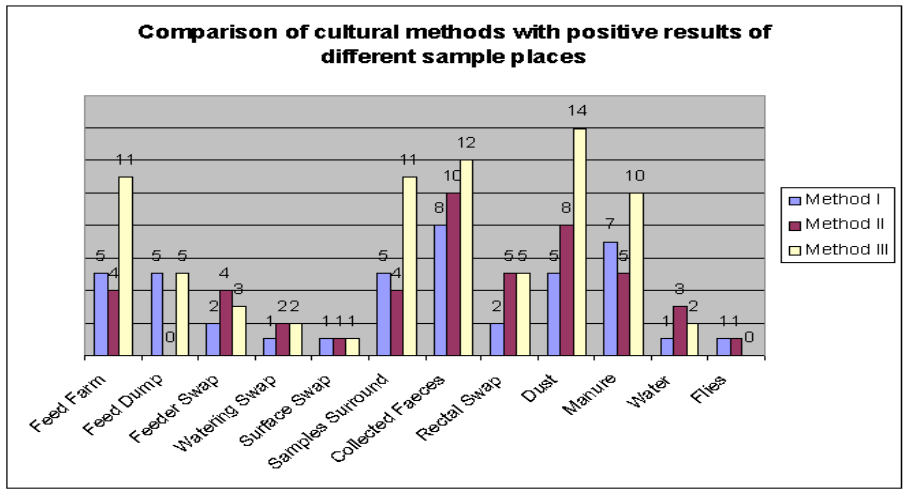


Fig. 2: Comparison of cultural methods with the positive results of different Sample places

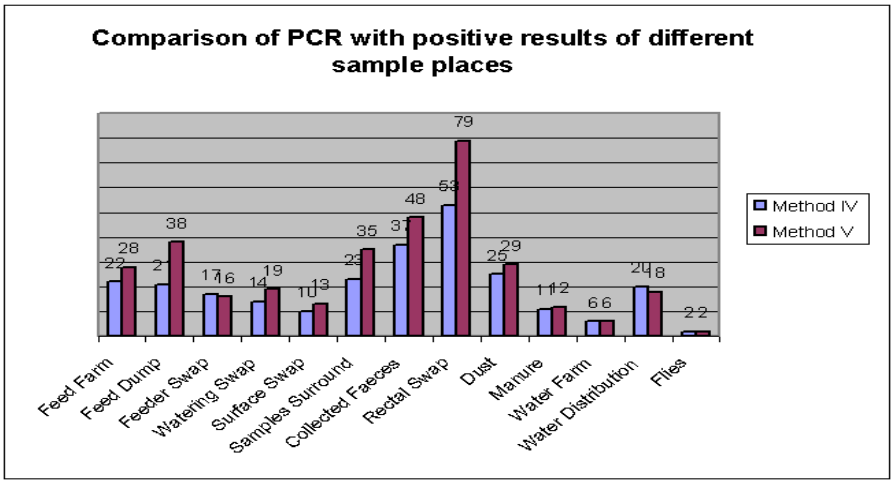


Fig. 3: Comprision of PCR with positive results of different Sampleplaces

Discussion and Conclusion

To ensure effective controlling salmonella in pigs should be carried out not only the retrospective data from the antibody status of pigs from meat samples shall be regarded, but active strategies of sampling and bacteriological investigations in the framework of an on farm quality assurance system (HACCP) shall be applied with priority. This is the only way to gather reliable information's on the current health situation in the farm and to organize subsequent preventive measures in the further steps of the food chain as slaughtering and meat processing. For the bacteriological-epidemiological examinations of the final fattening farms it indicated that feed samples from storage and delivery to the animals, mixed faecal samples from the keeping unit, dust and with some limitations litter shall be regularly investigated with method V. Even if rectal swabs in combination with the method V have proved to be the most effective it could not be recommended from the practical point of view.

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Experimental studies on dietetic concepts and epidemiological investigations regarding *Salmonella* prevalence in different stages of the pork production chain under field conditions

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Summary

This study was carried out on eight farms. On piglet producing farms (f1-4) *Salmonella* status of a total of 36 control (cg) and experimental groups (eg) with nearly 100 weaned pigs each was tested by cultural technique. The diets of the cg had grinding intensities generally used on the farms. In the eg the diets obtained a coarsely ground feed, acid compositions were also used.
On each of the fattening farms (f5-8) about 800 pigs were subjects of investigation and divided into a cg and eg. For the detection of the *Salmonella* serovars most occurring on farms and to test the effect of the dietetic concept (coarse grinding of feed combined with organic acids f5-7; f8: another question), at least 800 samples per farm were taken during the fattening period. At slaughter, samples of the tonsils, gall, caecal content, ileocaecal lymph nodes (iln) and carcass surface were taken from about 120 animals per herd for cultural analysis, a piece of muscle tissue to determine the antibody titre.
Salmonella spp. has neither been culturally detected in any feed (n=207) or water sample (n=147) nor during the suckling period in feces of sows (n=428) or suckling piglets (n=1700). Only on two farms *Salmonella* spp. was culturally found in feces of weaned pigs. Here *Salmonella* prevalence was reduced in eg on f1 about 68 % (cg: 69/1021, eg: 21/973) and on f3 about 86 % (cg: 28/432, eg: 4/432). In fattening pigs the exerimental diets resulted in a continuous reduction of *Salmonella* shedding up to the end of the fattening period and in a significantly lowered *Salmonella* prevalence in meat juice (farm 5-7). In the eg at slaughter *Salmonella* prevalence in tonsils of pigs from all three farms was significantly lower. The same was observed in caecal content and for iln in two of three farms. Overall *Salmonella* prevalence at fattening farms was lower than at slaughter (farm: 191/3418 = 5.58 % of fecal samples *Salmonella* positive, slaughter: 330/2494 = 13.2 % of samples *Salmonella* positive). At slaughterhouse *Salmonella* prevalence in the caecal content (24.9 %) was the highest (tonsils: 20.1 %; iln: 14.5 %; carcass surface: 4.6 %; gall: 2.2 %).
In samples at piglet producing farms 43.1 % of serotyped samples contained *S.* Typhimurium 1,4,5,12:i:1,2 DT 104B low, 24.5 % *S.* Derby 4,12:f,g:- and 20.6 % *S.* group B 4,5:i:-. The predominant serotype on fattening farms and at slaughter was *S.* Typhimurium (81.5 %). By means of phagetyping, the concordance between the serovars found on farm and in the different types of samples at slaughter was only similar in samples of f1 and in the corresponding iln obtained at slaughter (p=0.1188); in samples of f2 and the corresponding tonsils (p=0.1479) and in samples of f3 and the corresponding caecal content (p=0.3230) and iln (p=0.1921), respectively. Pigs harbouring *S.* Typhimurium 1,4,12:i:1,2; DT104L in tonsils or *S.* Typhimurium 1,4,12:i:1,2 DT 104B low in caecal content or iln, respectively, showed the highest optical densities in meat juice.

Introduction

Outbreaks of *Salmonella* infections in humans in the last twenty years were the main trigger for intensified efforts at slaughter hygiene and on-farm surveillance and control. The physical characteristics of the diet and feed additives can influence the susceptibility of pigs to *Salmonella* infection [1,3,4,6]. On the one hand the aim of the field study was to test whether an increased feed particle size in diets containing organic acids could influence the prevalence of *Salmonella* in pigs. On the other hand a further aim was to compare the prevalence and kind of *Salmonella* serovars on the farms and at slaughter.

Animals, Material and Methods

On piglet producing farms *Salmonella* status of a total of 36 cg and eg with nearly 100 weaned pigs each was tested by cultural technique several times (before and after weaning, at 18 kg body weight, before and after entering the fattening unit; approximately 150 rectal swaps and 50 pooled faeces samples per group). The diets in the cg had grinding intensities generally used, in the eg diets were coarsely ground. The ingredients and chemical composition were the same in both diets (Tab. 1).

The investigations in fattening pigs were performed on four farms (f5-f8) classified in category III according to the German *Salmonella* monitoring system. On each of the farms, about 800 pigs were the subject of these investigations, divided in a cg and eg. The botanical composition of diets for the cg and the eg on each farm was the same. On f5-7, pigs in the cg were fed a finely ground diet whereas pigs in the eg were fed a coarsely ground diet (Tab. 1). For f8 only epidemiological question were of interest here.

Tab. 1: Feeding concept in control and experimental groups in piglet producing and pig fattening farms used for *Salmonella* interventions strategy trials

farm**1	% particles >1.4 mm in diet		organic acids	
	control	experimental	control	experimental
1	19.7	32.1	f.a./l.a.	f.a./pdf
3	8.08	26.5	f.a./c.a./l.a./a.a.	f.a./c.a./l.a./a.a.
5	11.4	29.2	f.a./p.a.	f.a./p.a.
6	16.9	45.7	-	pdf**2
7	11.2	27.2	f.a./p.a.	pdf

f.a.= formic acid, l.a.= lactic acid, c.a.=citric acid, a.a.= acetic acid, p.a.= propionic acid, pdf= potassium diformate; overall acid concetration in diet: each with 0.7 – 1.2 %; 1** f2 and f4 not shown, because *Salmonella* spp. was not detected; 2** pdf only in finisher diet

The *Salmonella* status of the animals on each of these farms was assessed during the fattening period using rectal swaps (at 30 , 65 and 110 kg body weight; 140 samples per group and point of time) and pooled faeces samples (45, 60, 85 and at 100 kg body weight, about 100 samples at each stage of weight and group). At the three slaughterhouses from 120 pigs per farm samples of iln, tonsils, gall, caecal content and carcass surface were taken and analysed

by cultural technique. *Salmonella* antibody status was ascertained in meat juice of the same individuals.

Results

Salmonella detection in any feed (n=207) or water sample (n=147) failed on f1-8. During the suckling period *Salmonella* has never been culturally detected in feces of sows (n=428) and suckling piglets (n=1700). Only at f1 and f3 *Salmonella* was culturally found in feces of weaned pigs. In the eg on f1 *Salmonella* prevalence was reduced by about 68 % (cg: 69/1021; eg:21/973), on f3 about 86 % (cg: 28/432; eg: 4/432).

In fattening pigs the dietetic concept ("coarse diet") led to a continuous reduction of *Salmonella* shedding up to the end of the fattening period. An increased particle size of the diets (f5-7) resulted in a significantly lower *Salmonella* prevalence in meat juice (cut off 40), significantly reduced *Salmonella* prevalence in tonsils (f5-7), caecal content (f5 and f6) and in iln (f5 and f7). In general, from 3418 fecal samples that were taken at f5-f8 and analysed microbiologically, 191 were *Salmonella* positive (5.58 %), whereas from slaughtered pigs more samples were positive (330/2494 = 13.2 %). According to the *Salmonella* prevalence in different samples taken at the slaughterhouse, the one in the caecal content (124/499 = 24.9 %) was the highest, followed by tonsils (100/498 = 20.1 %), iln (72/498 = 14.5 %), carcass surface (23/501 = 4.6 %) and gall (11/498 = 2.2 %).

At piglet producing farms 43.1 % of serotyped samples contained *S.* Typhimurium 1,4,5,12:i:1,2 DT 104B low, 24.5 % *S.* Derby 4,12:f,g:- and 20.6 % *S.* group B 4,5:i:-. The predominant serotype on fattening farms and at slaughter was *S.* Typhimurium (81.5 %). By means of phagetyping, the concordance between the serovars found on farm and in the different types of samples at slaughter was almost always poor ($p<0.05$). Exceptions were the in most cases identical serovars found in samples of f1 and in the corresponding iln taken at slaughter ($p=0.1188$); in samples of f2 and the corresponding tonsils ($p=0.1479$) as well as in samples of f3 and the corresponding caecal content ($p=0.3230$) and iln ($p=0.1921$), respectively. With regard to the optical densities in meat juice samples in pigs harbouring *Salmonella* spp., the densities were highest in the case *S.* Typhimurium 1,4,12:i:1,2; DT104L was found in tonsils and *S.* Typhimurium 1,4,12:i:1,2 DT 104B low in caecal content or iln, respectively.

Discussion

It is known that organic acid inclusion in pig diets reduces the number of Enterobacteria in different sections of the gastrointestinal tract [4]. Feeding coarsely ground diets to pigs usually results in more starch entering the caecum [6]. Coarse grinding increased ($p<0.05$) the total volatile fatty acid concentration and molar proportions of propionic and butyric acids in the contents of large intestine [6]. So, possibly a modification of the gut flora in particular to produce more butyric acid could reduce invasive infections by *Salmonella* serovars in farm animals [2,5]. This may be the main mechanism that resulted in a lower *Salmonella* prevalence of pigs in the eg observed in the present investigation. During the fattening period, environmental contamination with *Salmonella* spp. is a potential source of *Salmonella* infections in pigs. Due to the long persistence of these bacteria in tissue of animals, *Salmonella* species, as soon as they have colonised an animal, might be a possible hazard for *Salmonella* contamination at slaughter.

Conclusion

The results of this field study clearly demonstrate that increasing feed particle size in diets containing organic acids reduces *Salmonella* spp. prevalence in pigs. Also a larger quantity of *Salmonella* serovars and a higher *Salmonella* prevalence at slaughterhouses all in all should not imply that farm intervention strategies aiming at a reduction of the *Salmonella* prevalence are dispensable. This is because the serovars once introduced into a slaughterhouse via swine maintain the resident slaughterhouse flora and at least add to it.

Acknowledgement

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Incidence and antimicrobial susceptibility of *Salmonella* in fattening pig herds in Northern Germany

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Introduction

This study is part of the research consortium "FBI-Zoo", which focuses on foodborne zoonotic infections of humans and animals.
Salmonella spp. are a leading cause of acute gastroenteritis in several countries, and salmonellosis remains an important public health problem worldwide [2]. Ubiquitous serotypes of *Salmonella enterica ssp. enterica*, such as *S. enteritidis* or *S. typhimurium*, which affect both man and animals, are an major cause of infectious human enteritis [8].
Each year approx. 50,000 cases of human salmonellosis are occurring in Germany [4]. Most of human *Salmonella* infections are caused by foodborne infections, only a small part is triggered by direct contact to infected animals or humans. Pork is known to be a vector in cases of human salmonellosis [3], an estimated 20 % of human salmonellosis are due to pig meat in Germany [5].
The European Union, national authorities and the pig industry are increasingly interested in the *Salmonella* status of the pig population in order to control food safety and to minimize foodborne human infections. Since 2007, a national rule was implemented in Germany to control the *Salmonella* status of slaughter pigs (Verordnung zur Verminderung der Salmonellenverbreitung durch Schlachtschweine). This ordinance is based on the serological status of the pig herds. Therefore knowledge about the association between serological testing on one hand and the bacteriological status of a herd on the other hand is important. Therefore these associations were determined within the study presented. Furthermore, the serovar distribution, the resistance of Salmonella isolates against a panel of antibiotics and the association between some potential risk factors and *Salmonella* infection in finishing pigs were investigated.

Animals, Material and Methods

Sampling. Sampling took place on an abattoir in Lower Saxony. 27 farms were selected, and samples were taken from July 2007 until June 2008. 30 animals per farm were tested serologically and from 20 animals of the same farm ileocaecal lymphnodes were cultured bacteriologically. Blood samples were taken during the sticking. Ileocaecal Lymphnodes were harvested on the gut conveyor band by tearing the plica ileocaecalis and drawing out of the connective tissue per hand.

Bacteriological examination. The bacteriological examination took place in the Department of Microbiology of the University of Veterinary Medicine Hannover. For serotyping, lysotyping and antimicrobial susceptibility

testing the isolates were sent to the NRL-Salm (BfR Berlin).
The shredded lymphnodes were cultivated on the selective growth media Xylose-Lysine-Desoxycholat (XLD)- agar and Brilliant Green-Phenolred-Lactose-Saccharose (BPLS) agar after growing in selective enrichment media Rappaport-Vassiliadis broth (RVB) und Mueller-Kaufmann-Tetrathionat-Novobiocin broth (MKTTn).
The presumptive *Salmonella* colonies were tested biochemically and were suspended in polyvalent *Salmonella* antiserum and examined for agglutination. The isolates were cryo-conserved and submitted to the NRL-Salm/BFR Berlin for further serotyping, lysotyping and susceptibility testing.
A herd was classified positive, if *Salmonella* was isolated from at least one ileocaecal lymph node.

Serological testing. Blood samples were sent to the Institute of Animal Hygiene and Veterinary Public Health of the University of Leipzig. Sera were analyzed for the presence of antibodies with Salmotype Pigscreen ELISA. For evaluation the producer's reference cut-off (OD 20%) was used. A herd was classified positive, if at least one animal showed a positive serum sample.

Antimicrobial suscebility testing. Isolated *Salmonella* strains were tested against 14 antibiotics in the NRL-Salm/ BfR Berlin by using the microdilution method according to the NCCLS guidelines.

Statistical Analysis. Data were imported into SAS (SAS systems for Windows, version 9.1) for statistical analyses.

Results

Serological and bacteriological testing. In 26% (7) of the farms, *Salmonella* spp. could be isolated culturally (between 5 % and 25 % positive samples per herd), while in 13 (48%) farms serologically positive animals were found (between 1 and 28 positive samples per herd).
In total 17 *Salmonella* strains were isolated of 540 lymphnode samples, i.e. 3.15% of all pigs tested were bacteriologically positive (table 1). Main serovar was Typhimurium, beyond this one serovar Brandenburg was detected. 12 (44%) farms have been tested serologically and bacteriologically negative, while 19% (5) were both bacteriologically and serologically positive. Seronegative pigs that were positive based on bacterial culture were found as well as culture-negative pigs that were tested serologically positive. On the one hand 30% of the farms were solely serologically positive, and on the other hand only 7% (2) of the farms were exclusively bacteriologically positive.

Antimicrobial susceptibility testing. Two strains were sensitive against all tested antibiotics. The other strains showed resistance against 2 to 8 of the tested antibiotics.
Resistance was high ($> 70\%$) in case of Ampicillin, Streptomycin, Sulphametoxazole and Tetracycline. The proportion of resistant isolates was moderate considering Chloramphenicol and Florfenicole (< 20 %) and in case of Cefotaxime, Ciprofloxacin and Nalidixic acid relative low ($< 10\%$). Whereas no isolate showed any resistance on Ceftazidime, Colistin, Gentamicin and Kanamycin respectively (table 2).

Table 1: Results of the bacteriological and serological testing for *Salmonella*

		herds		animals	
		bacteriology and serology :n=27		bacteriology: n=540 serology: n=810	
		positive	negative	positive	negative
bacteriology	n	7	20	17	523
	%	26	74	3,15	96,85
serology	n	13	14	160	650
	%	48	52	20	80

Table 2: Distribution pattern of resistance (The 2 Isolates not described are susceptible against all tested antibiotics, R=resistant)

Isolate No.		Ampicillin	Chloramphenicol	Florfenicol	Streptomycin	Sulfamethoxazole	Tetracycline	Trimethoprim	Cefotaxime	Ciprofloxacin	Nalidixic acid
01		R	R	R	R	R	R				
02		R			R	R	R				
03		R			R						
04		R			R						
05		R			R						
06		R				R	R	R			
07		R				R	R	R			
08		R				R	R	R			
09		R	R	R	R	R	R	R	R		
10		R			R	R	R				
11		R			R	R	R				
12		R			R	R	R				
13		R			R	R	R				
14		R			R	R	R				
15		R	R	R	R	R	R			R	R
Resistant Isolates	(n)	15	3	3	12	12	12	4	1	1	1
	(%)	88.2	17.7	17.7	70.6	70.6	70.6	76.5	5.9	5.9	5.9

Conclusion

Culture and serology are the two different approaches for determining *Salmonella* status at the individual or population level and the use of each technique depends on the research question [1]. Farzan et al. found a good association between serology and bacteriology on herd basis but not on individual animal basis[1]. In our study we only found a weak association on herd basis (kappa=0.25, p=0.0578). But it must be considered that the small sample size (N=27 herds) influenced our results. Bacterial resistance against Fluoroquinolones (Ciprofloxacin, Nalidixic acid) and against 3rd generation Cephalosporines (Ceftazidime, Cefotaxime) - substance groups that are discussed frequently in literature regarding the possible risk to man - was low [7]. The study could give a hint to develop strategies for reducing the prevalence of resistant *Salmonella* strains in livestock.

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Uptake of *Salmonella enterica* in Monocotyledonous and Dicotyledonous Model plants

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Summary

The investigations shall give more detailed informations on the quantitative aspects on the internalisation of Salmonella into plants and concentrated on two main questions. First is there a difference between monocotyledonous and dicotyledonous plants and second is there a relationship between the concentration of Salmonella in the soil and how does the time influence the amount of internalized bacteria? Maize (*Zea mays*) and garden cress (*Lepidum sativum*) were selected as model plants. A freshly isolated field strain of *Salmonella Typhimurium* was used for the contamination of the soil in two concentrations 10^4cfu and 10^7 cfu/g soil. The soil was contaminated prior and after germination and plants were harvested in regular intervals up to 28 days.5g of plant material were analyzed quantitatively for Salmonella with different methods in order to measure the superficial contamination separately from the Salmonella count in the plant tissue. First result indicate, that there are differences in the internalisation of Salmonella between monocotyledonous and dicotyledonous model plants. With the low level contamination also less tissue samples were positive (maize 25%, garden cress 37%) than with the high contamination level of the soil (maize 75%, garden cress 87%). The highest Salmonella count in both test plants was $6{,}9x10^2$ cfu/g.

Introduction

Since the publication of Cooley et al [2] it is known that plants can internalize gram-negative pathogens actively via roots. The publication of Barak et al. [1]demonstrated that the virulence factors are important for the internalization and the publication of Klerks et al [3] draw up some of the molecular mechanisms involved in the internalisation of Salmonella into lettuce.
Those and several other publications are showing a proof of principle but also big variations can be seen in the moment as quantitative aspects are coming into focus. Therefore this investigations are concentrating on two main questions besides the methodical aspects. First is there a difference between monocotyledonous and dicotyledonous plants and second is there a relationship between the concentration of Salmonella in the soil and how does the time influence the amount of internalized bacteria?

Material and Methods

Maize (*Zea mays*) and garden cress (*Lepidum sativum*) were selected as model plants. Both were cultivated on standardized TKS I – substrate (pH 5,5). A freshly isolated field strain of *Salmonella Typhimurium* was used for the contamination of the soil in two concentration levels 10^4cfu and 10^7 cfu/g soil. The soil was contaminated prior and after germination and plants were harvested in regular intervals up to 28 days.5g of plant material were analyzed quantitatively for Salmonella with different methods in order to measure the superficial

contamination. Table 1 is summarizing the experimental set up.
The harvested plants were investigated before and rinsing with sterile NaCl-solution as well as before and after disinfection of their surface as described by Klerks [3].

Table 1: Experimental set up with contamination of the soil before and after the germination

Day	Maize				Garden cress			
	Before		After		Before		After	
	10^4	10^7	10^4	10^7	10^4	10^7	10^4	10^7
1	sawing into the contaminated soil		sawing					
4					sawing into the contaminated soil		sawing	
7			contamination of soil				contamination of soil	
8	Sampling of soil							
12	Sampling of plant material							
17	Sampling of plant material							
22	Sampling of plant material							
27	Sampling of plant material							
28	Sampling of soil							

Results

In the experiments with cress in combination with sawing into the contaminated soil a time dependent decrease of the Salmonella count in and on the plant could be seen. The concentration of Salmonella in the plant was higher as in the contaminated soil. The experiments with garden cress in combination with soil contamination after germination A clear relationship between the level of soil contamination and the salmonella count on and in the plant material could be seen, table 2 is summarizing the results obtained in this experiments.
The results are indicating, that the level of contamination detremines the uptake of Salmonella by the plant. The results obtained with maize were similar, but it seems that the level of uptake is lower. It could be demonstrated that not all exposed maize plants were Salmonella positive, independent from the pretreatment by rinsing or disinfection. The same applies to the rinsing liquid itself as could be seen from table 3.

Discussion

This results have to be regarded as preliminary results demonstrating the proof of principle and the influence of the contamination level. It seems that there are differences between the used monocotyledonous and dicotyledonous model plants, but this has not been documented by statistical significance, because the number of maize plants was not high enough in every methodical variation.

Table 2: Amount of Salmonella which could be reisolated from the plant tissue.

Count of S. Typhimurium cfu/g soil	Count (cfu/g plant tissue) at day			
	12	17	22	27
$2{,}3x10^5$	-.	-	-	+
$2{,}3x10^4$	+	+	-	-
$2{,}3x10^7$	$6{,}9x10^2$	$2{,}7x10^2$	-	6,9x10
$2{,}3x10^7$	$1x10^2$	2,7x10	$6{,}9x10^2$	6,9x10

- = not detected +=positive but below counting level

Table 3: Number of maize plants and the related rinsing liquid beeing Salmonella positive after pretreatment (n = 8)

Contamination level of soil	10^4 cfu/g soil	10^7 cfu/g soil
Plants rinsed with NaCl solution	2	4
Rinsing liquid	2	6
Disinfected plants	2	4

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Prevalence of Salmonella in wastewater and sewage sludge

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Summary

The intention of this work was to provide an overview over the prevalence of Salmonella in wastewater and sewage sludge. Therefore wastewater and sewage sludge were analyzed qualitatively and quantitatively with different methods.
The study showed a high prevalence (80%) of Salmonella in wastewater and sewage sludge. However, the detected bacterial count was low. The highest count was 40 MPN/ml.The comparison of the methods confirmed the high selectivity of enrichment in buffered peptone water with Novobiocin in combination with Rappaport-Vassiliadis medium. It also was showed that "ONE-Bouillon Salmonella and Brilliance^{TM} Salmonella" is not applicable for the isolation of Salmonella in wastewater or sewage sludge.
The best variant for quantitative analyses depends particularly on the expected bacterial count, since the methods differ in their detection limits.
Method 1682 by EPA is preferable if low bacterial counts are expected. MPN is preferable if high or extremely high bacterial counts are expected.
In view of potential hygienical risks resulting from the use of sewage sludge on agricultural land, the need for general hygienic parameters for sewage sludge becomes apparent.

Introduction

Every inorganic or organic fertilizer used on agricultural land has to be hygienically safe according to the law [2]. This requirement should also apply to sewage sludge.
To get a general idea of the prevalence of salmonella, samples of wastewater and sewage sludge were tested qualitatively and quantitatively for salmonella.
Sewage treatment plants all over Germany were contacted for the study and provided samples from their plants for analysis.

Material and Methods

Sewage treatment plants all over Germany were contacted for the study and provided samples from their plants for analysis. Generally, every plant offered repeat samples, so that 54 samples of sewage sludge with corresponding samples of wastewater could be analysed.
Two methods were used in parallel for qualitative analysis. The first method was an enrichment in buffered peptone water with Novobiocin in combination with Rappaport-Vassiliadis medium, for detection of Salmonella in 50g/ml of sample. This method was carried out according to the Bioabfallverordnung [1], with Novobiocin used as an additive [5]. Second "ONE-Bouillon Salmonella and Brilliance^{TM} Salmonella" was used for the detection of Salmonella in 25g/ml of sample.
As quantitative methods, two procedures were

used for comparison. On the one hand, analyses were carried out using MPN based on Rappaport-Vassiliadis, on the other "Method 1682: Salmonella in sewage sludge (biosolids) by Modified Semisolid Rappaport-Vassiliadis (MRSV) medium" by the United States Environmental Protection Agency was used.

Results

Salmonella was detected in 80% of all wastewater and sewage sludge samples using enrichment in buffered peptone water with Novobiocin in combination with Rappaport-Vassiliadis medium (RVS). In contrast only 3.6% of the wastewater samples and 6.3% of the sewage sludge samples using "ONE-Bouillon Salmonella and Brilliance^{TM} Salmonella" tested positive for Salmonella. This means only 5.9% of all positive samples detected by RVS were affirmed by "ONE-Bouillon Salmonella and Brilliance^{TM} Salmonella"

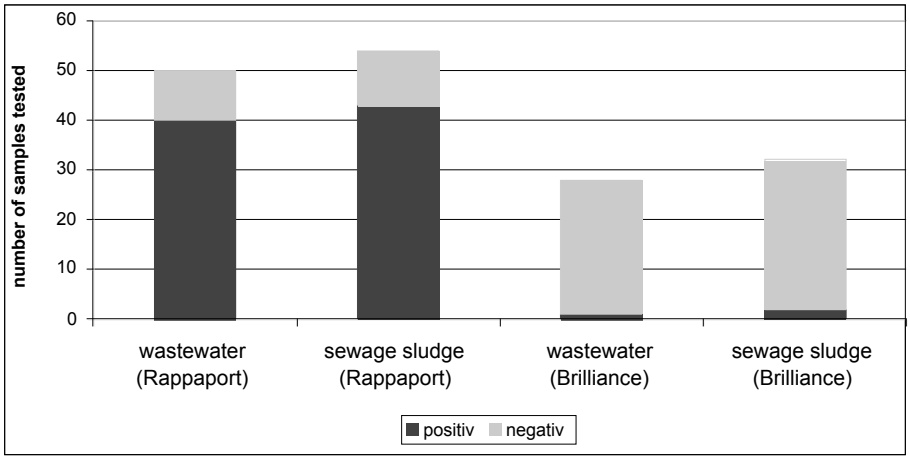


Fig. 1: Results of the qualitative analyses of wastewater and sewage sludge. Rappaport: enrichment in buffered peptone water with Novobiocin in combination with Rappaport-Vassiliadis medium, Brilliance: ONE-Bouillon Salmonella and Brilliance^{TM} Salmonella

Tab. 1: Results of the qualitative analyses

	Method 1: Rappaport		Method 2: Brilliance	
	Wastewater	Sewage sludge	Wastewater	Sewage sludge
number of samples tested	50	54	28	32
Salmonella positiv	40	43	1	2
Salmonella negativ	10	11	27	28
Salmonella positiv (in %)	80	79.6	3.6	6.3

Quantitative analyses using MPN showed a maximal contamination with Salmonella of 40 MPN/ml. In the magority of the samples (50% of wastewater and 52% of sewage sludge) the germ count was unascertainable by MPN.

Tab. 2: Percentage distribution using MPN

	n.n.	0.3-3.9 MPN/ml	4.0-40 MPN/ml
Wastewater	50.0%	38.0%	12.0%
Sewage sludge	52.0%	41.0%	7.0%

Tab. 3: Percentage distribution using Method 1682.

	n.n.	0.006-0.9 MPN/ml	0.9-1.6 MPN/ml	>1.6 MPN/ml
Wastewater	4.7%	52.4%	9.5%	33%
Sewage sludge	12.5%	58.8%	12.5%	16.7%

Due to the lower detection limit of the Method 1682 (0,006 MPN) there is a minor percentage of the samples in which Salmonella were undetectable. Contrast, 33% of the wastewater and 16.7% of the sewage sludge samples were above the detection limit. Most of the samples show a bacterial count up to 0.9MPN/ml (52.4% wastewater; 58.8% sewage sludge).

Discussion

The present results accentuate the need for selective detection methods for analysing wastewater and sewage sludge. The enrichment in buffered peptone water in combination with Rappaport-Vassiliadis medium is statutory according to the Bioabfallverordnung [1] for Salmonella detection in environmental samples. With Novobiocin as an additive the sensitivity of this method is increased [5,6].
The results of this study confirm this selectivity. Salmonella were isolated in 80% of all samples of wastewater and sewage sludge, in spite of the low count of Salmonella and the high bacterial count of other bacteria.
ONE-Bouillon-Salmonella in combination with Brilliance^{TM} Salmonella led to a very low Salmonella detection rate. This method is insufficient for the isolation of Salmonella in wastewater or sewage sludge.
Both quantitative methods led to a low bacterial count of Salmonella in wastewater and sewage sludge. The different detection limits complicates the direct comparison of the results. Because of the lower detection limit for Method 1682 bacterial counts could be evaluated by a higher number of samples. The best variant for the quantitative analyses depends particularly on the expected bacterial count, because the methods differ in their detection limits.
A high Salmonella count was not detected in wastewater or in sewage sludge. However, in nearly all samples, Salmonella could be detected by the qualitative method. In view of potential hygienical risks resulting from the use of this sewage sludge on agricultural land, it is to not the pathogen count but its presence that creates the risk, since bacterial pathogens may be able to accumulate in the environment or in hosts [4]. Using untreated sewage sludge on agricultural land is therefore a general risk. Furthermore, using sewage sludge allows pathogens to cross farm boundaries, increasing epidemical risks as well, the area of circulation of pathogens and since this increases possible chains of infection can dilate [3].
These risks are the subject of the discussion about the need for a requirement that sewage sludge be hygienically safe, which should be introduced with the amendment of the Klaerschlammverordung.

Conclusion

There is a high prevalence of Salmonella in wastewater and sewage sludge, but the bacterial count is low.
To assess the risk resulting from the use of sewage sludge as a fertilizer, analyses of the behavior of Salmonella in the environment fallowing low contamination the end product would be interesting.
The requirement that sewage sludge should be hygienically safe is supported. The use of sewage sludge must be adjusted accordingly

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Chapter 23

Block 8C

Spread of *Salmonella* spp. in the broiler production chain in Germany

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Summary

The results of two baseline studies on *Salmonella* spp. in the broiler production chain are shown. 17.5% of broiler flocks and 17.6% of broiler carcasses were positive for *Salmonella* spp. Serovar pattern in broilers and broiler carcasses was quite divers. *S.* Enteritidis and *S.* Typhimurium, which are the major causes of human salmonellosis in Germany, are not the dominating types in broilers. Different routes of transmission contribute to the overall level observed.

Introduction

Salmonellosis is an important cause of foodborne illness in humans with 42 789 cases reported in 2008. Food of animal origin is considered an important source of human salmonellosis. Regulation (EC) No 2160/2003 and Directive 2003/99/EC foresee the collection of harmonised data in the European Union and the implementation of an EU wide control strategy. In this context, two EU-wide baseline studies were carried out to estimate the prevalence of *Salmonella* spp. in broilers. To ensure a harmonised study protocol in all countries, all relevant details were fixed in Commission Decisions 2004/665/EC and 2007/515/EC respectively. The results of these studies will be used to set targets for Salmonella control in broilers in the European Union.

Material and Methods

The first study covered holdings with a minimum capacity of 5000 birds. 378 flocks of broilers were sampled within the last 3 weeks before slaughter during 1 October 2005 to 30 September 2006 in Germany. Stratification of the holdings by region and holding size was applied to ensure representative sampling of the broiler population in Germany. Furthermore, sampling was distributed over the year to cover all seasons. From each flock five pairs of sock samples were collected and investigated bacteriologically.

For the second study, slaughterhouses were selected to cover at least 80% of the slaughter capacity in each region of Germany. The number of batches to be sampled was distributed proportionally to the regional share of the slaughter volume of the previous year. From 1 January to 31 December 2008, 432 slaughter batches were selected and one bird from each batch was tested for the presence of Salmonella on the carcasse after cooling. In addition, caecal samples from 307 of these slaughter batches were investigated on a voluntary basis. The caecal sample from a slaughter batch consisted of a pool of 10 caeca, the carcass sample was 25g of neck skin.

In both studies, the samples were collected by official veterinarians and tested in official

laboratories regularly involved in Salmonella testing. Faecal samples were analysed by the method laid down in ISO 6579 Annex D (with semi-solid MRSV as only selective media). Neck skin samples were analysed by the ISO 6579 methods for foodstuffs. All isolates were confirmed and further typed at the National Reference Laboratory for Salmonella at the BfR.

Standardised questionnaires were used to collect information on the region, the size and the production type of the holding. For the slaughterhouses, the region, number of birds slaughtered in the previous year and the type of chilling was collected.

Results

At farm level, 17.5% of the tested broiler flocks were positive for *Salmonella* spp.. In most of the positive flocks, Salmonella could be isolated from several sock samples. In 27 (40.9%) of the positive flocks, all five sock samples were positive. No significant differences could be identified as regards production type and holding size. Most of the birds were kept in barn flocks with at least 10,000 birds capacity. The monophasic type *S.* 4,12:d:- was the predominant type, followed by *S.* Anatum, *S.* Paratyphi B d-tartrate positive (dt+) and *S.* Infantis. The number of positive flocks and isolates for each of the 18 different serovars detected are summarised in Table 1. Most important, 2.9% of the flocks were positive for *S.* Enteritidis or *S.* Typhimurium, the two serovars which will be covered by the control programm to be started in 2009.

Tab. 1: Salmonella serovars in broiler flocks at farm level

Salmonella serovar	Number isolates	Share of all isolates	Number flocks	Share of all positive flocks
S. 4,12:d:-	52	23.1	20	30.3
S. Anatum	33	14.7	13	19.7
S. Paratyphi B (dt+)	30	13.3	6	9.1
S. Mbandaka	21	9.3	7	10.6
S. Infantis	20	8.9	7	10.6
S. Typhimurium	19	8.4	6	9.1
S. Enteritidis	11	4.9	5	7.6
S. Heidelberg	7	3.1	2	3.0
S. Virchow	7	3.1	2	3.0
S. Ohio	6	2.7	3	4.5
S. Indiana	5	2.2	2	3.0
S. Livingstone	5	2.2	1	1.5
S. 6,7:r:-	2	0.9	2	3.0
S. Subspec. I rough	2	0.9	2	3.0
S. Tennessee	2	0.9	1	1.5
S. 4,12:i:-	1	0.4	1	1.5
S. 6,7:-:1,5	1	0.4	1	1.5
S. 3,10:-:1,6	1	0.4	1	1.5
Total	225	100.0	66	100.0

In the second study at slaughterhouse level in 2008, 7.5% of the pools of caeca collected from 307 slaughter batches were positive for *Salmonella* spp..

After slaughter, 17.6% of the carcasses were

positive for *Salmonella* spp.. Again, the monophasic type *S.* 4,12:d:- was most frequently detected. In contrast to the previous study, *S.* Typhimurium and *S.* Paratyphi B (dt+) positive were the next frequent serotypes. More details are shown in Fig. 1.

In slaughterhouses, using spraying for cooling, Salmonella were significantly more frequently detected compared to those using air for cooling.

In slaughter batches, tested for Salmonella in caeca and carcasses, 19.9% of the carcasses were Salmonella positive. There was a marked difference in the carcasse contamination depending on the presence of Salmonella in the caeca. 65% of the batches with Salmonella findings in the caeca and 16% of those without Salmonella findings in the caeca showed Salmonella contamination on the carcasse.

These results reflect that broiler carcasses can become contaminated from different sources. Salmonella may be introduced by the birds itself, when carrying them in the intestine. An additional spread to other slaughter batches may lead to the Salmonella contamination of broiler carcasses from Salmonella negative birds. This is further supported by the observation that single serovars were isolated repeatedly in single slaughterhouses only.

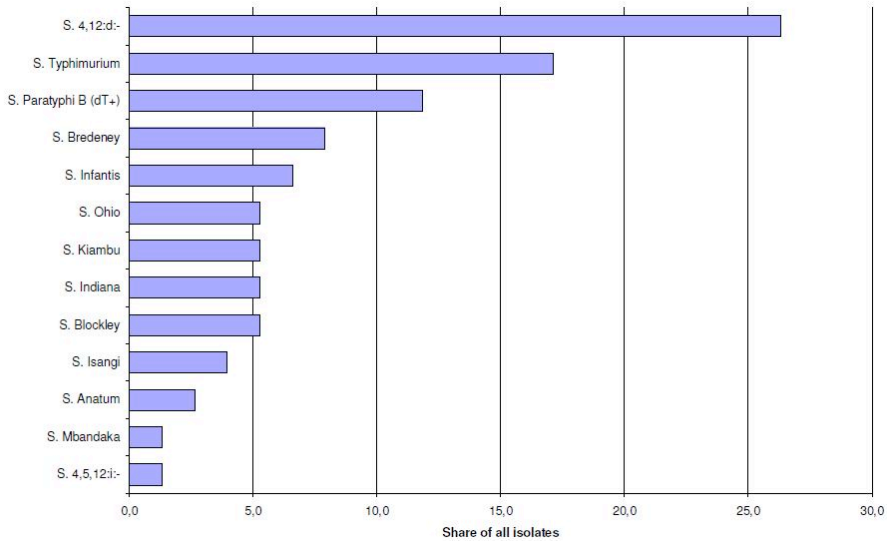


Fig. 1: Salmonella serovars in broiler carcasses after slaughter

Discussion

Both baseline studies could be successfully conducted in Germany and allow comparison of the situation in other EU member States. In the analysis of the first study at farm level, which has been reported on EU level too, the *Salmonella* spp. EU prevalence was 23.7% [1]. The EU prevalence of flocks positive to *S.* Enteritidis and/or *S.* Typhimurium was 11.0%. In comparison, the results in Germany within this study were clearly below the average, but higher than in some Member States. For the second study at slaughterhouse level, data on EU level are not yet available but a similar ranking can be expected. Comparing the *Salmonella* prevalence reported routinely in Germany, the result from the baseline survey is higher. An explanation could be the more sensitive sampling design applied in the baseline survey or the increased sensitivity

of the ISO 6579 Annex D analytical method used. The difference could also be explained by the restriction to the last three weeks before slaughter and to larger holdings which is not fixed for routine reporting.

The main objective of these studies is to establish the baseline for setting targets for Salmonella control. The Regulation (EC) No 2160/2003 foresees setting of the *Salmonella* reduction target for broiler flocks for only two serovars, *S.* Enteritidis and *S.* Typhimurium for a transitional 3 year period, as these are currently the two most frequently reported *Salmonella* serovars in human salmonellosis in the EU. By Regulation (EG) Nr. 646/2007 the Community target for the reduction of *S.* Enteritidis and *S.* Typhimurium in broilers was set to a maximum of 1 % or less by 31 December 2011. Furthermore, according to Regulation (EC) No 2160/2003, fresh poultry meat may not be placed on the market for human consumption unless it meets the criterion 'free of Salmonella in 25g' by 2011. Detailed rules for this criterion specifying in particular the sampling schemes and analytical methods should be laid down in 2010. As the starting point for the Salmonella control in broilers in Germany is 2.9%, the reduction target within three years seems to be feasible. In contrast, the requirement for freedom for all salmonella serovars in poultry meat seems difficult to achieve if the outcome of this study, a contamination rate of carcasses of around 18%, is considered. Further discussion is needed on how to establish acceptable rules for checking the fixed criteria in poultry meat.

Conclusion

This baseline survey has established a baseline prevalence for *Salmonella*, which is used to set the EU *Salmonella* reduction target, compare future trends and follow the impact of the control programmes.

The results reflect different mechanisms involved in the current Salmonella situation observed. Some serovars are consistently prevalent over several years, other types increased for a limited time period and disappeared again.

Acknowledgement

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Sanitation of Salmonella spp. in broiler farms

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Introduction

Lowering the prevalence of salmonella in poultry is a target of the European Community under the aspect of the protection of consumer health. The legal requirements hereto (EC 2160/2003; EC 646/2007) comprise a step-by-step approach, tailored to the stages in production, from the analysis of the prevalence to the control of the stocks and minimisation of the occurrence of the serovars by means of various measures.

Material and Methods

In broiler farms repeatedly found to be positive over several production cycles (S. Java, S. Livingstone, S. Virchow) extensive hygiene measures were carried out. These measures were determined on the basis of a hygiene analysis and microbiological analysis of the risk vectors. The hygiene analysis consisted of targeted questions, how chains of infection are interrupted and which controls on the efficiency of these measures are carried out. Based on the results of the questionnaire in connection with an inspection of the flocks a risk evaluation was carried out and appropriate measures deduced. Targeted analyses for Salmonella in the flocks complemented the monitoring and conduced to identifying the reservoir, substantiating the risk evaluation and evaluating the efficacy of hygiene measures on sanitising broiler farms.
Some detailed questions on cleaning and disinfection measures as important elements towards the interruption of chains of infection are shown in table 1. These measures were tested for individual complexes, such as poultry house and environment, pre-room, fodder silo and feeders and drinking water system.
By means of hygiene analyses various weak points in the individual farms could be identified. The main aspects in connection with Salmonella problems are the insufficient knowledge of the farmer and his advisors on epidemiological correlation and the evaluation of hygiene measures. Hygiene measures are inadequately considered as preventative measures against the introduction of infectious agents. This applies in particular to personnel hygiene measures in broiler farms meaning that a consistent hygiene sluice is inadequately developed and difficult to establish. The pre-rooms are often not structured, are untidy and still dirty even after disinfection. The changing of clothes and shoes as well as the disinfection of shoes and hands constitute further reserves.
Inconsistent pest control and monitoring was identified as the cause for the establishment of Salmonella in the stocks. The significance of rodents as a source of entry and possible reservoir for Salmonella is also often underestimated.
Insufficient cleaning and disinfection measures are identified as a further cause of salmonella persisting in the stocks. Cleaning and disinfection measures are often carried out inadequately, are not understood as separate consecutive hygiene measures, are not adapted to the specific needs and the efficiency rarely controlled.

Table 1: Spectrum of questions on the analysis of cleaning and disinfection measures in broiler farms

Cleaining	Yes / no
	Who cleans
	How is the cleaning done
	How often is the cleaning done
	What is used for cleaning (cleaning agent)
	Concentration of the cleaning agent
	Duration of the cleaning
	Removal of cleaning agents yes/no
	Duration until disinfection
	Control of the cleaning
Disinfection	Yes / no
	Who disinfects
	How is the disinfection carried out
	When is the disinfection carried out
	How often is the disinfection carried out
	Duration of disinfection
	What is used for the disinfection
	Concentration of the disinfectant
	Required amount of disinfectant
	Required amount of working solution
	Contact time of the disinfectant
	Control of the disinfection yes/no

Using examples it can be shown which measures can be necessary to sanitise a broiler flock from Salmonella. On the farms sanitised so far these measures centred on structural alterations, improvement of personnel hygiene, appropriate cleaning and disinfection of the house and equipment, drinking water and feeding systems as well as on consistent rodent control. The basis was provided by a thorough analysis of all farm-specific and epidemiological risks. Of particular significance are the continual documentation of all measures and the efficiency control of each measure. Within one service period reservoirs were successfully eliminated in farms repeatedly found to have been positive and thus the basis for the complete sanitation was provided. In other farms up to 5 service periods were needed to achieve for the first time a production process with no evidence of Salmonella. In these farms there was no consistent observation of all risks and/or the hygiene measures were not carried out successfully. By controlling the efficiency of the cleaning and disinfection measures it can be established that Salmonella can be eliminated with sufficient reliability with tried and tested disinfectants when used appropriately.

Discussion and Conclusion

For the sanitation of broiler stocks repeatedly found to be positive, complex hygiene measures, running simultaneously and encompassing all vectors and reservoirs are necessary. Besides organisational and structural changes, intensive cleaning and disinfection measures as well as rodent control measures are needed, the success of which must be controlled and documented continually. The more consistently each measure is implemented in the sanitation process, the faster and longer-lasting the success. If individual risks are not eliminated according to their significance, are underestimated or dealt with consecutively, the sanitation process is delayed considerably. A broiler farm which has repeatedly tested positive is considered to be sanitised when no evidence of the serovars concerned can be found in the weekly sock swab samples over 3 production cycles.

The sanitation measures involve considerable material, financial and personnel expenditure. An effective and short-term sanitation of broiler farms is possible during continual

husbandry of the farm. It is not recommended that the houses remain unoccupied for longer periods (over several weeks) as individual reservoirs – such as supplying utilities, can be better analysed and evaluated during the production phase.

In connection with the sanitation of the stocks the veterinarian in charge as well as the farmer and his advisors must conjointly decide on the individual steps and work on putting these into practice. The running hygiene measures within the production process are just as important for the sanitation process as lowering the pressure of infection and eliminating the pathogens during the service period.

According to current experience with the sanitation of broiler farms repeatedly found positive, it is more important to prevent new entry, identify reservoirs and eliminate them than to assume an increased tenacity of Salmonella or resistance of these pathogens to disinfectants. Further analyses will examine these epidemiological correlations more closely.

References

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Prevalence of Salmonella in German battery cages and alternative housing systems

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Introduction

Between 2001 and 2008 the share of German laying hens kept in cage systems dropped from about 85 % to 59 % while non-cage systems rose to 41% [1]. This developement will continue since battery cages are no longer allowed from 2002 and furnished cages from 2009 with a transition period (requiring special permission) for the latter up to 2020. This move to alternative housing systems and its impact on the *Salmonella* spp. prevalence and egg safety is of great interest for the consumer and the egg producers. About 50.000 confirmed clinical cases of salmonellosis are reported per year in Germany [2]. Important transmission routes are eggs and egg products [3]. Particularly *Salmonella enterica* serovar Enteritidis which is able to contaminate the eggshell as well as the egg content is frequently associated with human infections [4]. To assess the potential risk of transmission of this and other pathogenic *Salmonella* serovars from laying hens housed in battery cages and alternative housing systems into the food chain, a field study was conducted within the EU-project SAFEHOUSE. This paper reports about the occurrence of *Salmonella* in 92 German laying hen flocks sampled between May 2007 and June 2008.

Material and Methods

Sampled flocks. 78 farms participated voluntarily in the German cross–sectional study of the SAFEHOUSE project. 92 flocks (27 in battery cages, 23 in floor raised systems, 25 in free range systems and 17 in organic free range systems) of these farms with more than 1000 hens were sampled. Questionnaires about the farms (e.g. age, size, system, management) and the sampled flocks (e.g. number and age of hens, race, *Salmonella* status, medical treatment, vaccination) were filled in by the farmers.

Sampling. Samples were taken in the month prior to depopulation. Five pooled faecal samples with at least 250 g of faecal material, 250 ml of a pooled dust sample from 20 separated locations within the house, 40 cloacal swabs, three pairs of boot swabs, 10 swabs from egg belts (if present) and approx. 500 red mites (if possible) were sampled from each flock. All samples were transferred in a cool box at 4-8 ‘C to the laboratory.

Laboratory methods. 25 g of the faecal samples, 25 ml of the dust samples and each pair of boot swabs were homogenised and shaken for one minute respectively in 225 ml buffered petone water (enrichement medium) by using a Stomacher 400 circulator (Seward, England). Swabs were transferred in steril tubes with 9 ml buffered peptone water and shaken for one minute on a MS1 Minishaker (IKA®Works Inc., Wilmington, USA). 200 mites were homogenised in 5 ml enrichement medium and futher 200 mites were washed firstly in 10 ml 95% ethanol and secondly in 10 ml diluted kickstart 2 solution (7,5% hydrogen peroxide, Cid lines, Belgium) before homogenised in 5 ml enrichment medium. For pre-enrichement of *Salmonella* all prepared samples were incubated at 37 'C for 18 ± 2h. *Salmonella* detection was conducted by using the amended international standard ISO 6579:2002. Typing and phage typing of *Salmonella* isolates were performed at the National Reference Laboratory for the Analysis and Testing of Zoonoses (*Salmonella*) in Berlin.

Statistical analysis. Odds ratios and 95% confidence intervals were calculated with R version 2.6.2, 2008 (The R Foundation for statistical computing).

Results

The Salmonella findings showed one or more positive *Salmonella* samples in 28 (30%) of all sampled flocks. 20% of all flocks were tested positive by using boot swabs, 18.5% showed positive faecal samples and 15% had positve dust samples. 4% of the flocks were found to be positive by testing cloacal swabs and 4% of all tested egg belts were also positive. Mite samples were all negative. A considerable lower occurrence of *Salmonella* was found in the alternative housing systems compared to the conventional battery cages (Fig. 1). The odds ratios (OR) and the 95% confidence intervals (CI) (battery cage vers. floor raised system: OR = 9,73, CI from 2,73 to 43,11; battery cage vers. free range system: OR = 8,82, CI from 2,45 to 39,29; battery cage vers. organic free range system: OR = 13,32, CI from 2,89 to 107,56) indicate clearly a higher risk of contaminated samples in the convetional battery cage system.

112 Salmonella strains were isolated from all samples. Serotyping resulted in a high amount of the serovar *Salmonella* Enteritidis (83% of all strains, Tab. 1). This serovar was detected in all positive flocks except of one free range system. The predominant phage types were PT4 and PT8. In some cases more than one serovar or different phage types were found in the same flock.

Discussion

The results show that there is a high prevalence of *Salmonella* in German laying hen flocks. The total prevalence is in good agreement with earlier results of a comprehensive study conducted by the Federal Institute for Risk Assessment in Berlin from October 2004 to September 2005 [5]. *Salmonella* Enteritidis (mainly PT4 and PT8), which was resposible for the most human *Salmonella* infections in recent years in Germany [6, 7], was detected in 96% of the positive tested flocks. According to article 1 of the regulation EC No 1168/2006 this means that Germany has to reduce the total prevalence of zoonotic *Salmonella* from nearly 30% to at least 21% within one year. Radical measures such as killing of infected flocks and a stringent implementation of hygiene rules can reduce the *Salmonella* prevalence effectively [8]. Vaccination and competitive exclusion treatment may also be options to achieve a reduction of zoonotic *Salmonella* serovars in laying flocks [9, 10]. In this

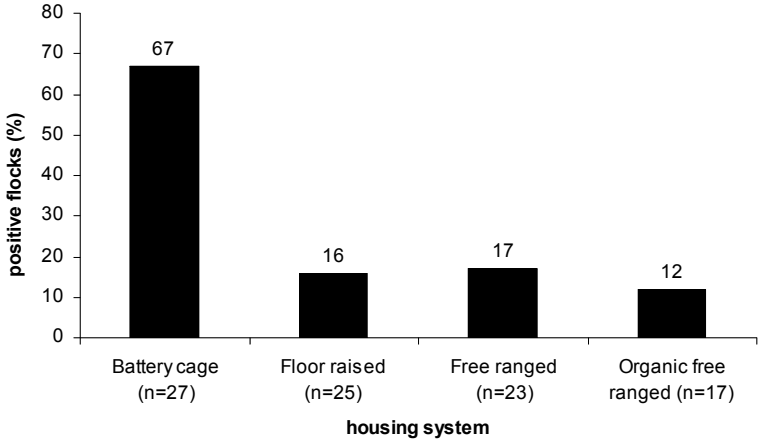


Fig. 1: Salmonella positive flocks in % relative to the number of sampled flocks (n) of each housing system.

Tab.1: Results of serotyping and phage typing of strains from positive samples.

no of strains	serovar	phage type	% of all strains
93	*Salmonella* Enteritidis	64 x PT4 22 x PT8 3 x RDNC 2 x PT21c 2 x nt	57.1 19.6 2.7 1.8 1.8
6	*Salmonella* Tennessee	-	5.4
5	*Salmonella* spp.	-	4.5
2	*Salmonella* Typhimurium	-	1.8
2	*Salmonella* München	-	1.8
1	*Salmonella* Infantis	-	0.9
1	*Salmonella* Livingstone	-	0.9
1	*Salmonella* Thompson	-	0.9
1	*Salmonella* Ouakam	-	0.9

context Germany passed a new regulation in April 2009 for a better control of *Salmonella* in laying hen flocks. The enforcement of this regulation and the continuous move from battery cages to alternative systems may contribute to a decrease of the *Salmonella* prevalence but it remains questionable wether a reduction of 9% can be reached within one year.

In this study a relatively low contamination rate was detected in the alternative housing systems. This is in agreement with a number of other studies and may be the result of factors such as: large flocks, old farms, poor disinfection measures associated with the conventional battery cage system [3, 8,11,12]. A risk factor analysis on the presented data will be conducted in future to verify these assumptions.

Conclusion

There is still a high prevalence of *Salmonella* in German laying hen flocks which poses a risk to the consumer. New regulations which will enhance prevention and control measures and the move of laying hens to alternative systems seem to have the potential to reduce this risk in future.

Acknowledgement

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Risk factors for Salmonella in flocks of laying hens in Germany

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Introduction

Salmonellosis still belongs to the most frequent zoonoses in Germany and in the European Union, where S. Enteritidis is the predominant serovar. The source of infection is food of animal origin, especially eggs and poultry meat.

In order to follow the target of Regulation No (EC) 2160/2003 to detect and to control salmonella and to reduce the prevalence, an EU-wide baseline study was carried out to estimate the prevalence of Salmonella in flocks of laying hens. In Germany, 533 flocks were investigated in this cross-sectional study (Oct. 2004 to Sept. 2005). The raw prevalence of *Salmonella* spp. was 29.8% (CL: 25.9-33.6); accounting for regional allocation and farm size, prevalence was 25% [1].

Within a baseline analysis first hypotheses on possible population-based risk factors for the transmission of Salmonella were generated. Detailed statistical-epidemiological analyses of that data focussing on serovar specific analyses were conducted in order to generate further risk hypotheses.

Material and Methods

Unifactorial logistic regression modelling was conducted in order to identify possible factors that influence the occurrence of Salmonella in laying hens on a population level. Doing this, the possibility of spread of Salmonella on the farm given the influencing factor was modelled [2]. All factors in the model were categorical. The most frequent category of exposition was chosen as reference in order to assure stability of risk estimation.

Analysing possible risk factors additional modifying influences have to be considered. Therefore, logistic regression modelling was conducted including stratification variables. This was done systematically to generate hypotheses on possible modifying influences on any of the salmonella risk factors.

In order to analyse the influence of the region, in which the farm was located, Germany was divided in four regions accounting for poultry and farm density as well as for size of farms in that areas. Farm size was classified in small (<3,000 hens), medium (3,000 to 30,000 hens) and big farms (>30,000 hens).

To conduct serovar specific analyses the most frequent serovars or serovar groups were analysed separately. Therefore, four comparisons of Salmonella affected farms and not-affected farms were conducted, each considering another Salmonella serovar (all Salmonella serovars, S. Enteritidis, S. Typhimurium and other Salmonella serovars). S. Typhimurium and other S. serovars could only be analysed descriptively as too few farms were available with these serovars for statistical testing.

Results

S. Enteritidis and S. Subspec. I rough form are the predominant types; as their patterns are similar (see figure 1 and 2), both types have been analysed together.
Serovar-specific analyses were restricted by the fact that S. Enteritidis was diagnosed in most of the positive flocks. Therefore, Serovar-specific risk patterns cannot be differentiated clearly. However, accumulations of serovars could be found. S. Enteritidis including rough strains can be found in immunised barn flocks and in immunised big farms predominantly. Whereas, S. Typhimurium occurs in barn flocks in region "West" and free range systems in the South as well as in not immunised farms of moderate size. Other Salmonella serovars, however, can be found in barn flocks in the Northwest and in all cage systems as well as in big and not immunised farms.

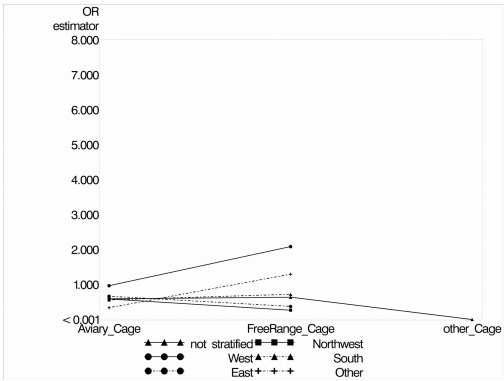


Fig. 1a: Effect of Region on Housing system (all serovars)

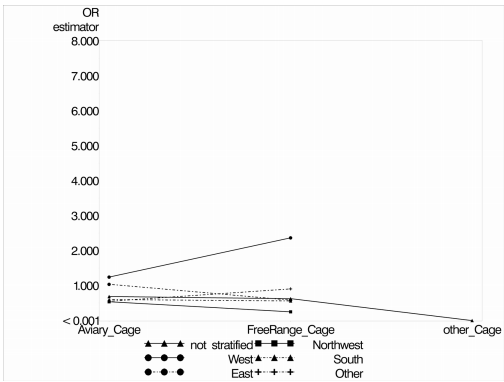


Fig. 1b: Effect of Region on Housing system (S. enteritidis)

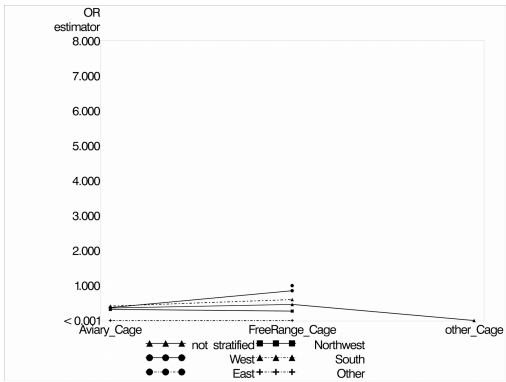


Fig. 1c: Effect of Region on Housing system (S. rauform)

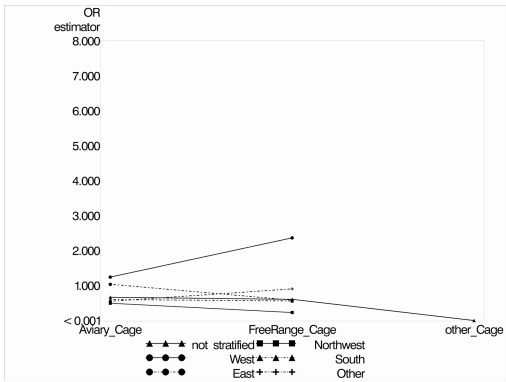


Fig. 1d: Effect of Region on Housing system (S. enteritidis and rauform)

Regardless of serovar detected, differences of prevalence concerning region, farm size, type of housing and immunization status could be confirmed. As the regional allocation is a stratification variable rather than a risk factor, differences between regions reflect different management and structure profiles. In the unifactorial approach the highest risk of Salmonella could be found in the Northwest of Germany, in cage systems and on big farms (see figure 1 and 2). Immunization status does not seem to influence salmonella prevalence or any of the analysed risk factors notedly.

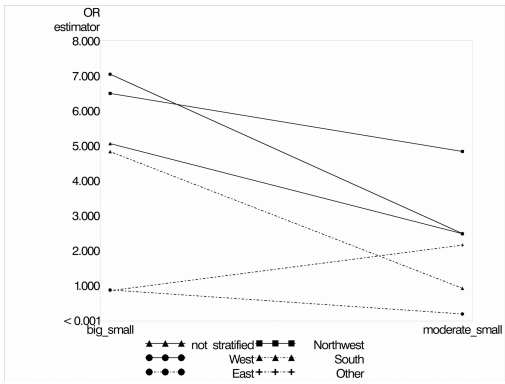


Fig. 2a: Effect of Region on farm size (all serovars)

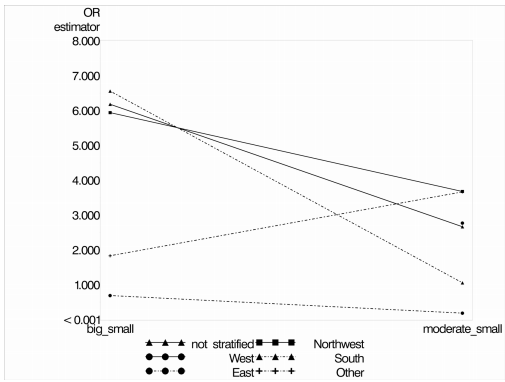


Fig. 2b: Effect of Region on farm size (S. enteritidis)

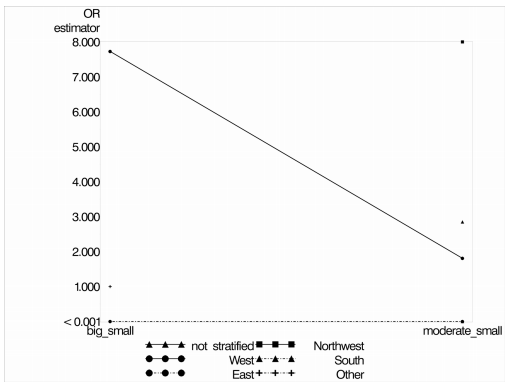


Fig. 2c: Effect of Region on farm size (S. rauform)

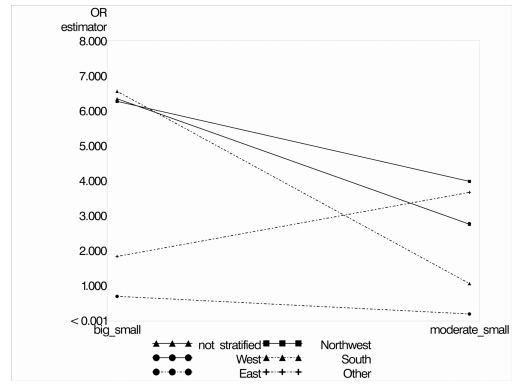


Fig. 2d: Effect of Region on farm size (S. enteritidis and rauform)

A multitude of systematic analyses has been conducted. Analyses were to explore data and generate hypotheses. Therefore, results should be interpreted without any meaning of statistical significance. Especially, when interpreting serovar specific analyses, the size of groups and subgroups is too small to ensure statistical significance.

Results indicate interactions between the analysed risk factors that are complex and cannot be separated easily. This indicates influence of other risk factors that were not collected in the study and therefore not analysed here.

Conclusion

The analysis of the baseline study indicates that Salmonella prevalence depends on interacting risk factors. Correlations, however, are complex and difficult to separate. Risk factors are highly interactive associated, which indicates influence of further risk factors not addressed in the EU-wide baseline study.

Due to the predominance of S. enteritidis and its occurrence in combination with other Salmonella serovars, serovar specific risk patterns cannot be concluded. Nevertheless, different risk

structures are evident. In either case, it is important to document the Salmonella serovar/s for every farm.

Due to interactions with the region itself and its strong modifying effect on other risk factors, monitoring should consider regional structures in future. Above this, instruments for data collection should consider further possible risk factors characterizing the farm management.

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The reservoir for *Escherichia coli* strains causing fatal systemic infections in poultry is the chicken itself

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Introduction

Extraintestinal *Escherichia coli* (ExPEC) infections are among the most significant infectious diseases in production birds. Still, the term avian pathogeneic E. coli (APEC) is ill–defined and little knowledge exists about the reservoir of these bacteria [5, 6, 7, 8]. The aims of this study were to characterize fecal and environmental E. coli strains by molecular typing and to select a variety of well defined strains from clinically healthy birds to assess their putative zoonotic potential and their pathogenicity in a systemic chicken infection.

Animals, Material and Methods

E. coli strains isolated from internal organs of diseased chickens (n=121) were compared with those isolated from the faeces (Afecal; n=211) and the environment (Aenviron; n=35) of clinically healthy chickens by determining virulence gene profiles (VAGs; [3, 4, 6]), phylogenetic backgrounds (Multilocus sequence typing; MLST; [9]), and responses to chicken serum [2]. In vivo pathogenicity was assessed in a chicken infection model, using 5– week old SPF White Leghorn chickens [1].

Results

By examining 46 VAGs, specific fecal and environmental isolates were detected that had a virulence gene profile indistinguishable from that of outbreak strains. In addition, a substantial number of phylogenetic EcoR group B2 strains, known to include human and animal ExPEC, were identified among APEC (44.5%) and among Afecal strains (23.2%). Intratracheal infection of chickens corroborated the pathogenicity of several non– outbreak strains. MLST revealed that most strains, being virulent in chicken infection experiments, belonged to sequence types (STs) that are almost exclusively associated with extraintestinal diseases in birds and in humans, like septicemia, urinary tract infection, and newborn meningitis, supporting the hypothesis that not the ecohabitat, but phylogeny and virulence gene profile of E. coli strains determines virulence. The data are summarized in Figure 1.

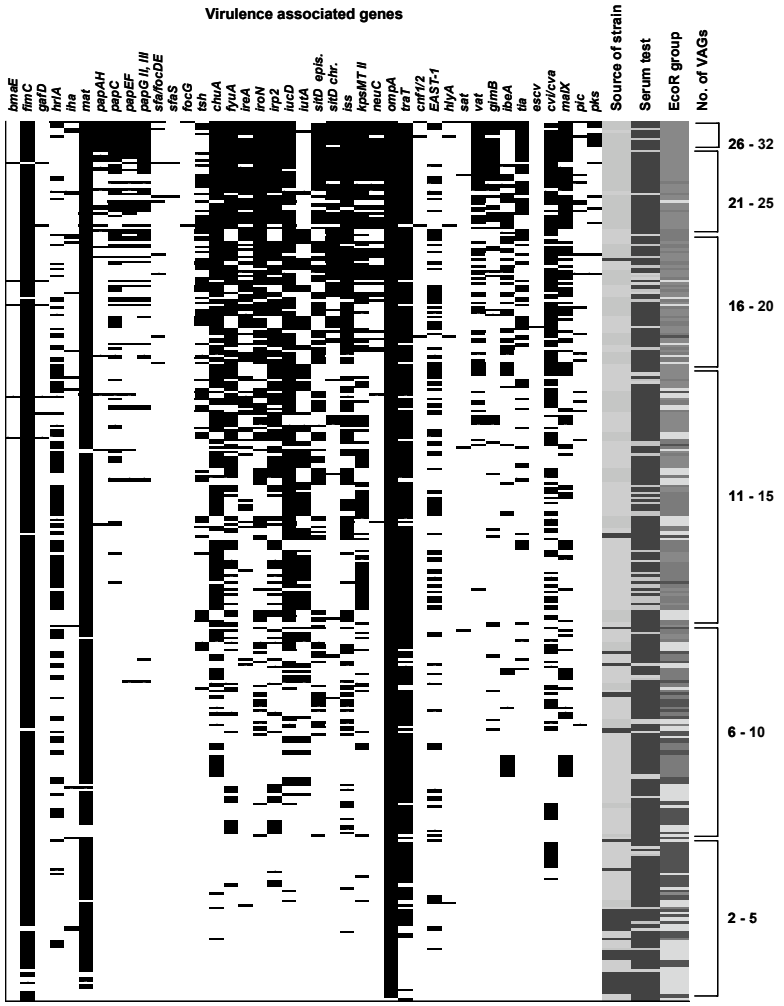


Figure 1: Virulence gene patterns, sources of strains, assignments to phylogenetic groups and results of serum tests of 347 *E. coli* strains isolated from diseased as well as healthy chickens and their. Legend: Virulence-associated genes (VAGs): presence of a gene in a particular strain = black; Source of strain: A_{fecal} = light grey; $A_{environmental}$ = dark grey; APEC = light green; EcoR groups: A= orange, B1:=blue; B2:=red, D=green; Serum test: resistant = dark blue; intermediate = light grey; sensitive = cyan Genes completely absent among strains (afa/dra, bfp, nfaE, stx1, and stx2) and are not illustrated in the figure.

Discussion

Our data provide strong evidence for an avian intestinal reservoir hypothesis, which is helpful in developing intervention strategies. In conclusion, virulence genotyping and phylogenetic data, including EcoR and MLST results, show that certain non-outbreak strains, in particular Afecal strains originating from the intestine, isolated from clinically healthy poultry bear a zoonotic potential either by being directly transferred from birds to humans or as a genetic pool for extraintestinal pathogenic *E. coli* strains. A rigorous epidemiologic study, unveiling the diversity of the *E. coli* population in the chicken intestine is currently being

performed in our lab to analyse the frequency of such strains in the chicken gut envisioning the development of intestinal intervention strategies as a future perspective.

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Chapter 24

Block 9C

Rabies virus neutralizing antibodies in occupationally exposed humans in a Nigerian University

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University of Ibadan, Nigeria

Summary

Sera from people occupationally exposed to rabies were assayed for Rabies virus neutralizing antibodies to determine their levels of protection against the disease. Specimens were obtained from zoological garden workers, veterinarians, and veterinary students at the University of Ibadan, between September 2008 and February 2009. A total of 60 individuals were screened using the Enzyme Linked Immunosorbent Assay method. Out of the 60 persons, 39 (65%) were not protected against rabies virus (Ab $<$ 0.5Eu/ml). Eleven out of 18 zoological garden workers (61.1%), 8 out of 12 veterinarians (66.7%), and one out of 30 veterinary students (3.33%) were protected against rabies virus. Seroprevalence was higher within the older segment of the study population (veterinarians and zoological garden workers) (77.3-96.8%) than in the younger veterinary students (3.33%). Almost all staff that had spent at least 10 years in their units had more vaccination compliance and became protected through bi-annual anti-rabies vaccination. The overall prevalence rate of 35% (21/60) and average prevalence rate of 43.7% per group were indicative of low anti-rabies protection among occupationally exposed individuals at the University of Ibadan. This study shows an overdependence of employees on their employers for anti-rabies protection. The impacts of these results on rabies control in Nigeria and prevention of occasional international cases of human rabies among travellers to Europe and the Americas is discussed.

Introduction

Human anti-rabies vaccination in south-west Nigeria. Fatal cases of human rabies are still presented at clinics and hospitals in towns and villages of south-west Nigeria yearly. This is because rabies has not been controlled and eradicated in Nigeria. Many of the modern rabies vaccines provide nearly 100% protection with high degree of safety (2). The combination of late presentation of infected dog bite cases to the hospitals for post-exposure vaccination and non-compliance with recommended pre-exposure vaccination among high risk occupationally exposed humans in villages and cities of south-west Nigeria appear to largely account for human deaths due to rabies in this part of the country (1). Fatal human rabies in an immigrant from West Africa to the United Kingdom was a recent evidence of the scenario. In observing World Rabies Day 2008, i.e. September 28th, the Department of Veterinary Public Health and Preventive Medicine at the University of Ibadan in collaboration with the University Health Center organized an investigation of pre-exposure vaccination seroconversion in occupationally exposed people in the University of Ibadan. Numerous safe, but relatively high cost and effective vaccines for man have become available in the developing countries after the first rabies vaccine that was developed by Pasteur over 110 years ago. With these, human and animal health authorities hoped that the disease would be eliminated or at least drastically

reduced even in Africa where deaths due to rabies are yearly recorded.

Veterinary and medical literature in Nigeria is clear on dog bite cases presented to selected hospitals and their management by post-exposure vaccination (3), but limited in anti-rabies seroconversion in human patients. The World Rabies Day 2008 with the theme "Working together to make Rabies a history" was a unique opportunity for joint effort of physicians and veterinarians at the University of Ibadan, Nigeria to investigate the success of bi-annual rabies pre-exposure vaccination of staff in rabies high risk areas of the University.

Rabies is one of the oldest known diseases of mankind and has not been controlled in Nigeria. Over 40,000 people world-wide die from rabies each year. Mass vaccination has been used successfully in Western Europe and North America [5], illustrating that the disease can be controlled and eliminated by vaccination of reservoir animal populations. Japan was the first country to implement mass vaccination of dogs, and the country successfully eliminated rabies in 1956 [Kaare et al 2009]. The University pays for staff anti-rabies vaccination in the Faculty of Veterinary Medicine, the Veterinary Teaching Hospital and in the Zoological Garden, but students in these departments bear the cost of their own immunization. This paper is an evaluation of rabies neutralizing antibody profile in the sera of veterinarians, technologists and students of veterinary medicine and of zoo workers at the University of Ibadan, Ibadan, Nigeria.

Material and Methods

A sample of 60 occupationally exposed students and staff of the University was screened. This comprised of 30 Veterinary Students, 12 Veterinary Faculty Staff and 18 Zoological Garden workers. Some 2.5mls of blood was collected through the cephalic vein from each person (by medical technologist at University Health Services (UHS) Center. Sera were collected from the clotted blood samples. Each serum was centrifuged at 3,000rpm and supernatant harvested. These were temporarily stored at -4^{0}C until test was conducted within a week. The Platelia^{TM}Rabies II kit (Bio-Rad, France) (4) a quantitative Indirect-Enzyme Linked Immunosorbent Assay (I-ELISA) for classical rabies neutralizing antibodies was used. Platelia^{TM}Rabies II kit is an immune-enzymatic technique for the detection of rabies virus anti-glycoprotein antibodies. The test is based on the use of a solid phase enzyme immunoassay technique referred to as indirect ELISA. A microplate is coated with rabies glycoprotein extracted from the inactivated and purified virus membrane. The enzyme conjugate consists of a protein A from *Staphylococcus aureus* coupled with peroxidase. Positive controls, calibrated against WHO standards, allow the quantitative determination of anti-rabies antibody titter in the serum tested. Serial dilution of the standard positive control was done. This was used according to manufacturer's specification (4). A minimum antibody titer of 0.5Eu/ml was considered protective against rabies virus.

Personal information, occupational and rabies immunization records were gathered through structured questionnaire administered in form of participatory interview with each person tested. Assessment criteria in forms of frequency, percentage, mean and product moment correlation coefficient were calculated.

Results

Eight out of 13 (61.5%) veterinary staff were serologically protected (seroconverted), and 12 out of 17 (70.6%) zoo workers were seroconverted. Seroconversion ranged from 0.5 Eu/ml to 4.1 Eu/ml. All veterinary students sampled were second year clinical students (in the 6th year of a 6 year Doctor of Veterinary Medicine programme). Some 7.1% (3/42) of the youngest exposed people (1-5 years on the job) were seroconverted (Table 1). Thus about 92.9%

(39/42) of the 1-5 year occupationally exposed individuals were seronegative. The 7 individuals with 6-10 years work tenure and 7 with 11-15 years on the job were 100% seroconverted respectively (Table 2). Overall seroconversion in veterinary students was 3.3% (1/30). Thus 29 of the 30 (96.7%) sampled veterinary students were unprotected. Antibody titer among veterinary students ranged from 0.0 Eu/ml to 0.2 Eu/ml. Overall seroconversion increased from 1-5 years on the job to 25-30 years on the job (Figure 1). Thus, the seroconversion is lowest in veterinary students, veterinarians and zoo workers that were new on the job, followed by workers 11-20 years on the job and highest among the oldest staff of veterinary medicine and the zoological garden (Table 2).

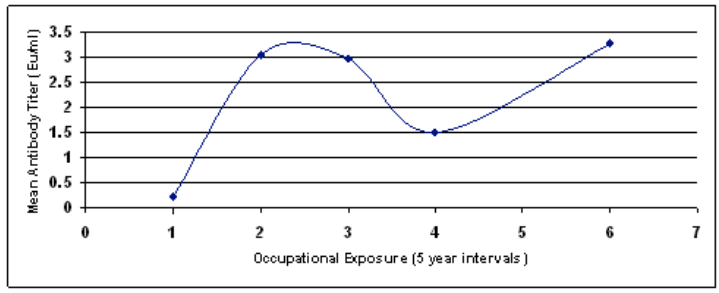


Fig.1: Anti-Rabies seroconversion in exposed staff and students, Ibadan

Tab. 1: Anti-Rabies antibody titer in veterinarians and zoo workers in Ibadan

Group	No	No Protected	Mean ±SD	Male			Female		
				No.	No protected	Mean ±SD	No.	No protected	Mean ±SD
Veterinary Students	30	1	0.90 (0)	20	1	0.90(0)	10	0	0(0)
Veterinarians	13	8	3.89 (0.23)	11	8	3.89 (0.23)	2	0	0(0)
Zoo workers	17	12	2.46 (1.14)	11	7	2.01 (1.03)	6	5	3.08(1.06)
Total	**60**	**21**	**2.93 (1.2)**	**42**	**16**	**2.88 (1.27)**	**18**	**5**	**3.08(1.06)**

Tab. 2: Effect of length of exposure on anti-rabies antibody titer in Ibadan

Years of Occupational Exposure	No	No Protected	Mean ±SD	Male			Female		
				No.	No protected	Mean ±SD	No.	No protected	Mean ±SD
1-5	42	3	2.7(1.57)	29	3	2.7(1.57)	13	0	0(0)
6-10	7	7	3.04 (1.12)	5	5	3.18 (1.09)	2	2	2.7(1.1)
11-15	7	7	2.46 (1.14)	5	5	2.94 (1.52)	2	2	3.05(1.06)
16-20	1	1	1.5(0)	1	1	1.5(0)	0	0	0(0)
21-25	0	0	0	0	0	0	0	0	0(0)
26-30	3	3	3.27(1.27)	2	2	2.95(1.63)	1	1	3.9(0)
Total	**60**	**21**	**2.93 (1.2)**	**42**	**16**	**2.88 (1.27)**	**18**	**5**	**3.08(1.06)**

Discussion

The free bi-annual anti-rabies immunization of veterinary and zoological garden staff were effective in conferring seroconversion and adequate protective levels of rabies neutralizing antibodies on the University staff. It was noteworthy that only one new staff was seroconverted, indicating pre-employment immunization out of 12. This situation is similar to the results shown by students that did not receive

immunization against rabies virus in spite of two years of active clinical exposure to dog and cats in the Veterinary Teaching Hospital. These findings indicate a situation whereby occupationally exposed individuals appear not willing to pay for anti-rabies immunization on their own (1). The students were all agreed in their knowledge of the risks involved in handling animals when unprotected, and the higher cost involved in post-exposure vaccination. They also agreed on a relatively high cost of the vaccine which was N2,500:00 in 2008 (about USD22.7 at N110 = 1 USD). All the students desired that the University authority should fund the immunization of veterinary students.

Conclusion

This low level of compliance with rabies vaccination in man in the University of Ibadan may very well represent the situation in the south-west part of Nigeria. It may be concluded that occasional international cases of human rabies among travellers from West Africa to Europe and Americas may be due to this factor.

Acknowledgement

The authors acknowledge the collaboration of the University Health Services Center.

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Avian Influenza Viruses in Aquatic Biosystems

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Summary

Water appears to play an important role in the transmission of avian influenza viruses (AIV). Many factors in the aquatic environment may be capable of harboring and transmitting these viruses, including the water itself as well as sediment at the bottom of contaminated lakes. Invertebrates living in the water may take up virus during feeding and in turn be eaten by susceptible water birds. The results of this study show that AIV survive for months in water and sediment at low temperatures and that some invertebrates are capable of taking up virus from the surrounding water. Water, sediment and invertebrates are therefore capable of transmitting AIV over time.

Introduction

Aquatic birds are considered the natural reservoirs of AIV. The viruses are maintained in wild bird populations through the fecal-oral route. Environmental transmission has recently been shown to play an important role in recurrent AIV epidemics (Breban et al., 2009) and water is thought to be an important factor in virus transmission. There is little information available on the survival of AIV in water over a wide range of temperatures and none on the role of invertebrates in the aquatic environment on the distribution of AIV. The present study aimed to evaluate the tenacity of AIV in lake water and sediment from a lake as well as their uptake by invertebrates in surface water.

Material and Methods

Viruses. Two low pathogenic AIV (H4N6 and H5N1) were used in all tests. Both viruses were provided by PD Dr. Timm Harder, Friedrich-Loeffler-Institut, Insel Riems, Germany. The viruses were propagated in the allantoic cavity of 9-11 day old specific pathogen free embryonated chicken eggs (Lohmann, Cuxhaven, Germany). Infective amniotic allantoic fluid (AAF) was used in the tests.

Virus detection and quantification. For the tenacity studies in water and sediment, virus was detected and quantified by endpoint titration on Madin Darby canine kidney cells (MDCK). Virus replication was confirmed by hemagglutination test. For studies on virus accumulation in zebra mussels and water fleas, viral RNA was quantified by quantitative real-time RT-PCR (Spackman et al., 2002). In the trials with zebra mussels, virus was also quantified by titration in embryonated chicken eggs.

Animals. Zebra mussels (*Dreissena polymorpha*) were collected from wild populations of Lake Constance (provided by Dr. Hans Güde, Institut für Seenforschung, Landesanstalt für Umwelt, Messung und Naturschutz, Baden-Württemberg, Langenargen,

Germany). Mussels were stored in natural lake water without supplementation of nutrients. Water fleas (*Daphnia magna*) were obtained from a pet store.

Tenacity studies in surface water and lake sediment. Tenacity studies were carried out at –10, 0, 10, 20, and 30 C. For the studies in surface water, water was collected from Lake Constance. AAF containing virus was diluted 1:100 in water and incubated at each temperature. Virus was quantified in the water by titration on MDCK at regular intervals. For the tenacity studies in lake sediment, sediment was collected from Lake Constance. Virus was introduced into the sediment adsorbed onto sandwich carriers and incubated at the same temperatures.

Accumulation in mussels and water fleas. Experiments with water fleas were carried out at room temperature, experiments with zebra mussels were carried out at 4 and 19 C. Virus was added to water containing zebra mussels or water fleas. Water fleas were left in virus containing water for 24, mussels for 48 hours. After incubation, animals were removed from water containing virus, washed 3 times with virus free water and placed in fresh (virus free) water.

Results

The survival time of viruses in water and sediment was inversely proportional to the incubation temperature. In surface water, viruses remained infectious for a few days at 30 and 20 C, for a few weeks at 10 C and for months at 0 and –10 C. In comparison to surface water, virus in sediment remained infective for longer periods of time and higher D-values were calculated for both viruses in sediment than in surface water.

The studies on virus accumulation in zebra mussels and water fleas showed that both animals were able to accumulate virus from the surrounding water. After washing the animals and placing them in fresh water, virus was detectable in both the water and the animals, but virus amounts in the animals were generally higher than in the water and remained higher over time. Virus was detectable in both water and animals for several days in all tests.

Discussion

Water is known to play an important role in the epidemiology of AIV. Following excretion by infected birds, AIV may be readily diluted in volumes of flowing water, but in the stagnant water of small ponds or lakes where a large number of birds gather, virus can clearly spread to susceptible birds. At the very low temperatures of 0 and –10 C both AIV survived for several months. This explains how some frozen lakes may harbor virus over winter as hypothesized previously (Webster et al., 1992; Zhang et al., 2006). Sometimes fecal material passed by infected ducks near the banks of water bodies does not disolve, but is deposited at the bottom, and lake sediment may be contaminated. Dabbling ducks would then also come into contact with virus. The results of the studies with sediment from a lake show that virus deposited at the bottom of lakes may survive for longer than virus suspended in the water. Since virus in sediment is also less susceptable to dilution factors, this may be an important way for AIV to survive in the environment over time. One factor that may help explain the longer survival of viruses in sediment than in surface water is their

adsorption on carriers for the tenacity studies carried out. Virus adsorbed on carriers may be less susceptable to environmental factors and therefore survive for longer periods of time. This corresponds to the situation in nature, where viruses may be protected by fecal material.

Both water fleas and zebra mussels were able to accumulate virus from the surrounding water in this study. Both of these animals serve as food animals for various vertebrates, including birds. Invertebrates may therefore serve as vectors for AIV.

Aquatic environments are complex and many different factors can play together to affect the persistence of AIV in these environments. Both biotic and abiotic factors can affect this. In cold climates, AIV can persist in water for extended periods of time. Accumulation of virus in invertebrates can lead to an increased exposure of birds that eat invertebrates to virus in water and possibly counteract the effects of dilution in the water. Further studies are needed to fully describe the effects of these factors on the epidemiology of AIV.

Conclusion

1. Avian influenza viruses remain infectious in surface water and sediment over long periods of time (months) at low temperatures.
2. Both water fleas and zebra mussels can accumulate influenza viruses from water.

Acknowledgement

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Serological Evidence of Avian Influenza Viruses in Pigs in South-Western Nigeria

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Summary

In order to assess the involvement of pigs in the waves of Highly Pathogenic Avian Influenza (H5N1) outbreaks (HPAI) in recent years in Nigeria, one hundred and fifty (150) sera samples were collected from 10 pig herds in Ibadan and Lagos within graded distances from HPAI virus infected poultry farms. By purposive sampling method, twenty five (25) cloacal and pharyngeal swabs were collected from two herds reared along with poultry at less than 1km apart. All the sera samples were tested for presence of Avian Influenza A virus (AIV) species, using polyclonal antibodies by competitive immunochromatographic assay while the H5N1 specific antigen was used for further typing and confirmatory diagnosis of HPAI from the pharyngeal and cloacal swabs. Prevalence of 48.5% (n=73) of AIV polyclonal antibodies was obtained, while 0% prevalence of H5N1 antigen was recorded in pigs examined. Highest prevalence (57.1%) of AIV antibodies was recorded in pigs reared less than 5km to poultry farms and the lowest prevalence (1.4%) was recorded in pigs more than 15km to poultry flocks. The zero prevalence of H5N1 antigen was an indication of lack of active infection with HPAI among the pigs. The high prevalence of AIV antibodies, however confirmed exposure of the pigs to different strains of low pathogenic avian influenza virus strains. These findings could be attributed to the common practice of mixed poultry and piggery management in south-western Nigeria. Thus, there is need to include screening of pigs in AIV surveillance exercise for effective control, monitoring and public health protection.

Introduction

Between January 2006 and January 2007, about one hundred and sixty-six outbreaks of HPAI were confirmed and well over 828,000 poultry including commercial layers, breeder stocks of chicken, turkeys, ostriches, broilers, ducks, geese, local chicken and guinea fowls, had died of the disease or were culled in Nigeria [4]. The H5N1 subtype which is known to be occasionally pathogenic to human was identified in each of the outbreaks. All of these outbreaks received adequate regulatory attentions such as depopulation, biosecurity and appropriate compensation paid. However, instances of pre-depopulation sales and disposal of birds occurred on some farms, indicating that the virus have been able to escape to other premises before the confirmation or diagnosis, depopulation and biosecurity measures could be applied. The first and yet only human H5N1 case was however confirmed in January 2007 in Lagos State, south-western Nigeria 12 months after the first outbreak of the virus in the poultry population in the country [4]. Recent study [1] has confirmed the presence of trans-species spread of Influenza A viruses from pig to man and form from man to pig in Ibadan, Nigeria. Thus, classical swine viruses and avian-human reassortant viruses have been isolated from pigs across the world [2, 3 and 7]. In view of the critical role of pigs and the mixed husbandry including co-ownership practice of poultry and piggery production system in south-west Nigeria, this study investigated the presence of antibodies and antigens of avian influenza viruses including HPAI strains in pigs at graded distances from poultry farms

that were confirmed infected with the HPAI in Lagos and Oyo States. Geographic distribution of the primary outbreak of HPAI in Nigeria showed that Lagos and Ogun States in south-western Nigeria were among those with highest numbers of poultry farms affected, while Oyo and Ondo states were least affected in this part of the country [4].

Material and Methods

Using purposive sampling method, in which pig farms within graded distance of less than 2.5km, 5km, 10km and 15km from HPAI confirmed poultry flocks, serum specimens were collected from 150 pigs within Ibadan and Lagos, Nigeria through the cranial venacava and 25 pharyngea and cloacal swabs were also obtained from April to June, 2008 and tested at the Department of Veterinary Public and Preventive Medicine, University of Ibadan (U.I). The sera were tested with Anigen Rapid AIV Ab Test kit^R(Animal Genetics, Inc.,Korea), for any of the polyclonal antibodies using the principle of "competitive chromatographic immunoassay. While the swabs were tested for H5 AIV subtype with Anigen Rapid AIV Ag Test kit^R (Animal Genetics, Inc.,Korea). All samples were tested in replicates.

Results

A total prevalence of 48.5% (n=73) of AIV polyclonal antibodies was obtained, while 0% prevalence of H5N1antigen was recorded in pigs in this study. There was higher prevalence (57.1%) of AIV antibodies among the pigs reared at less than 5km to poultry farms confirmed HPAI infected, while in pigs reared at more than 15km distance from such poultry flocks recorded the lowest prevalence of 1.4% (table 1). There was no evidence of respiratory infections among the pigs tested. Feeding and watering equipment were sometimes shared by pigs and poultry as they were owned by the same farmers.

The spatial distribution of the sampled farms and relationship with the HPAI infected were presented in figure 1.

Discussion

The results showed that although there was no active infection of avian influenza viruses among pigs tested, the high prevalence of AIV antibodies confirmed the exposure and circulation of different strains of avian influenza viruses among the pigs in this region of the country which may not be the highly pathogenic strain as indicated by none detection of H5 antigen. These findings can be attributed to the common practice of mixed poultry and piggery management and the sales disposal culling approached by some poultry farmers during the HPAI outbreak.

Conclusion

Considering the role of pigs in the genetic re– assortment of different strains of influenza viruses,there is a need for broad suveillance of influenza A viruses among human, poultry and pigs to forestall threat of human pandemic emanating from south– west Nigeria.The efforts of the animal health services thus needs more support from within and outside the country to effectively and efficiently control and stamp out the disease in Nigeria.

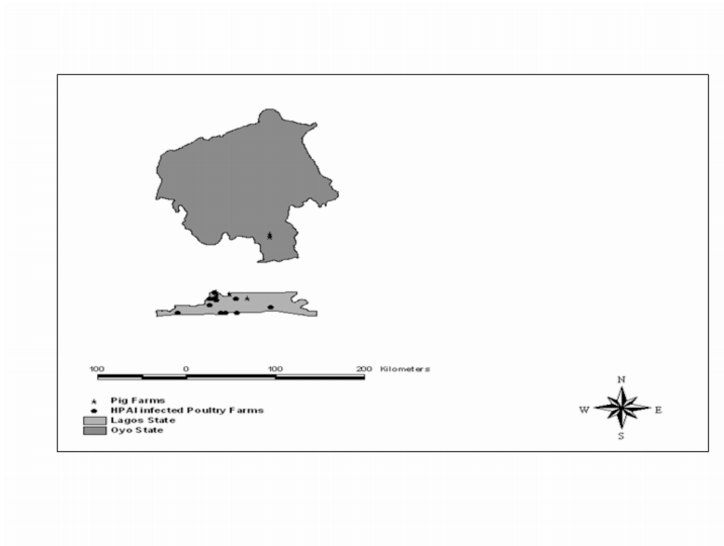


Fig. 1; Study areas in southwestern Nigeria

Tab. 1: Pig farms surveyed at five graded distances with sera AIV Ab prevalence rate

Name of pig farm	Graded distance from HPAI infected poultry farms (km)	Cordinates	Number of samples for polyclonal antibodies	Positives (%)	Negatives (%)
Oke-Aro Phase I	< 2.5km	6.6949N; 3.3206 E	20	13(65.0)	7 (35.0)
Oke-Aro Phase II		6.6907 N 3.3255 E	15	8 (53.3)	7 (46.7)
Oko-Oba Farm	< 5.0km	6.6480N; 3.3184 E	18	12 66.7)	6 (33.3)
Agege Farm		6.6265N; 3.3242 E	14	9 (64.3)	5 (35.7)
Ikorodu Farm I	< 10.0km	6.6685N; 3.34498E	12	8 (66.7)	4 (33.3)
Ikorodu Farm II		6.6663N; 3.4494 E	17	9 (52.9)	8 (47.1)
Pig-Estate Complex I	< 15.0km	6.6064N; 3.6324 E	20	8 (40.0)	12 (60.0)
Pig-Estate Complex II		6.6077 N; 3.6368 E	12	5 (41.7)	7 (48.3)
University Teaching And Research Farm	Above 15.0km	7.4380N; 3.8570 E	10	0 (0.0)	10 (100.0)
Alibode Farm		7.4680N 3.8650 E	12	1 (8.3)	11 (91.7)
Total			150	73(48.7%)	77(51.3)

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Control of Avian Influenza in Syria

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Overview

Avian influenza has become a disease of great importance for animal and human health. Millions of animals have died, and concern is growing over the loss of human lives and management of the pandemic potential. Syria was surrounded by outbreaks in human and animal (Turkey, Iraq, Jordan, Palestine and Egypt), the country still free of the disease and many effective measures were taken to keep it free. In this paper we review the intersectoral control measures undertaken for the prevention of the avian influenza infections in Syria.

Avian influenza (AI), which emerged from the animal reservoir, represents one of the greatest recent concerns for public health. Compared with the number reported for the past 40 years, the number of outbreaks of AI in poultry has increased sharply during the past 5 years. The number of birds involved in AI outbreaks has increased 100-fold, from 23 million from 1959 through 1998 to >200 million from 1999 through 2005 (1). Since the late 1990s, AI infections have assumed a completely different profile in the veterinary and medical scientific communities. Some recent outbreaks have been minor, but other epidemics, such as the Italian 1999–2000, the Dutch 2003, the Canadian 2004, and the ongoing Eurasian, have been more serious. They have led to devastating consequences for the poultry industry, negative repercussions on public opinion, and, in some instances, created major human health issues, including the risk of generating a new pandemic virus for humans through an avian-human link.

Avian influenza virus is a segmented, negative-strand RNA virus that is placed in the family Orthomyxoviridae in genera Influenzavirus A. Influenza A viruses are the only type reported to cause natural infections of birds and are further divided into subtypes according to antigenic characteristics of the surface glycoproteins hemagglutinin (H) and neuraminidase (N). At present, 16 hemagglutinin subtypes (H1–H16) and 9 neuraminidase subtypes (N1–N9) have been identified. All subtypes and most possible combinations have been isolated from avian species.

High Pathogenic Avian Influenza (HPAI) is a lethal infection in certain domestic birds (e.g., chickens and turkeys) and has a variable clinical effect (may or may not cause clinical signs and death) in domestic waterfowl and wild birds. The potential role of wild birds and waterfowl as reservoirs of infection by HPAI strains has been described for only the Asian HPAI virus H5N1. The ecologic and epidemiologic implications of this unprecedented situation are not predictable.

Theories suggest that HPAI viruses emerge from H5 and H7 Low Pathogenic Avian Influenza (LPAI) progenitors by mutation or recombination (2,3), although >1 mechanism is likely. This theory is supported by findings from phylogenetic studies of H7 subtype viruses, which indicate that HPAI viruses do not constitute a separate phylogenetic lineage or lineages but appear to arise from nonpathogenic strains (4,5); this indication is supported by the in vitro selection of mutants virulent for chickens from an avirulent H7 virus (6).

Prevention of Avian Influenza. AI viruses are introduced to domestic poultry primarily through direct or indirect contact with infected birds. Transmission may occur

through movement of infected poultry; movement of contaminated equipment, fomites, or vehicles; and exposure to contaminated infectious organic material. Airborne transmission over long distances between farms has not yet been demonstrated. For these reasons, if biosecurity measures are implemented at the farm level, AI infections can be prevented.

Outbreaks that involve large numbers of animals are characterized by the penetration of infection into the commercial circuit; that is, industrially reared poultry and all other poultry that is traded, including those from semi-intensive and backyard farms. Biosecurity (encompassing bioexclusion and biocontainment) represents the first and most important means of prevention. If biosecurity measures of a high standard are implemented and maintained, they create a firewall against infection penetration and perpetuation in the industrial circuit. However, breaches in biosecurity systems do occur. Good epidemiological surveillance system is the main source of a functioning early warning system, which is an important control tool for AI.

SYRIAN Measures for the prevention and control of Avian Influenza. Since 2005 the ministry of Agriculture established a high committee for the control of the disease, very restricted measures were taken to avoid disease transmission to Syria. In cooperation between the ministries of agriculture, health, local affaires, trade and economy and faculty of veterinary medicine of Al Baath university a very effective information system was established to spread knowledge about the disease. Other measures like movement restrictions of poultry, closing bird markets inside towns and cities, prohibiting unhygienic disposal of dead poulty, prohibiting all kind of hunting and distributing up to date information about the global progress of the disease were undertaken.

The poultry industry in Syria were 80% decreased through this measures, and many breeders were out of this business by such procedures.

Once the WHO declared emergency situation concerning the avian influenza, the Ministry Of Health dedicated all possible resources to prevent the disease entry to Syria.

A governmental committee, chaired by the Minister of Health, was established, and it had his deputies and relevant directors as members.

The objective of this committee was to develop a national plan for Avian Flu surveillance and control, and to follow up the measures taken inside the ministry along with other relevant ministries and sectors as well.

The governors headed subcommittees on governorate levels to follow up the implementation of the national plan.

The MOH has assigned suitable places in public hospitals equipped with trained staff and respirators. It also issued proper instructions regarding the diagnosis and management of cases, and disseminated these instructions to health directorates.

Furthermore, the MOH printed and distributed posters addressing the standard definition of Avian Flu and the definition of the suspected case.

A pile stock of suitable drugs (Amantadine, Remantadine, and Tamiflu) was set up.

The MOH and the MOA (ministry of agriculture) issued the instructions of reporting mechanism regarding human and avian suspected cases, and how to do the follow up on behalf of their directorates.

The instructions concerning lab diagnosis were set up (collect, transport and examine specimen. . . ..).

A guideline manual for suspected case surveillance and investigation was published. It has also the prevention and control procedures concerning human and bird cases.

Health directorates were requested to send daily reports to the ministry. These reports contain the epidemiological situation in human and birds (including zero level case report) and the measures taken by the directorates and other related sectors in this field.

At the same time, seasonal Influenza cases are the subjects of surveillance follow up in public hospitals in Damascus and Damascus Countryside. The Namru-3 lab in Egypt has its role in detecting the prevalent virus type and how to control it. In addition the MOH has issued proper instructions about procedures

that should be implemented on border centers (airports, seaports...) in case of passengers coming from known infected areas.
Many workshops have been conducted in the ministry and the health directorates focusing on disease management, surveillance, epidemiological investigation, and control procedures. The targeted audience was the physicians and health workers in this field.
Messages to raise awareness about the diseases were prepared. They targeted the public (including travelers from and to the country) according to social classes, and contained how to protect the people against the virus.
These messages were disseminated through various media types (TV, radio, posters, brochures, etc...).
MOH has exchanged valuable data with other concerned countries and international organizations, it requested that the relevant ministries and sectors take the following actions:

(1) A ban on bird and their product export from infected areas.
(2) A ban on bird hunting.
(3) Disease surveillance in poultry.
(4) Implementation of preventive measures in poultry breeding places and markets.
(5) A ban on bird trade in inhabited places.
(6) Monitoring of bird migration and water surfaces.

In 2006 WHO mobilized a rapid response mission of team from WHO headquarters, regional and country offices with the support of experts from GOARN and other international agencies to Syria. This mission assessed the capacity of national and local authorities and facilities to detect and respond to potential cases of human avian influenza. They provided a better understanding of the alert and response mechanisms in place including the capacities for national early warning and verification. The mission assessed the local health infrastructure, availability of resources, clinical management and containment measures in place and the capacity of local laboratories to sample, handle and diagnose the H5N1 virus.

FAO regional project on emergency assistance for early detection and prevention of avian influenza in the Middle East region. This project was started in different countries of the region including Syria. The target institute of this project was ministry of agriculture. The project oriented its activities according to the animal human statistics of the region.

The main outputs of the project:

- Baseline data on migratory birds and domestic poultry, mapped for use in targeted surveillance and HPAI control;
- Strengthen disease surveillance and monitoring for HPAI in domestic and migratory birds;
- Documentation of wild bird trade and other movement of wild bird species;
- Strengthen Laboratory capacity to support HPAI diagnosis;
- Improving regional early warning disease intervention, technical information and technology transfer by timely, regional disease information exchange;
- Developing national action plans to form the framework for national HPAI control plans and a regional strategy in line with the FAO/OIE Global Strategy for the progressive control of HPAI.

The project impact is in three fold: improved regional disease information exchange and strengthened HPAI early warning and control measures; national strengthening of the public sector involved in livestock agriculture, natural resources and tourism, to address potential HPAI outbreaks, and locally increased health security and food safety for consumers, and production security for commercial and non-commercial poultry producers.
The European Community Regional Project : ConFluTech - Capacity Building for the Control of Avian Influenza Through Technology Transfer and Training
the partners of this consortium including Syria working to:

(1) organise technical workshops to facilitate technology transfer particularly in the field of molecular diagnostic tools for pathogen detection and differentiation, to reinforce epidemiological analysis for monitoring and modelling of avian influenza especially and to respond to outbreaks of infectious diseases of livestock in general;
(2) provide training through organisation of seminars and short-term courses in well qualified laboratories of a number of EU Member States;
(3) organise technical workshops, courses and training in the INCO target countries to improve the technical experimental level of the staff and laboratories in charge of livestock infectious diseases.

The first workshop of this project has the following objectives:

(1) Present the current situation of Avian Influenza in the region in both Human and animals.
(2) Discuss and improve surveillance and diagnosis local capabilities
(3) Modern technology transfer and training

Main topics discussed in this workshop was:
Aetiology and Global Epidmiology of Avian Influenza in Poultry.
Transmission and Clinical Manifestation of Avian Influenza in Birds.
Transmission and Clinical Manifestation of Avian Influenza in human
Molecular and other diagnostic techniques for avian influenza.
Bio-security in poultry hatcheries
Survey of Avian Influenza in Syria
Control of Emerging outbreaks
Designed studies to evaluate the efficacy and safety of Avian Influenza Vaccines
Strategic National Control Programs of Emerging Epidemics.
Avian Influenza National Control Programs, Preparedness and Response
Evolution of Avian Influenza in Humans - Clinical Review
All this projects and measures were led in very collaborative cross sectoral harmony and evaluated regularly by different local authorities and regional and international agencies.

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Recent Events Relating to H5N1 Highly Pathogenic Avian Influenza in the Hong Kong Special Administration Region of the People's Republic of China (2008-2009)

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Summary

Hong Kong Special Administrative Region of the Peoples' Republic of China (HKSAR) has an intensive active and passive surveillance system for avian influenza. The program covers poultry farms, poultry markets, and dead birds (any species) detected by members of the public or government agencies. HKSAR has also implemented measures to minimize the risk of infection in poultry, including modifications to marketing and farm practices, strict controls on sources of poultry and universal preventive vaccination for all poultry destined for sale in markets in HKSAR.

Since the first recorded HKSAR cases of highly pathogenic avian influenza caused by viruses of the H5N1 subtype (H5N1 HPAI) in poultry and humans in 1997, a range of H5N1 HPAI viruses has been detected through the surveillance program. Yet, despite the intensive surveillance, no H5N1 HPAI viruses were found in live poultry markets from December 2003 until June 2008 or in farms from early 2003 until December 2008. Dead bird markets from December 2003 until June 2008 or in farms from early 2003 until December 2008. Dead bird surveillance conducted during this period has resulted in the detection of H5N1 HPAI viruses, predominantly Clade 2.3.2 and Clade 2.3.4, in a range of species, with most cases detected in the cooler months of the year. In 2008, all but one of the viruses from the dead bird program belonged to Clade 2.3.2; the exception was a novel Clade 2.3.4 virus from a dead peregrine falcon in March 2008. In June 2008, the active surveillance program in retail markets (faecal sample swabs from cages) detected a Clade 2.3.4 virus, similar to the falcon isolate, in several retail markets stalls.

The viruses from different stalls scattered across HKSAR were essentially identical suggesting a common source; tracing studies did not determine how the virus got in to the retail markets. In December 2008 an outbreak of H5N1 HPAI occurred on single local poultry farm. Investigation of the farm revealed a number of potential routes of introduction, but the actual source of infection was not confirmed. The Clade 2.3.4 virus isolated shared a common ancestry with, but was not derived from, the June 2008 poultry market viruses and the March 2008 peregrine falcon virus. Both poultry viruses (June and December 2008) were antigenically dissimilar to earlier Clade 2.3.4 isolates, reacting poorly with antisera from poultry vaccinated with vaccines currently used in the field. From January to March 2009 dad domestic chicken, geese and ducks were detected on beaches in western Hong Kong (January) and at Tung Ping Chau (March) an island some 12 km east of mainland Hong Kong. All of the viruses from dead poultry in January were Clade 2.3.2 viruses. The virus isolated from a dead chicken at Tung Ping Chau was a Clade 2.3.4 virus closely related genetically to those found in poultry in Hong Kong in 2008.

The findings demonstrate that Clade 2.3.2 and Clade 2.3.4 viruses, including potentially significant antigenic variants, are circulating in the broader region and that there is an on-going threat of infection in HKSAR.

Chapter 25

Poster

Integrated monitoring system as an instrument to combat zoonotic agents in poultry flocks

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Introduction

Quality assurance and quality control processes belong to the standard daily procedure in many sectors of industry. In the various stages of the food chain these processes, in the form of HACCP, QS systems and ISO 9001, are varyingly developed and widespread. Due to the inclusion of primary production the transposition of the EU food hygiene regulation into national law will, for the first time, lay down uniform rules with regard to hygiene requirements in all elements of the food chain. A high level of personal responsibility will thus be transferred to the primary producers. This will not only lead to established monitoring procedures in food manufacturing and processing being tested for their suitability in primary production, but also to the need for new risk analyses and risk evaluations being developed. As a result of the zoonoses regulation and its implementation in poultry stocks, monitoring- and risk evaluation are becoming increasingly significant and can thus make a contribution towards minimising risk and combating zoonoses.

Material and Methods

Extensive observation and monitoring measures have been developed to record the sources of entry and distribution channels of zoonotic agents and have further been checked in field work for their suitability as instruments for the sanitation of broiler flocks.
The following measures have been incorporated:
1. Formulation of a questionnaire to record the business specifications
This questionnaire includes 15 complexes on external and internal risks for the entry and spreading of infectious agents, in particular zoonotic pathogens, in poultry flocks. The questionnaire is to be answered together with the primary producers and thus provides an insight into the sequence of operations and processes which are in operation on the farms either continually or problem-oriented
2. Hygiene analysis and quantitative evaluation of the hygiene status
The hygiene analysis complements the questionnaire by providing a quantitative evaluation of the hygiene status in poultry flocks. In 7 complexes a total of 50 indicators which correspond to the critical control points of the HACCP concept are evaluated. The indicators are quantitatively evaluated (evaluation 0, 3, 5) in the companies as planned (periodic, e.g. 1x per annum), or not planned (in case of problematic situations such as evidence of zoonotic pathogens or increased used of antibiotics as a result of increased morbidity or mortality) and then multiplied by the weighting factor (1, 2, 3). The resulting score enables the hygiene status (very good, good, poor, very poor) to be objectively estimated and evaluated.
3. Control and evaluation of the cleaning and disinfection measures
In addition to the process analysis of the cleaning and disinfection measures in poultry

flocks, efficiency control constitutes a significant part of an integrative monitoring system. This involves surface swab samples being taken at critical points and analysed for total germ count and indicator germ groups. Using these quantitative microbiological analyses weak spots in the processes become clear.

4. Monitoring and evaluation of the rodent and insect control

The detailed analysis and evaluation of the occurrence of rodents constitutes an important part of an integrative monitoring system. To achieve this, the risk-oriented positioning of the bait boxes is defined and controlled regularly. The use of suitable rodenticides and exact documentation of the amounts used enable the risk of entry of infectious agents via rodents to be monitored and make a problem-oriented control possible.

5. Targeted, systematic controls for zoonotic pathogens

Systematic controls for zoonotic pathogens in connection with the sanitation of poultry flocks also constitute a significant part of an integrative monitoring system. These controls are carried out both on the animals (e.g. faecal samples) and in the environment both during the service period as well as continuously during the production process. Thus, the sources of entry and reservoirs can be identified and subsequently target control measures can be introduced. This concept is currently established for salmonella, whereby it is based strongly on the methods of analysis as per EU legislation.

6. Analysis of performance data, losses, animal health and outlay for veterinary drugs

Results

The broiler farms evaluated so far had a hygiene status rated as good.

In some cases it was also possible to demonstrate a connection between an improved hygiene status and higher performance whilst using less drugs on broiler farms. (table 2)

The complexity of the systems and their constant use are necessary to achieve a fast and safe sanitation of the flocks and to protect the flocks on a sustained basis.

Table 1: Hygiene status using the example of various farms

	Farm H	Farm DK	Farm DL	Farm GR	Farm K
Complex 1	56	63	63	47	43
Complex 2	63	80	80	69	81
Complex 3	99	94	73	100	85
Part I	76	81	72	75	73
Complex 4	73	75	75	65	64
Complex 5	90	95	83	87	84
Complex 6	100	91	91	100	82
Part II	89	90	83	86	79
Part III Complex 7	91	100	100	91	83
Farm	83	86	79	81	77
Hygiene status	good	good	good	good	good

Discussion and Conclusion

On sanitising flocks shortcomings in personnel hygiene, deficits in feed and drinking water hygiene, lack of success with the cleaning and disinfection of the poultry houses and deficiencies in rodent control were identified as being the key aspects. As a result Salmonella was particularly able to establish itself in broiler

Table 2: Example of performance parameters depending on hygiene measures

Parameter	Unit	Farm G		Farm A	
		Prior to sanitation measures	After sanitation measures	Prior to sanitation measures	After sanitation measures
Total losses	% of animals placed	5,15	3,84	4,78	2,47
Of which losses in week 1	% of losses	42,94	33,55	35,98	31,78
Daily weight increase	g/fattening day	60,05	58,00	60,05	60,83
Veterinary costs	€ Cent/animal	5,46	3,18	7,32	3,36

farms. By implementing targeted and appropriate cleaning and disinfection measures as well as a controlled consistent rodent control it was possible to eliminate reservoirs for infectious and zoonotic pathogens.

The monitoring system which has been further developed and extended is suitable with its individual components for the evaluation of the risk of spreading zoonotic agents, for the recognition of paths of infection, and for the derivation of appropriate measures. Success was achieved in improving the hygiene status, sanitising the flocks and minimising the risk of re-entry. Farms rated as having a poor hygiene status prior to sanitation could be rated as good afterwards. Even after sanitation there are still reserves with regard to the constant implementation of hygiene measures on the farm and shielding the farm from outside.

The observation and monitoring methods developed will be used and optimised in field work in further production sectors. The aim is to establish the system beyond sanitation measures as an inherent part of risk minimisation, and thus make a contribution not only towards animal health and performance but also to consumer protection. The complex and consolidating system of risk analysis and evaluation represents a new instrument, along the lines of the HACCP concept, for the monitoring of broiler farms. For the evaluation of the risk of introduction of zoonotic pathogens into broiler farms the quantitative evaluation of the hygiene status as a preventative instrument in particular shall be further developed.

References

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Airborne moulds, dust and endotoxins in four alternative housing systems for laying hens

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Summary

Airborne moulds and endotoxins as well as inhalable dusts were monthly measured during the course of one year using impingement (AGI 30) and filtration-elution-method in four different housing systems: a floor keeping system, a usual aviary, a floor keeping system with outdoor-access (free range) and the german 'Kleingruppenhaltung' (a small group system). Highest concentrations of endotoxins were regularly found in the floor keeping system (about 9000 EU/m^3), followed by the aviary, free range and 'Kleingruppenhaltung' (about 2000 EU/m^3). The concentrations of inhalable dusts ranged from below 1mg/m^3 to 9 mg/m^3 with the highest levels in the systems with integrated litter space. The impact of the housing type on the concentration of airborne moulds seems lower than the seasonal influence. The results indicate an urgent need for improving the work environment of farm workers e.g. by breathing masks in order to prevent negative health effects.

Introduction

Airborne particles like mould spores, dust and endotoxins in and from the animal husbandry are associated with a multiplicity of health effects on the human and animal organism. Comparatively high concentrations can be found in the poultry keeping units, especially in broiler barns [4, 5]. With the move to alternative housing systems for laying hens in the EU (1999/74/EC), in order to improve animal welfare, new systems have been introduced which allow the hens more free movement than in the former battery cages. However, little is known about the air pollution in these systems and the possible health effects on the people working in these atmospheres. For this reason a study, funded by the German Ministry of Food, Agriculture and Consumer Protection (BMELV) through the German Federal Agency for Agriculture and Food (BLE), was carried out in four different alternative keeping systems for laying hens to learn more about the concentrations of airborne bacteria in these environments.

Material and Methods

The collection of mould spores was conducted by impingement (All-Glass-Impinger type AGI 30). This method is known to show the highest yield of micro-organisms in stables under regular conditions [1, 2]. For each measurement there were six samples collected in three turns with two impingers running parallel. The impingers were charged with phosphate buffer (after Soerensen) to avoid a change in pH-value due to a drag-in of ammonia. Collecting of air samples took place in 150 cm height in the centre of the barn and middle of the daily maintenance patrol.

During sampling impingers were kept in temperated and isolated aluminium tubes to allow for constant sampling conditions over the year. After sampling the impingers were stored and transported to the laboratory at 4 °C. Estimation of airborne moulds was done by aerobic plate count method (APC) on DG18-Agar (Oxoid, Wesel, Germany). Colony forming units (CFU) were counted after incubation at 25 °C and 36 °C for 72 hours. Dust was collected on glass fibre filter in I.O.M. filter heads on SKC-pumps with an airflow of 2.5 l/min. The amount of airborne total inhalable dust was estimated by calculating the weight gain of the filter. After weighing the glass fibre filter were further processed to assess the concentrations of airborne endotoxins by LAL-Test (Kinetic QCL, BioWhittaker, East Rutherford, USA).

Results and Discussion

Figure 1 shows the concentration of airborne moulds during the course of the year. There is a clear seasonal course with highest concentrations in the late summer and autumn month for all investigated housing systems. Mould spore concentrations in ambient air show a similar course during the year [3], indicating that introduced moulds from outside have a great effect on the burden of airborne mould spores in the air of the housing systems. The highest concentrations of mould spores can be found for the floor keeping system with outdoor access presumably due to a higher exchange with the ambient air and dragged-in mould-bearing floor coverings. Lowest values were found for the german 'Kleingruppenhaltung'.

This is the same for the airborne inhalable dust (figure 2). Whereas the values of dust concentrations exceed the threshold limit value of 4 mg/m^3 (MAC, maximum allowable concentration) in the air of the aviary and the floor keeping system regularly - especially in the winter months when air exchange is lowered to reduce heat losses -, the dust concentrations in the air of the 'Kleingruppenhaltung' do not exceed 2 mg/m^3.

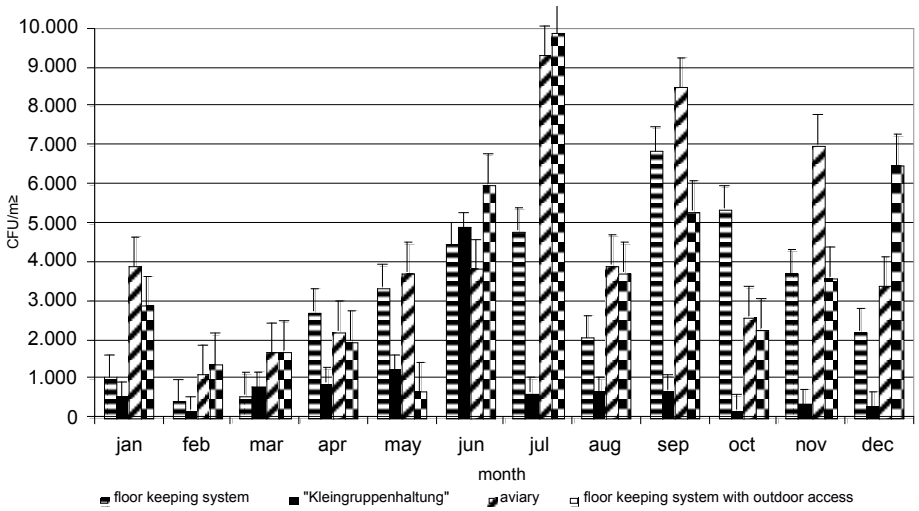


Figure 1: The mean concentrations (n=18) of airborne moulds in the air of four different housing systems for laying hens during the course of one year

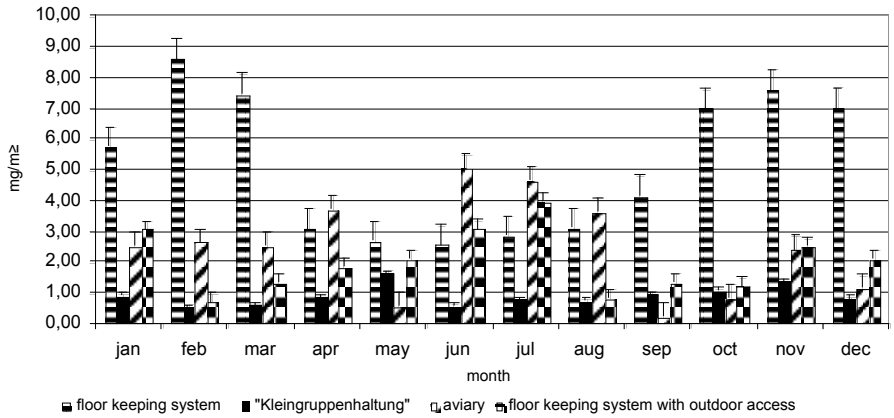


Figure 2: The mean concentrations (n=3) of airborne inhalable dust in the air of four different housing systems for laying hens during the course of one year

Ventilation dependent concentrations can also be found for the airborne endotoxins (figure 3). Again the highest values can be found for the floor keeping system, followed by the floor keeping system with outdoor access and the aviary. Lowest concentrations of airborne endotoxins among the four housing systems for laying hens were detected in the 'Kleingruppenhaltung'. In contrast to the three other housing systems the concentrations of endotoxins in the air of 'Kleingruppenhaltung' show an adverse seasonal course with slightly higher values in the 'warm' (summer) month whereas aviary, floor keeping system and floor keeping system with outdoor access have a higher burden of airborne endotoxins in the 'cold' (winter) month. This is again - as for inhalable dusts – presumably due to a reduced ventilation in cold weather.

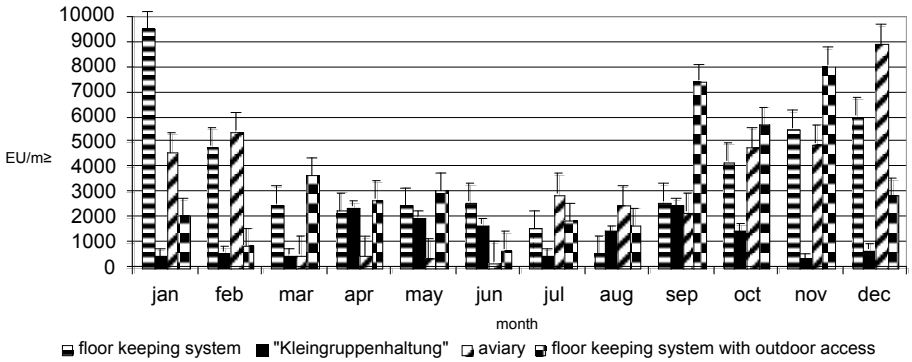


Figure 3: The mean concentrations (n=3) of airborne endotoxins (EU= Endotoxin Units) in the air of four different housing systems for laying hens during the course of one year

Conclusion

Endotoxin respectively dust concentrations in the air of alternative housing systems for laying hens exceed the natural concentrations in ambient air by a factor of up to 100 respectively 10. There is a strong influence of housing type and season on endotoxin and dust particle counts. The results indicate an urgent need to protect the respiratory health of farm workers also in

alternative laying hen houses e.g. by breathing masks in order to prevent negative health effects.

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The annual dynamic in new giardiasis cases at children with ages between 1 and 14 years

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Summary

In Romania, the incidence of giardiasis in children, varies in respect with the origin of the candidates, those coming from families having an incidence of 1.5-21%, whereas those who would come from a colectivity will have a more increased percentage, of 78%.
The establishment of the annual giardiosis new cases dynamic was performed using the data base provided by the National Center of Organisation and Insurance of the Informatic and Informational System, of the Health Department from Bucharest,Rromania. The data base represents the number of the new giardiosis cases found each year, between the year 2000-2005, in children 1 up to 14 of age , regarded as the population with maximal responsiveness.
2004 is the year of maximum incidence of giardiosis, when 3427 new cases/100000 capita were registered, meaning 3,42%of the population was affected. At the beginning of the study, we find that 2.38% was the lowest incidence registered in the beginning of the study, from the year 2000. Processing statistical data shows that there is no significant variation regarding the incidence value during the study. For this reason, during the 6years of study, only 2-3% of the population at risk maximum, showed symptoms characteristic to giardiosis.

Introduction

Giardiosis is a digestive disease with both occurance in man and animals, mainly found in young candidates, with tipical findings such as diarrhea [2]. The man is considered the main host and reservoir of parasites. Latelly it has been proven that the infestation can come from the animals as well, the man being the direct provider. This fact proves once again that giardiasis is not host specific, the animals representing as well possible sources of infestation for human beings, especially whithin the teritories know to have animals at large (dogs, cats, bovines) among population. Referring to the more developed countries, pets can have the same role as previously mentioned [1].
Both transmissibility and patogenity of giardiosis along with the rather difficult determination of species found within the feces collected from the ground, determination based on the morphological characters of the chists,represent an important aspect of the giardiosi's epidemiology.
Regarding the international impact of the disease, both the incidence and the reoccurence of giardiosis are very challenging aspects, wich mainly depend on the nominalization of the disease among the reported ones.Thus, France declaires a 1% incidence for giardiosis in human. Denmark, even though it's presenting itself as having the giardiosis among the OIE comunicable diseases, list B, has no records of the incidence or occurance in humans, only mentioning a few cases, imported accidently from abroad [6]. Germany includes the presence of giardiosis in it's legal sistem, as being part of the O.I.E. B list, with indications of periods of constant monitoring of the prelevance of the disease, both year 2004, and 2005, with only few incidence variations, depending on the geographic areas that were investigated

[3]. The maximum of receptivity was shown in children 1-4 years of age. Italy introduces whithin the B list only the agent of the disease *Giardia sp.*, but not the disease itself, making sure though that notes of the human and animal incidence of the disease are made every year. The prevalence varies with the age.

Material and Methods

Establishing the giardiosis new cases annual dinamic in human population was based on the informations provided by the National Center of Organisation and Insurance of the Informatic and Informational System, of the Health Department from Bucharest,Rromania.
The on trial material was represented by the annual number of new giardiosis cases for a period of 6 years (200-2005) from the population with the largest receptivity (children - 1-14 years of age). The interpretation of the results was based only on one epidemiological indicator - their incidence. Reporting the new shown giardiosis cases is made nationally by the personal practitioner's clinics.
The working method is actually the epidemiological survey wich was obtained through a retrospective analysis using analytical investigations (statistical processing of obtained data) from the quantitative operational surveys, such as the epidemiological modeling (modern method of epidemiological investigation, performed on the mathematical representation of the zoonoses's characteristic) and their dinamical profile. It is realised by computerized specifical programms, allowing also the biological stimulation of the pathological conditions, by experimenting on lab subjects.

Results

The data base has been panneled according to the on use method of evaluation, for which case an annual pannel was made for each year from 2000-2005, regarding the same target of high risk population (children 1-14 og age). The national distribution of giardiosis cases shown to appear every year in children between 1-14 of age. According pannel number one, we can point out the percentual incidence of every year's bases experiment (Fig. 1, Tab. 1).

Table 1: The national distribution of giardiosis cases shown to appear every year in children between 1-14 of age.

Year	*No.new cases/100.000 habitans (children 1-14 years)*
2000	*2385 (2.38%)*
2001	*2979 (2.97%)*
2002	*2913 (2.91%)*
2003	*2765 (2.76%)*
2004	*3427 (3.42%)*
2005	*3195 (3.19%)*

Discussion

Evaluating the graphic, no obviouse variation of the incidence of the giardiosis cases was noticed, therefore, during the 6 years of trial, only 2-3% of the population showed clinical signs of infestation based on which, a diagnostic was made and comunicatios regarding the national registering of giardiosis which were nationally comunicated.
The highest year on giardiosis incidence was 2004, (about 3427 new cases/100.000 subjects/ 3.42% out of the affected population). The lowest incidence was found at the beginning of the survey.
The already presented informations from the graphic no.1, are offering only the disease percentage(actual frequency shown to affect children and due to the low ascending or descending variations (due to the very similar values of the annual variation), it was difficult to tell

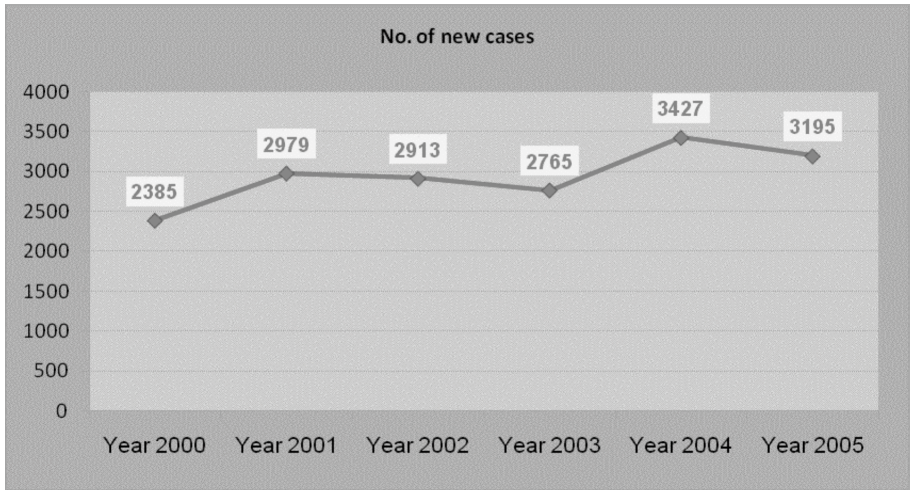


Figure 1. The annual distribution of the new case appeared , in children between 1-14 of age

whether the actual tendency is either to decrease the risk of disease among population with high risk receptivity, or if the profilactic measures are well applied.

Also, there are limitations to the epidemiological survey for it studies one part of population known to have indeed the highest risk of disease , but there can be variations of the incidence value, if the age sector is limited and if it's possible to evaluate the epidemiological situation of the zoonoses in children, in relation to the environment itself (rural/urban environment), and if they are coming from families/collectivitys.

Due to a missing major variation of the incidence, in a large period of time, we can conclude that giardiosis is a zoonoses , epidemiologicaly stable, especially that the incidence values are not very big, therefore the risk of disease among human is very low.

Conclusion

1. The prevalence and the incidence of giardiosis varies in relation to the age and environment of provenience of the children (urban/rural), family ground (family/institution), cultural and development aspect of the country.

In Romania the high risk population is represented by the children between 1-14 years of age after the 6 year epidemiological survey, we seem to find ourselves a relatively constant evolution of the zoonoses. During the 6 year study, all incidence values were very close related.

2. In the beginning of the investigation period, giardiosis incidence had the lowest values, other variations such as increased or decreased episodes being noticed along the survery course. With a maximum in the year 2004.

3. Mentaining a high value of the giardiosis's incidence in our country, comparing to other european states is up to a certain point understandable, if considering the low financial possibilities of the countr, the limited cultural/educational development, the increasing number of institutionalized children, the high risk of contamination through the contaminated water in the absence of an efficient and accessible filtration water system for the entire romanian population.

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Active surveillance for leptospirosis. Research for antibodies in bovines, equines, canines and humans in a Brazilian diagnostic routine from 2004 a 2007

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Summary

Leptospirosis is an important zoonosis to humans and several animals' species. The aim of this study was to evaluate the frequency of Leptospira spp. antibodies and the most prevalent serovars in blood samples from different domestic animal species and humans by microscopic agglutination test (MAT) at the Zoonosis Diagnosis Service, Botucatu, SP, Brazil, from 2004 to 2007. 1221/3605 (33.9%) of all samples reacted. Most of them (769/2037; 37.8%) were from bovine of Botucatu region farms, SP, Brazil. Strong statistical correlation was observed only between the years 2004 and 2007 (r=0.97; CI95%=0.62-1.00; P<0.05) that shows similar profile of reagents samples, when considered all the year, independently of the animal species. This result shows the importance to adopt reference serological tests, mainly in labs that make serological diagnosis in routine. In this way, the study emphasizes the serological surveillance and the diagnosis of leptospirosis in herbivorous, dogs and humans, mainly based on the epidemiological profile of a studied region, contributing to the public health and controlling this important zoonosis.

Introduction

Leptospirosis is a worldwide anthropozoonosis with an emerging and reemerging profile [10]. It presents high epidemiological importance in rural and urban areas of tropical and subtropical countries [3, 7]. The etiological agent is a bacteria Leptospira spp. [5].

Many domestic and wild species are susceptible to leptospirosis, some of them becoming reservoirs, shedding the bacteria in the environment by urine. The diagnosis may be carried out by clinical signs, and confirmed by laboratorial tests, as detection of antibodies for each serovar by microscopic agglutination test (MAT), the gold-standard test for leptospirosis diagnosis in animals and humans [11, 4]. Thus, this study aimed to evaluate the frequency of Leptospira spp. antibodies and the most prevalent serovars in bovines, equines, canines and humans from Southeast Region from Brazil during 2004 to 2007.

Material and Methods

3065 sera samples (850 canines, 2037 bovines, 642 equines and 71 humans) received at the routine of Zoonosis Diagnosis Service (ZDS), College of Veterinary Medicine and Animal Science, UNESP, Campus of Botucatu, SP, Brazil, were researched for antibodies to 27 different Leptospira spp. serovars by microscopic agglutination test (MAT). Sera were initially diluted at 1:100. The serum was considered reagent when at least 50% of leptospires agglutinated. The final titer was obtained by

two-fold serial dilution until the last reagent dilution.

The frequency of antibodies was determined by year and from each species. All frequencies, percentages and confidence interval 95% was calculated by hand. Pearson correlation coefficient of each two years was calculated adopting the significance level ($P<0.05$) with BioEstat 5.0 program.

Results and Discussion

1221/3605 (33.9%) of all samples reacted. Table 1 shows the distribution of all samples by year, specie and number of reagent samples. It can be observed a reduction of canine and bovine assayed samples until 2007. A peak of reagent samples was observed to canines in 2007 (41/150; 27.3%), bovines and human beings in 2006 (252-518; 48.6% and 6/15; 40.0%, respectively) and equines in 2005 (111/231; 48.0%). Dog samples reacted most frequently to Canicola and Copenhageni serovars, while bovines to Hardjo, Hardjo-CTG and Hardjo Prajitinum, equines to Copenhageni and human beings to Patoc and Grippothyphosa.

Tab. 1: Frequency of Leptospira spp. antibodies per year and species assayed in Zoonosis Diagnosis Service, FMVZ, UNESP, Brazil.

Species	2004			2005			2006			2007		
	R[a]/N[b]	%[c]	CI95%[d]	R/N	%	CI95%	R/N	%	CI95%	R/N	%	CI95%
Canine	29/266	10.9	7.7-15.2	57/302	1.9	14.9-23.7	25/137	18.2	12.7-25.6	41/150	27.3	20.8-35.0
Bovine	208/722	28.8	25.6-32.2	246/633	38.9	35.1-42.7	252/518	48.6	44.4-53.0	63/164	38.4	31.3-46.1
Equine	75/214	35.0	29.0-41.7	111/231	48.0	41.7-54.5	15/39	38.5	24.9-54.2	84/158	53.2	45.4-60.8
Human	3/15	20.0	7.3-45.6	3/28	10.7	3.9-27.4	6/15	40.0	19.8-64.6	3/13	23.1	8.4-50.8
Total	315/1217	25.9	23.5-28.4	417/1194	35.0	32.3-37.7	298/709	42.0	38.4-45.7	191/485	39.4	35.1-43.8

[a] R: reagents; [b] N: total sample assayed; [c] %: percentage; [d] CI95%: confidence interval 95%

The most samples assayed in this study were from bovines (2037/3605; 56.5%) and 769/2037 (37.8%) were considered reagent, what is higher than 166/1253 (13.25%) and the most prevalent serovars were Icterohaemorrhagiae and Pomona found in Londrina/PR, Brazil [9], what is different to the present study. 82/138 (59.4%) of reagent bovines was found in Mossoró/RN, Brazil with the most prevalent serovars Hardjo and Wolfi [2], a higher prevalence and different to this study (Figure 1).

285/642 (17.8%) of equine samples were seropositive to Leptospira spp. antibodies in the present study, what is lesser than 82/182 (45.0%) found in Goiânia/GO, Brazil, that found Icterohaemorrhagiae and Pomona as the most prevalent serovars [6].

152/855 (17.8%) of canines samples were seropositive to Leptospira spp. antibodies in the present study, what is similar [8] to 119/775 (15.4%) in dogs from Botucatu/SP, Brazil. The most prevalent serovars were Canicola e Pyrogenes, what partially corroborates with found in the present study.

15/71 (21.1%) of human beings samples assayed were seropositive, which is of extremely importance in public health due to the early diagnose can enhance the chance of the treatment success.

Strong statistical correlation was observed only between the years 2005 and 2007 ($r=0.97$; CI95%=0.62-1.00; $P<0.05$) that shows similar profile of reagents samples, when considered all the year, independently of the species samples. This results shows the importance to adopt reference serological tests, mainly in labs that

makes serological diagnosis in routine.
Thus, the study emphasizes the importance of the serological surveillance and the diagnosis of leptospirosis in herbivorous, dogs and humans, mainly based on the epidemiological profile of a studied region, contributing to the public health and controlling this important zoonosis.

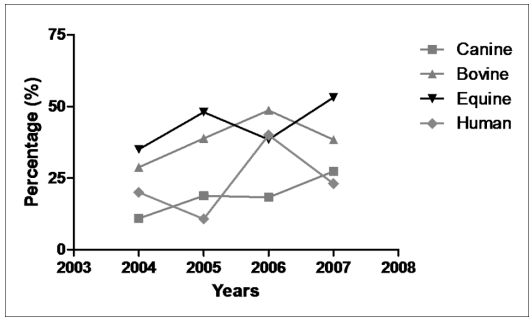


Fig. 1: Serological profile of Leptospira spp. antibodies per species since 2004 to 2007 assayed in Zoonosis Diagnosis Service, FMVZ, UNESP, Brazil.

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Morphological study and external characters analysis of Old World screw worm fly *Chrysomyia bezziana* Villeneuve 1914 in south western Iran

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Summary

Geographical races of *C. bezziana* producing Cutaenous myiasis were studied in Khoozestan province in 2006.Totally 986 specimens were examined and three geographical races of screw-worm fly were identified on based of morphological characters by using diagnosis key of Spradbery,1991.

Introduction

The old world screw - worm fly (SWF), *Chrysomyia bezziana* Villeneuve is warm-blood animals obligate myiasis parasite including human and livestock. Maggots of flies caus economic losses in livestock production in some countries where the fly occur. This species has public health hazards in man .Infestiation of human by larvae of *Chrysomyia bezziana* was reported by Djalayer et al in 1978. *C. bezziana* distributed in most tropical and subtropical areaes of Africa, Indian subcontinent, south-east Asia and New Guinea (Norris & Murray 1964, Sutherst et al 1989). The old Screw-worm fly also occurs is some countries like Iran (Navidpour et al, 1996), Kuwait, Bahrain and Qatar (Rajapaska and Spradbery, 1989). Spradbery (1991) proposed three geographical races of the old world screw-worm (Africa, Arabian and South-East Asian) in regard to anatomical difference between population of *C. bezziana* from different parts of its geographical range. There is no report about geographical races of *C. bezziana* in Iran. The present paper is the first research related to geographical races of *C. bezziana* population on based of morphological studies in south-west of Iran.

Material and Methods

The present study concentrates on adult flies that reared in the Entomology Research Center of Razi Research Institute (Ahvaz- Iran). The larvae of *Chrysomyia* collected from infested animals (Mostly sheep) and were incubated under suitable substratum in the Entomology laboratory.

In this study totally 986 specimens (656 Females and 330 males) were examined. All studies were carried out under a stereomicroscope (X60 magnification).We used some morphological characters for identifying distinctive geographical races of old world screw worm as Spradbery used them in 1991.

Results

Morphological analysis were made on adults using all external characters that had been identified previously as being of value in separating geographical races of Old World screw worm(Spradbery, 1991) . The three morphological races proposed by Colless (in Spradbery, 1991) were based on samples from just three countries, i.e. PNG (=South east Asia), The United Arab Emirates (UAE) (=Arabian) and Zimbabwe (African). Morphological characters of adult flies (from different parts of Khoozestan province, South western Iran) especially wings , frontal setulae and hairs of thorax showed that ; wing base is completely clear, soft hairs of the thorax (pleura) are pale, lower (posterior) squama is brilliantly white, with long white hairs, a few of setulae below vibrissa are black and finaly genal groove below compound eye not heavily indented. These results clearly separated the Iranian flies from PNG and African races and the same with Arabian race characters ,were explained by Spradbery , 1991.

Discussion

Morphological studies of geographical races in *Chrysomyia bezziana* are useful for epidemiological aspects of infestation.

Based on morphological studies, it has been proposed that there are three geographical races of the old world screw-worm Fly: Arabian, African and south - east Asian races (Colless in Spradbery, 1991).

Hall and others (2001) used both the morphological and molecular analyses for demonstrating regional differences in populations of old world screw - worm. Morphological analysis suggested that African and Most Asian populations were more similar to each other than Papua New Guinea (PNG) populations. Nevertheless, the Molecular evidence indicated that the Asian race was more closing related to those of PNG. However, both analyses described that the PNG populations were distinctive.

Besides, some studies that did on morphology by Colless in Spradbery, (1991), pervious investigations to differentiate population of *Chrysomyia bezziana* have used cuticular hydrocarbons (Brown et. al. 1992), cytology (Bedo, 1991) and enzyme electrophoresis (Strong & Mahon, 1991).

Navidpour et al, 1996, reported the first out break of myiasis caused by *Chrysomyia bezziana* from southern and south - western of Iran. Before this report there are only a few sporadic record of infestation in human (Djalayer, 1976) and animals south of Iran.

The present study demonstrates that the outbreak of*Chrysomyia bezziana*in Iran related to populations that originated from Arab countries of the Persian Gulf like United Arab Emirates; because morphological characters of Iranian population flies were more similar to Arabian races reported by Hall et al, 2001.

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Discrimination of *Campylobacter jejuni* strains by rep-PCR method in comparison with AFLP analysis

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Summary

This study was undertaken to compare the ability of AFLP and $(GTG)_5$- PCR methods for genotyping of *C. jejuni*. In total of 54 strains were tested by the two methods.
Despite the different grouping of *C. jejuni* strains, the discrimination ability of the techniques was comparable. In total 36 genotypes out of 54 strains of *C. jejuni* were generated by AFLP genotyping and 34 genotypes by rep- PCR genotyping.
Rep- PCR method showed good discriminatory power in terms of strain differentiation, therefore we suppose that rep- PCR method can be used as fast and cheap method and is sufficient for genotyping of *C. jejuni*.

Introduction

Campylobacter spp. are widespread bacteria in the environment and gastrointestinal tract of various species of birds and animals, and it is the leading cause of bacterial food borne diarrhoeal diseases throughout the world. The ingestion and handling of contaminated poultry meat is supposed to be major infection route for humans [2].
Due to high genetic diversity of *C. jejuni* it is very important to use methods with high discriminatory power particulary for epidemiological studies. The most common methods (AFLP, PFGE, MLST) applied for genotyping of *C. jejuni* are expensive and time consuming. In addition some routine laboratories could lack equipment needed to operate these methods. We suggest that the one of the cheap, simple and fast $(GTG)_5$- PCR typing method previously [4] applied for typing of *Campylobacter concisus* may also be suitable for typing of *C. jejuni* strains. The polytrinucleotide $(GTG)_5$ present in multiple copies in the genomes of most Gram-negative and several Gram-positive bacteria. Such sequence can be included in the category of interspersed repetitive sequences [3].
The aim of this study was to evaluate the ability of rep- PCR method to discriminate *C. jejuni* strains in comparison to the detected relationships of the same strains by AFLP method.

Material and Methods

In total 54 *C. jejuni* strains representing seven broiler farms sampled in period of 2007-2008 were randomly selected for this study. Strains were stored at – 80°C in freezing broth. Before testing they were grown on blood agar base at 37°C for 48 h microaerophilically. Subsequently DNAs were extracted using GenElute^{TM} Bacterial Genomic DNA Kit (Sigma- Aldrich). Extracted DNA was stored at -20°C until further analysis.

The AFLP analysis was performed as described by Bojesen et.al. (2003) [1], using the restriction enzymes *Bgl*II and *Bsp*DI The final products were electrophorised on a denaturing polyacrylamide gels on an ABI PRISM^{TM} 377 DNA automated sequencer (Applied Biosystems, Foster City, CA, USA) and the internal standard GeneScan-Rox 500 (Applied Biosystems) was applied for normalization. The electropherograms generated were compared using GeneScan 3.1 software (PE Biosystems).

Rep- PCR analysis was performed by procedure described by Matsheka et al, (2005) [4] with slight modifications. After PCR amplification using the GTG_5 primer, PCR products were separated by electrophoresis in 1.3% containing 0,5μl/ml of ethidium bromide solution and the gel was visualised on an UV board. The GeneRuler^{TM} Express DNA Ladder (MBI Fermentas) was used as the molecular size marker.

The relatedness between the strains for both methods was analyzed with GelCompar II program using the Dice similarity coefficient and clustering by UPGMA. Band position tolerance was set to 0.5%, optimization coefficient to 0.8% for AFLP and 2.0% and 1.5%, for rep-PCR respectively. The cut- off value for both of methods was set to 90%.

The reproducibility of AFLP and rep- PCR band patterns was investigated by additional testing of 10% *C. jejuni* strains examined in this study.

Results

Overall 36 distinct genotypes were generated by AFLP method after genotyping of 54 strains of *C. jejuni*. Thirty strains of all tested were clustered as different AFLP genotypes. Six clusters containing more then one strain with identical band patterns were created. The largest cluster representing type A31 included twelve strains, the cluster representing type A37- four strains of *C. jejuni*. Clusters representing types A10, A37, A38 and A47 were composed of two strains each.

Rep- PCR typing of the 54 strains yielded 34 distinct genotypes. Twenty four strains out of 54 analyzed had different rep- PCR profile each and were represented by only one strain. Remaining 30 strains were grouped into ten clusters having more then one strain. Each of clusters IV and X contained five strains, cluster III- four strains, clusters VII and IX- three strains each. Clusters I, II, V, VI and VIII included two strains of *C. jejuni* respectively (Fig. 1).

Discussion

The ability of $(GTG)_5$- PCR method to discriminate strains of *C. jejuni* was compared with the ability to type the same strains by AFLP method. Twenty out of thirty *C. jejuni* strains representing different AFLP types were also discriminated as different types after genotyping by rep- PCR method. Remaining ten strains were included into clusters created after rep- PCR typing.

The strains 98 and 1237 were grouped together by rep- PCR method, while AFLP typing showed only about 67% similarity between these strains. Nine strains of *C. jejuni* representing nine different AFLP types were included into three clusters (II, III and VIII) after typing by rep- PCR method.

Rep- PCR method clustered together the strains 370, 369 and 380 (cluster VII), whereas AFLP method strains 369 and 380 clustered together and strain 370 was grouped as distinct genotype.

Twelve strains of *C. jejuni* representing AFLP type A31 were subdivided by rep- PCR method. Five of those strains were included in cluster X, three strains – in cluster IX, one strain felt into the same cluster (VI) with strain 364. The AFLP method strain 364 grouped together with strain 372.

Five strains included in the cluster IV after

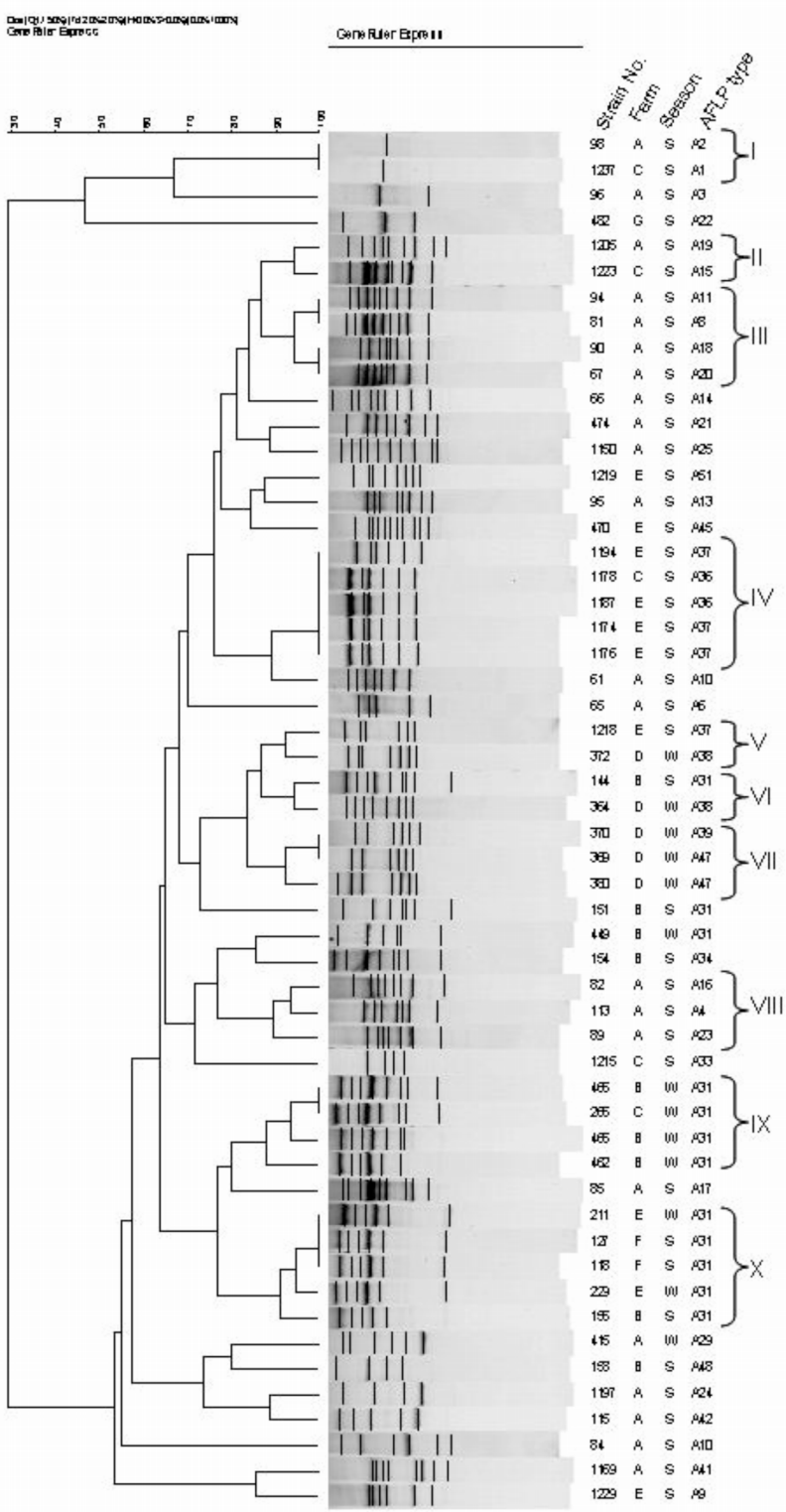


Fig. 1: Cluster analysis of 54 strains of C. jejuni genotyped by rep- PCR method. Clusters from I to X C. jejuni strains with identical band patterns.

typing by rep- PCR method were differently grouped when they were genotyped by AFLP method. After AFLP analysis 2 out of five strains formed AFLP type A36 and 3 together with the strain 1218 formed type A37. After rep- PCR analysis the strain 1218 was clustered together with strain 372 (cluster V). The low number of bands (1 to 11 bands) were generated using $(GTG)_5$- PCR method in comparison with AFLP method (60- 70 bands), however the method was able to discriminate most of the strains used in this study. Rep-PCR is much quicker and cheaper than AFLP, and does not require a complex aparatus, like DNA sequencing machine. However, poor reproducibility has been reported in the study of Matsheka et al. (2006) [3]. The profiles produced in our study during first testing and after repeated analysis of 14 strains were stable. We suppose that good reproducibility of $(GTG)_5$- PCR genotyping was achieved by using high quality DNA templates obtained with genomic DNA isolation and purification kit.

Conclusion

Comparison of typing ability of rep- PCR and AFLP methods for genotyping of 54 strains of *C. jejuni* showed that both methods were able to discriminate it similarly and generated 34 and 36 genotypes respectively.

It is concluded that $(GTG)_5$- PCR typing is useful as a genotyping tool but additional analyses with other methods would be advisable to properly define interstrain relationships.

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The serological Salmonella monitoring in German pork production: How can progress be observed?

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Introduction

In Germany, more than 52,000 human Salmonella cases were reported officially to the authorities in 2006. Pork is known to be one of the main sources for human Salmonella infections. Although pigs normally do not show clinical symptoms when infected with Salmonella spp. [8], the Salmonella burden should be reduced for public health reasons.

The Salmonella prevalence in the pig population can be controlled by serological investigation [9]. Several commercial ELISA tests produce comparable results [15].

The European Directive 2003/99 [1] and the Regulation (EC) No 2160/2003 [2] were transferred into German law in 2007 [3]. Therefore since March 2007, all German farms with at least 200 fattening pigs are obliged to investigate their pigs Salmonella seroprevalence through a sampling scheme.

Following the "regulation on the reduction of the Salmonella distribution in slaughter pigs" 2007, most of the pork producing farms now have to conduct a Salmonella monitoring [3]. Since 2002, the QS Qualitaet und Sicherheit GmbH in Bonn/Germany (QS) has carried out the serological Salmonella monitoring in German finishing pig herds [4]. This non-governmental voluntary programme certifies member farms and their products. At the moment the QS company has the largest database concerning Salmonella in the German pork production.

An epidemiological data analysis with this data was carried out from year to year. Since the participating rate increased enormously over the years, the decision whether changes of prevalence in the dataset were due to changes in the Salmonella burden of the German pork population or due to the changes of the participating cohort is difficult. Therefore, we selected data for analyses from farms which participated for a long time.

Material and Methods

The QS Company offers a certificate at the point of sale for agricultural products which passed through the value chain within QS [10, 11]. Depending on farm size, every farm has to take up to 60 blood serum or meat juice samples per year. The samples are taken on the farm by a veterinarian (blood serum samples) or at the slaughterhouse (meat juice samples) and sent to a laboratory, where they are analysed serologically. The number of samples per slaughter is given by the Qualiproof database (Qualitype AG, Dresden/Germany) following a consistent distribution scheme over the whole year [12]. Four ELISA-tests are registered within the Salmonella monitoring system (Enterisol, Herdcheck, Salmotpye Pigscreen, Salmotype Fleischsaft). For each sample, the laboratories

report the antibody activity in OD% (optical density relative to reference) as well as the decision "positive"/"negative" (cut-off: 40 OD%) to QS.

Farms are classified into the categories I, II, or III by their percentage of positive samples per year: category I, if less than 20% of the samples are positive; category II, if 20-40% of the samples are positive; and category III, if more than 40% of the samples are positive. The initial classification is given after the delivery of the first 60 samples. The classification is updated quarterly based on the last 12 months data. For farms with few samples or if the last sample was taken more than 6 months ago, the classification is not updated and farms are labelled "below quota" and "sampling gaps", respectively.

For this study the data were analysed statistically using SAS, version 9.1 TS level 1M3 [13]. Absolute and relative farms or samples frequencies with similar attributes were determined. Furthermore, weighted prevalences were estimated in reference to districts structural data (animals and farms numbers per district) [14]. To evaluate differences between the categorisation within different years Bowker's test of symmetry was used [5].

Results

Since 2003, 3,772,156 datasets of 20,791 farms were entered into the database, which contains information of 261 slaughterhouses and 40 laboratories.

In 2007-IV period, 10,789 farms were in category I, 1,835 farms were in category II and 577 farms were in category III. For farms in category I, II, or III, the distribution of the farms varied only slightly among quarters. About 80–84% of the farms are in category I, between 12and15% in category II, and approx. 4–6% in category III (figure 1).

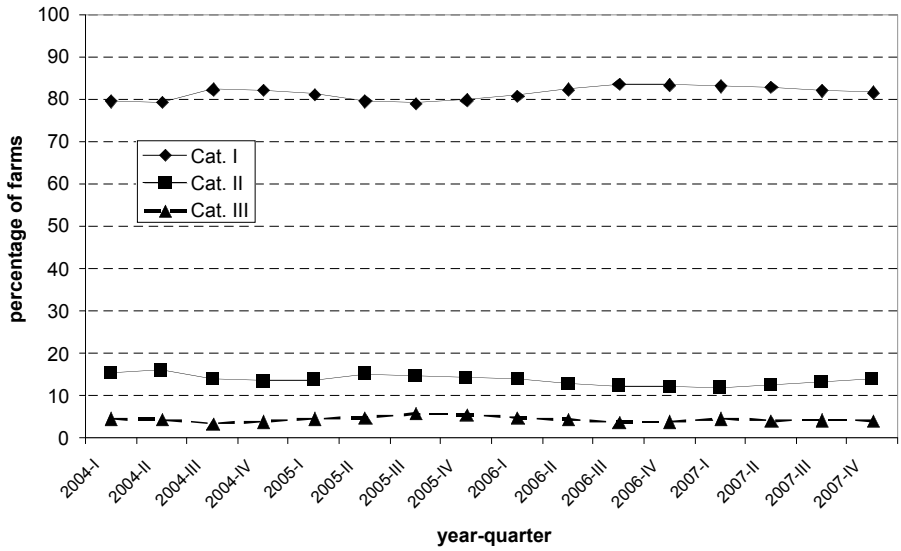


Figure 1: Percentage of farms per category and quarter; Categories based on percentage of positive samples per year: Cat I: <20%, Cat II: 20 – 40%, Cat III: > 40%

To detect changes between the categorisation over time, data from farms that had a valid category in 2005-IV as well as in 2007-IV

Table 1: changes between the categories I, II, and III from quarter 2005-IV to quarter 2007-IV; displayed is the number of farms

Category in 2005-IV	Category in 2007-IV I	II	III	Overall	%
I	4,061	339	75	4,475	80.18
II	499	234	60	793	14.21
III	153	112	48	313	5.61
Overall	4,713	685	183	5,581	100.00
%	84.45	12.27	3.28	100.00	

were analysed (table 1). In this subgroup, the percentage of category-I-farms increased from 80.18% in 2005-IV to 84.45% in 2007-IV. Correspondingly, the percentages of farms in category II and category III decreased during this period. Bowker's test of symmetry showed statistical significant differences regarding the frequencies of the categories between the quarters ($p<0.0001$).

Discussion

Categories I, II, and III farms' distribution seems to be stable since the beginning of the monitoring program. But regarding the fraction of farms that participated in IV-2005 as well as in IV-2007, the farms category has been slightly enhanced. More farms upgraded than downgraded, i.e. the Salmonella seroprevalence in QS monitoring participating farms has decreased. This effect has been masked by the continuous influx of farms which have a higher Salmonella burden. Farms which entered the monitoring system several years ago and which had category II or III underlay measures of consultations that may have caused positive effects on the Salmonella status.

Non corrected seroprevalences within the farms of the Salmonella monitoring were higher than the estimation of the adjusted seroprevalence in Germany. This is related to the fact that the coverage of the programme is higher in districts with greater Salmonella seroprevalence than in other districts. The high programme participation rate in districts with high animal densities is possibly linked to competition pressure.

Conclusion

To analyse monitoring data, the increase or change of participating subjects has to be taken into consideration. Progress can only be observed by a collective of long-participating farms. The results of the new participants can cover the success of measures.

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The isolation prevalence of bacteria belonging to yersinia genus isolated from carcasses and organs of wild boar

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Summary

The study follows the isolation frequency of Yersinia germs from different wild boar organs and carcasses samples, revealing the probability of contamination with this bacterial species, pathogen for human beings through alimentary toxic infections, manifested by austere diarrhea syndrome.
There have been collected and bacteriological analyzed samples of muscle from different anatomical parts of wild boar carcasses, sanitary swabs loaded on peritoneal surfaces and parietal pleura and also different portions of organs (liver, spleen, kidney, mesenteric lymphatic centers, intestinal portions). We had in view the hunting area from the southern and south-eastern part of the country, including the region of Danube Delta. A number of 4095 samples were harvested between 2004 and 2008. Laboratory processing followed both work stages provided by ISO techniques (SR ISO 10273, SR 12925, STAS 2356-82) and work techniques used in present day by different microbiology laboratories.
Results shows a low incidence at portage of Yersinia genus bacteria (maximum 2,34%), but they could be determined by the inhibition effect caused by the other bacteria populating the intestine. The highest isolation frequency was observed at serous surfaces level, which demonstrates the carcass post-mortem contamination either because of wild boar evisceration in improper conditions or the carcass faecal contamination. There haven't been noticed isolations from parenchimal organs and from muscle samples there has been made a single isolation.
After collection and processing the samples, there have been isolated 24 bacterial stems which were identified as belonging to Yersinia genus; The statistical analysis of the obtained results established a total prevalence of 0.49%, with annual relativelz low variations (1.02 - 0.1%); The highest value of isolation frequency was for the pleura and peritoneum sanitary swabs (1.96% for the entire period), the other ones being considered accidental; The bacterial species from Yersinia genus can contaminate the wild boar carcasses, the products and by-products obtained after processing, these carcasses being considered the origin of food toxic infections.

Introduction

The main provenance of Yersinia food borne diseases worldwide is represented by vegetal food products (salads, fruits, green vegetables), mixed products (meat along with salad or cheese), rarely by animal products (milk and milk products being considered unsafe), contaminated via non-hygienically processing stages or manipulation [3, 6, 10, 11].

Material and Methods

The samples were obtained from different anatomical parts of wild boar carcasses, sanitary swabs loaded on peritoneal surfaces and parietal pleura and also different portions of organs

(liver, spleen, kidney, mesenteric lymphatic centers, intestinal portions), being collected only from recently hunted wild boars (maximum 60 minutes after the blast), in some cases by the authors and in others by the hunters, especially prepared to perform this operation. The sample collection was executed between 2004 and 2008 gathering a total number of 4905 samples (table 1).

Tab.1 - The identification of sample types collected annually and processed through bacteriological techniques

Type of sample	Number of samples collected by entire period					
	2004	2005	2006	2007	2008	Entire period
Samples of muscle from carcass	156	224	194	256	278	1.108
Samples of liver portions	96	124	114	124	148	606
Samples of spleen portions	96	124	114	124	148	606
Samples of intestinal portions	107	128	114	128	156	633
Samples of mesenteric lymphatic centers	107	128	114	128	156	633
Samples of kidney	96	124	114	124	148	606
Sanitary swabs	115	129	142	19	188	713
Total samples	773	981	906	1.023	1.222	4.905

The processing of samples in the laboratory complied with the work ISO techniques work stages (SR ISO 10.273, SR 12925, STAS 2356-82) and the ones used nowadays by different microbiology laboratories to establish a diagnosis of this bacterial species.

The stages of the work methods comprised several stages:

(1) - sampling from animal food products in strict aseptic conditions. From each samples a 10 g quantity is taken (meat or tissues), introduced afterwards in the enrichment media, and obtaining a 1/10 or 1/100 dilution (% m/v or v/v).

(2) - enrichment in 2 different selective liquid media: 10 g/ml sample are introduced in 90 ml of PSB medium (peptone-sorbitol-biliar salts broth), obtaining a 1/10 dilution, followed by incubation at 25°C for 5 days; another 0,1g/ml sample are introduced in 9 ml of ITC medium (irgasan-ticarcylin-potassium chlorate broth), obtaining a 1/100 dilution, followed by incubation at 25°C for 2 days.

(3) - isolation and identification - performed by passing a loop from PSB broth culture directly on the surface of CIN agar and by transferring another 0,5 ml from the same medium in 4,5 ml of KOH saline solution 0,25 %, and homogenized for 10-20 seconds, afterwards a loop being passed directly on the surface of CIN agar [1, 2, 4, 5].

In parallel, there has been performed a passing through a loop from enrichment broth ITC culture to SSDC gelose (Salmonella-Shigella, with sodium desoxycholate and calcium chlorine), afterwards incubated at 30°C, in aerobic conditions, for 24 h. After 24 h, follows the magnifying glass examination or using an oblique light for recognizing the characteristic colonies of Yersinia enterocolitica. On the surface of CIN gelose, the characteristic colonies are small (1 mm), flat, with a red spot in the center, surrounded by a pink area, semitransparent (having the aspect of an "ox's eye"), and bile precipitate, and, after examining on oblique light, they are tiny granulated and not iridescent [7, 8, 9, 12]. On Wauters (SSDC) gelose, the characteristic colonies are small (1 mm) and grey, with a thin margin, and by examining on oblique light, they are tiny granulated and not iridescent. To confirm, a number of 5 characteristic colonies is selected, reintroduced in nutritive gelose dishes (to obtain pure cultures for biochemical assays). The dishes with sowed nutritive agar are incubated at 30°C for 24 h. The identification-confirmation stage was realized by using API 20E galleries.

Results and Discussion

The statistical analysis of data resulted on this research topic, performed on a total number of 4905, concluded the low variable values of annual incidence. The data showed a low incidence at portage of Yersinia species (maximum 2,34 %), o cause for this result being the preferential growth of other species which populate the gut. The highest values of frequency isolation was observed on serous surfaces (pleura and peritoneum), demonstrating the carcass

post-mortem contamination. There were no germs isolated from parenchimal organs, and only one case from muscle samples (through contamination by oral way).
Though obtaining low values of total prevalence (0,49), the conclusion is that the possibility of wild boar carcasses contamination is present, the products obtained after low temperatures processing representing a source of contamination for consumers.

Tab.2 - Identification of the number of positive samples and the isolation frequency of bacteria belonging to Yersinia genus, from analysed samples

The type of samples	The number of positive samples and the isolation frequency (%) / period											
	2004 nr. / %		2005 nr. / %		2006 nr. / %		2007 nr. / %		2008 nr. / %		Entire period nr. / %	
Samples of muscle from carcass	0	0	1	0,45	0	0	0	0	0	0	**1**	**0,09**
Samples of liver portions	0	0	0	0	0	0	0	0	0	0	**0**	**0**
Samples of spleen portions	0	0	0	0	0	0	0	0	0	0	**0**	**0**
Samples of intestinal portions	2	1,87	3	2,34	1	0,88	0	0	1	0,64	**7**	**1,11**
Samples of mesenteric lymphatic centers	1	0,93	1	0,78	0	0	0	0	0	0	**2**	**0,32**
Samples of kidney	0	0	0	0	0	0	0	0	0	0	**0**	**0**
Sanitary swabs	3	2,61	5	3,88	3	2,11	1	0,72	2	1,64	**14**	**1,96**
Total samples	**6**	**0,78**	**10**	**1,02**	**4**	**0,44**	**1**	**0,10**	**3**	**0,25**	**24**	**0,49**

Conclusion

After collection and processing through techniques and bacteriological assays a total number of 4905 samples of muscle, different organ parts and sanitary swabs pleura and peritoneum, there have been isolated a number of 24 bacterial stems which were identified as belonging to the species included in Yersinia genus.
The statistical analysis of the obtained results on a 5 years period permitted the establishing of a total prevalence of 0.49. The annual variations of the isolation frequency were situated between relatively low values (between 1.02% and 0.1%).
From the total number of analyzed sample types, the highest value of isolation frequency was observed for the sanitary swabs of pleura and peritoneum (1.96% for the total period), the other isolations being considered accidental.
The bacterial species form Yersinia genus can contaminate the wild boar carcasses, the products and by-products obtained after processing, these carcasses being considered the origin of food toxic infections.

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Yersinia enterocolitica RAPID METHOD FOR ISOLATION AND IDENTIFICATION FROM FOOD PRODUCTS

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Summary

The research was performed to optimize the methodology of *Yersinia enterocolitica* diagnosis from different categories of samples. In the first stage the general technique of collecting and processing the samples (intestinal mass integrally collected, organs collected integrally – liver, kidney, gizzard and fowl hearts, muscle tissue, samples harvested by stippling of the surface and interior of the carcasses, milk samples) was adapted and optimized. In a second stage of research there have been artificially contaminated different types of samples with *Yersinia enterocolitica* stems, checking the efficiency and accuracy of optimized method. The last stage of research was conducted to create a methodological scheme to assure rapidity and identification diagnosis for *Yersinia enterocolitica* species. In comparison with ISO 10273/1994 method, for the optimized method is noticed rapidity in obtaining a presumptive diagnosis, in 48 h from the collection of sample being determined the aparthenence to Yersinia genus. When using the identification galleries API 20E in the first phase is necessary to obtain several primary pure subcultures, requiring a minimum period of 48 h in which it is realized the primary sample processing, the incubation in a liquid enrichment medium, the passing over on a solid medium surface, the forming of subcultures from isolated colonies (presumptively Yersinia). For the optimized method, species confirmation is done by using a battery of biochemical tests including a series of stages and in which it is revealed also the pathogenic traits of the isolated stem. The presumptive diagnosis of bacterial species pertaining to Yersinia genus is of 48 hours maximum in case of using the optimized method, comparative with the classical methods (over 72 h); For presumptive diagnosis the optimized method offers a higher results accuracy for species confirmation comparing to ISO 10273 method and for revealing of pathogenity traits, comparing to API 20E; The rapid scheme for identifying *Yersinia enterocolitica*species from different types of samples assures a rapid diagnosis and offers the possibility of therapeutical intervention in an optimal period of time.

Introduction

In the last decade of the passed millennium, numerous researches pulled the attention that the bacterial species in *Yersinia* genus are frequently involved, in case of human beings, in production of alimentary toxic infections manifested by diarrheas acute syndrome [3].

Material and Methods

The methods used for collecting and processing the samples were adjusted taking into consideration the biological type of the products intended for analysis. From the intestinal mass, there was collected a culture loop from the caecal content, seeded directly on the CIN and SSDC media, thereafter being followed by incubation at 30 °C for 24-48 hours. The samples were diluted in 10 ml of physiological serum, being afterwards homogenized and introduced

in 9 ml of KOH solution 0,25 % in 0.85 % NaCl and agitated for 10 seconds. The loop content is transferred on the surface of CIN or SSDC media (for selective isolation), and the dishes are incubated for 24-48 hours at 30 °C in aerobic conditions.

The whole organs and muscle tissue: 10 g are transferred in 10 ml of diluent, being grind and homogenized for 2-3 in a stomacher; from the mixture are taken 10 ml and transferred into 90 ml of PSB media (1/10). Incubation is performed at 25 °C, for 5 days, afterwards adding the selective agents and incubating again at 12 °C for 24 hours. The incubation of the selective isolation media takes place in aerobiosis, at 30 °C, for 24-48 hours, and, at the end, each enrichment medium has a small content transferred on a solid media (CIN and SSDC).

In case of milk samples, the first step is to adjust the pH to 7,4, afterwards these being introduce in a centrifuge, at 2500 rpm, for 15 minutes. The supernatant is discarded, and the deposit is transferred in a selective isolation media. The treated milk (through temperature or adding chemicals) is enriched at 1/10 dilution, the rest of the stages being similar.

The last situation, considering carcasses, is performed like this: by stippling with swabs, introduced in 5 ml PSB media test tubes, hitting the test against the palm for 30 times, and afterwards a loop of test tubes content is transferred in a selective media (CIN, SSDC), incubating 24-48 hours at 30 °C. An alternative is to enrich the same swabs for 5 days (in PSB) and for 24 hours, adding the selective supplements, incubating again for 24 hours at 12 °C, in case of using ITC medium. After enrichment step, a small culture content is transferred on a selective isolation media surface. The dishes are incubated 30 °C for 24-48 hours, and after 48 hours and examination step is performed. Taking into account the characteristic colonies, the Gram dyeing method is applied. The pure selected cultures are subjected to biochemical tests: test of urease and ornitin-decarboxylase, the absence test for lysin-decarboxylase and oxidase, the absence test of citrate utilization and for producing H_2S, the test of esculin hydrolyze, the test for using some saccharides (glucose) and the absence of using others (lactose) on Kligler medium and the test of using saccharose, salicin and rhamnose [1, 2].

Results and Discussion

Each sample is made up as such - 10 g of muscle tissue (meat), 10 ml (milk or milk products) – followed by a enrichment stage, in 2 liquid media: PSB medium (1/0) and ITC medium (1/100).

For isolation and identification, a transfer to CIN agar is performed with a loop, and 0,5 ml of culture from PSB is added in 4,5 ml KOH, saline solution 0,25 %, homogenized for 10-20 seconds. In parallel, a culture loop from ITC is transferred on SSDC gelose. The incubation is realized at 30 °C, in aerobiosis, for 24 hours, afterwards, the dishes being examined with a magnifying glass or in oblique glass, for the presence of *Y. enterocolitica* characteristic colonies.

To confirm, a number of 5 characteristic colonies are selected, reintroduced on the surface of several dishes with nutritive gelose, for obtaining a pure culture for the biochemical tests. The dishes with seeded nutritive agar are incubated at 30 °C for 24 hours. From the nutritive agar dishes, a number of 5 colonies are chosed and tested for: morphology, the indole production, the urease production, oxidase production, the usage of saccharides on Kliger medium, all these for the prezumtive tests.

For the biochemical tests were selected only the colonies which presented: urease positive, indole production negative, acid from glucose positive, gas from glucose negative, acid from lactose negative, H_2S production negative, oxidase negative. There have been performed the usual tests: ornitin-decarboxylation, the usage of the citrate as unique source of carbon on Simmons medium and lysine-decarboxylation.

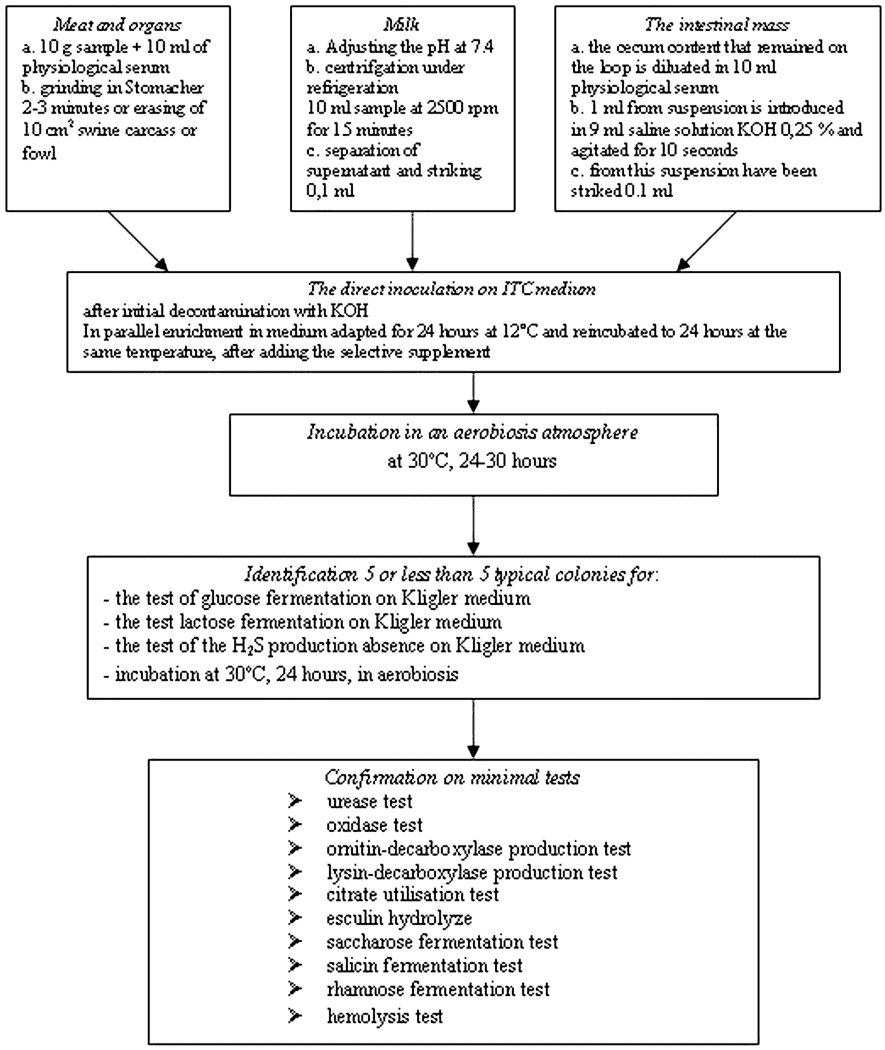


Fig. 1. - The rapid scheme for identifying *Yersinia enterocolitica*

The presumptive test of pathogenity comprised the verification of the following issues: the fermentation of salicin and the hydrolyze of esculin.

Conclusion

The collecting and primary processing of the samples for presumptive diagnosis of bacterial species pertaining to Yersinia genus, is of 48 hours maximum in case of using the optimized method, comparative with the classical methods which need a period of time relatively larger, of over 72 hours.

For a presumptive diagnosis of confirmation for the species, the utilization of the optimized methods offers a higher accuracy of the results in comparison with the ISO 10273 method and the revealing of pathogenity traits, in comparison to API 20E.

The rapid scheme for identifying *Yersinia enterocolitica* species from different types of samples, by using the obtained results after the research made by the authors, assures a rapid diagnosis and offers the possibility of therapeutical intervention in an optimal period of time or the withdrawing in real time from commerce, processing or direct consume of the products contaminated.

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Follow-up study of piglets in a serologically *Yersinia* -positive farrow-to-feeder herd.

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Summary

Yersinia (Y.) enterocolitica is a widespread zoonotic agent with high prevalence in pigs. Therefore, it is assumed to lead to human gastroenteritic infections by pork and pork products. The knowledge of the infection route is the first step to eliminate the agent from herds in order to reduce the consumers risk. In this study, eight sows and piglets of their litters from a farrow-to-feeder herd were tested serologically and bacteriologically in order to monitor the occurrence of *Yersinia* infections at the early stage of life. Over a period of seven weeks the presence of maternal antibodies, their decrease and time of possible seroconversion in relation to bacteriological findings from faeces were investigated in the piglets. Although sows and suckling piglets were tested serologically *Yersinia*-positive, the agent could not be isolated. In the piglets, antibodies decreased during the seven weeks of observation and there was no hint of seroconversion. The results suggest that the production of *Yersinia*-free pigs from serological positive sows could be possible.

Introduction

Y. enterocolitica, especially O:3/biovar 4, is often isolated from pigs [4, 8], and presents a common cause of human gastroenteritis. In order to identify the asymptomatic infected pigs as carriers of this zoonotic agent, serological and bacteriological analyses are common. In general, the organisms are present in the oral cavity, especially in pigs' tonsils, and to a lesser degree in the intestine, the faeces and lymph nodes of slaughtering pigs [9, 10]. Antibodies were detected more frequently in animals with bacteria in their tonsils than in those with faecal carriage [11]. Faecal shedding may stop soon after the ingestion of the bacteria, whereas pigs may carry *Y. enterocolitica* in their tonsils for months [8].

The purpose of the presented study was to demonstrate the decrease of maternal antibodies against *Yersinia* spp. in piglets and the time of seroconversion in comparison to bacteriological findings during breeding in a natural infected farrow-to-feeder herd.

Animals, Material and Methods

The study took place in a farrow-to-feeder herd with 80 sows, which were known as serologically *Yersinia*-positive from a previous investigation. The herd practised all-in/all-out routines in the farrowing and in the nursery units. Piglets were weaned five weeks after birth.

Eight sows and two to five piglets of the litters were examined over a period of seven weeks. Sows were tested within four days after birth (SD 1) and one day before weaning (SD 3). During the first time, blood and faeces were sampled, at the second time only blood samples were taken. Suckling pigs were examined within four days after birth (SD 1) by taking blood samples. Afterwards, the signed piglets were tested again after seven days (SD 2), one day before weaning (SD 3) and two weeks later (SD 4) in the nursery unit by taking blood and faecal swabs.

Additionally, tonsils of three necropsied weaners were tested culturally. Ten faecal samples from sows during gestation period were taken and investigated by culture and PCR method.

Cultural method.
Yersinia ssp. were grown in ITC-bouillon, isolated on CIN-agar, and identified with the API 20E system.

Serological investigation.
Yersinia-antibodies were detected with an ELISA, based on *Yersinia* outer proteins (Pigtype Yopscreen ELISA, Labordiagnostik Leipzig, Germany). A basic cut-off of optical density (OD %) 20 was used.

Multiplex PCR-method.
Template DNA was prepared out of faeces using the PSP®Spin Stool DNA Kit (Invitek, Berlin, Germany) and identified by PCR [12].

Statistical analysis.
Data were evaluated according to the programme for statistics SPSS version 16.0 (SPSS, Chicago, IL, USA).

Results

Yersinia spp. was not detectable by cultural growing or PCR technique from all faecel samples. Even the tonsils of the necropsied weaners were negative. However, all piglets (n=25) were serological *Yersinia*-positive in the first days of life, corresponding to the results of the sows. Seven weeks later, weaners were serologically negative (Fig. 1). The decrease of maternal antibodies in the piglets was statistically significant between each sampling day ($p \leqslant 0.05$, T-test for matched-paired samples).

Discussion

Although great efforts were made to prove *Yersinia* spp. in faeces or tonsils of the pigs in the herd neither cultivation nor PCR method were successful. Generally, asymptomatic carriers shelter only a small number of pathogenic strains of *Yersinia* [5], and these strains were found rather in the tonsils of the pigs than being shed with the faeces [8]. Bowman et al. [2] tested less than 1 % of suckling piglets and 1.4 % of nursery pigs bacteriologically positive by taking both faeces and oral-pharyngeal swabs. The agent was never detected in farrowing sows, whereas 9.1 % gestating sows tested positive. In light of these findings, the presented negative bacteriological results for *Yersinia*, especially in the farrowing stage, are not surprising. In the present study, all suckling pigs and farrowing sows were serologically positive

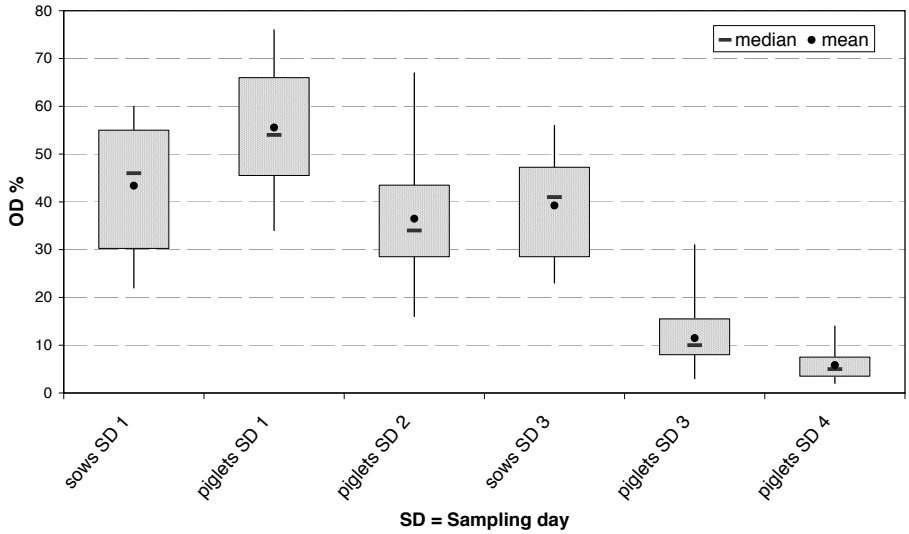


Fig. 1: Serological results of the ELISA for detection of antibodies against *Yersinia* spp. at the four sampling days

within the first week after birth, indicating that all sows might have come in contact with *Yersinia* spp.. Serological testing of suckling pigs within the first days of life reflects the immunological status of the sow by demonstrating maternal antibodies, which may protect the organism from infection within the first stage of life. Analysis of the immunogenicity of selected Yops showed the earliest immune response by detecting immunoglobuline A on day seven, followed by immunoglobuline G on day ten after infection [6]. Investigations into the persistence of antibodies against *Yersinia* spp. in pigs were not performed. Therefore, a specification of the date of infection of the sows is not possible. For humans the persistence of antibodies over years is known [7]. The low serological prevalence of *Yersinia* spp. during the last sampling day at the nursery stage and the absence of the agent in all bacteriologically investigated samples may indicate that the weaners were not infected by this time. At the age of seven and eight weeks, Bothe et al. [1] already observed seroconversion simultaneously with the first bacteriological findings. If the piglets in our study stayed uninfected up to the time of transport to the fattening unit, a *Yersinia*-free fattening pig herd might be established. Analogical results were achieved by investigations into *Salmonella* infections in farrowing units. It could be demonstrated that raising *Salmonella*-free finishers from sows in infected herds was possible [3]. Nevertheless, the assumption of achieving fattening pig herds free from *Yersinia* spp. from seroreacting sows has to been proven by further studies.

Conclusion

The serological negativity of the weaners and the absence of the agent may indicate the possibility to establish a *Yersinia*-free fattening herd from those pigs although the presence of seroreacting sows indicate a certain level of *Yersinia* exposure in the sow-herd.

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Follow-up study of the development of antibodies against *Campylobacter* spp. in piglets in a farrow-to-feeder herd.

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Summary

Campylobacter (C.) spp. is a widespread zoonotic agent with high prevalence in pigs. Maternal *Campylobacter* strains are the primary source of infection for piglets, which was proven in other studies by bacteriological findings. The aim of the presented study was to monitor the occurrence of antibodies against *Campylobacter* spp. in relation to bacteriological findings by testing five sows and piglets of their litters over a period of seven weeks. *Campylobacter* spp. was isolated from the faeces during the whole investigation period with *C. coli* as the predominant species. The results of an in-house ELISA for the detection of *Campylobacter* antibodies led to the assumption that seroconversion started between the fifth and seventh week of life.

Introduction

Campylobacter spp. is a major cause of diarrhoeal illness with an incidence rate comparable or even higher than *Salmonella* spp. in many countries. Accounting for about 20 % [3], *C. coli* is the second agent of campylobacteriosis behind *C. jejuni*. Pigs have been demonstrated to be an important reservoir of *C. coli*. Maternal *Campylobacter* strains are the primary source of infection for piglets within their first days of life [1] and the prevalence may rise to 100 % by the time of weaning [9]. The description of *Campylobacter* prevalence in pig herds is commonly based on bacteriological findings, especially from faecal samples. Serodiagnosis of *Campylobacter* infections is not routinely performed in Germany, not even in human medicine. However, its application is described especially in the diagnosis of the Guillain-Barre-Syndrome in humans, a postinfectious autoimmune polyradiculoneuropathy, which may occur after a *C. jejuni* infection [7]. Serological investigations of *Campylobacter* infections in pigs are rare. Von Altrock et al. [8] examined the serological prevalence in fattening pig herds by using a method based on immunoblot analysis. Kley [5] described the development of an enzyme-linked-immunosorbent-assay (ELISA) test system to enable the detection of antibodies to porcine *Campylobacter* in serum and meat juices. Nonetheless, investigations into the time of seroconversion in piglets naturally infected with *Campylobacter* were not performed.

The purpose of this study was to demonstrate the decrease of maternal antibodies against *Campylobacter* spp. in piglets and the time of seroconversion by using an in-house-ELISA in comparison to bacteriological findings during breeding in a natural infected farrow-to-feeder herd.

Animals, Material and Methods

The study took place in a farrow-to-feeder herd with 80 sows. The herd practised all-in/all-out routines in the farrowing and in the nursery units. Piglets were weaned five weeks after birth. Five sows and two to three piglets of the litters were examined over a period of seven weeks. Sows were tested within four days after birth (SD 1) and one day before weaning (SD 3). During the first time, blood and faeces were sampled, at the second time only blood was taken. Suckling pigs were examined within four days after birth (SD 1) by taking blood samples. Afterwards, the same piglets were tested again after seven days (SD 2), one day before weaning (SD 3) and two weeks later (SD 4) in the nursery unit by taking blood samples and faecal swabs. Swabs were stored in Cary-Blair-Medium (BBL Culture Swab, Cary-Blair Agar, Becton Dickinson, Heidelberg, Germany).

Multiplex PCR-method. Template DNA was prepared out of faeces using the PSP- Spin Stool DNA Kit (Invitek, Berlin, Germany). PCR for detecting *Campylobacter* in faecal samples was performed using the primers and the protocol described by Persson and Olsen [6].

Serological investigation. *C. coli* major outer membrane protein (MOMP) [10] was cloned and expressed using the pTrCHis Topo-TA Expression Kit (Invitrogen, Carlsbad, CA, U.S.A) according to the manufacturer's instructions. The expressed recombinant His-tagged antigen was purified using Protino-Ni-TED columns (Macherey-Nagel, Dueren, Germany). Purified protein was precipitated overnight using 10 % [v/v] TCA at 4 °C. The precipitate was centrifuged at 15000 x g for 30 min, supernatant removed and the recombinant protein washed using 80 % [v/v] acetone, followed by centrifugation at 15000 x g for 30 min. The recombinant His-tagged MOMP was resuspended in 8 M urea. Subsequently protein concentration was determined using the Micro BCA®Protein Assay (Pierce, Rockford, U.S.A.) according to the manufacturer's instructions. Protein concentration was adjusted to 2μg ml-1 in 50 mM carbonate/bicarbonate buffer. Polysorp®microtiter plates (Nunc, Roskilde, Germany) were coated overnight at 4 °C using 100 ml of the antigen suspension per well. Afterwards the coated plates were washed three times with PBS buffer containing 0.05 % Tween-20. ELISA was performed according to routine laboratory protocols. OD % values were calculated as percent positivity (PP) values using a defined positive serum from a *Campylobacter* infected pig as reference.

Statistical analysis. Data were evaluated according to the programme for statistics SPSS version 16.0 (SPSS, Chicago, IL, USA).

Results

C. jejuni was not identified neither from the faeces from the sows nor from the piglets. From the time of weaning, all piglets excreted *Campylobacter* spp., but isolation of *C. coli* succeeded mostly at the time of weaning (SD 3: 85.7 %). *Campylobacter* strains, which could not be identified as *C. jejuni* or *C. coli*, predominated at the nursery stage (SD 4: 57.1 %).

Comparing the serological test results of all piglets during the four sampling days (Fig. 1), the decrease of antibodies between the first three sampling stages were statistically significant ($p \leqslant 0.05$, T-test for matched-paired

samples), but stagnated between SD 3 and SD 4 ($p > 0.05$).
The mean OD % values of each litter steadily decreased up to weaning (SD 3) (Fig. 2). Afterwards, an increase of the antibody titers could be observerd in litter 3 and slightly in litter 4, although statistically the increase could not be proven.

Tab. 1: Number of *Campylobacter*-positive faeces samples from sows and faeces swabs from piglets at different sampling days

Number of positive faeces samples for *Campylobacter* / number of animals tested (proportion of positive animals in %)

	pigs being sampled	number of samples	number of *Campylobacter* positive samples (%)	number of *C. coli* positive samples (%)
SD 1	sows	5	3 (60.0 %)	1 (20.0 %)
SD 2	piglets	14	12 (85.7 %)	8 (57.1 %)
SD 3	piglets	14	14 (100 %)	12 (85.7 %)
SD 4	piglets	14	14 (100 %)	6 (42.9 %)

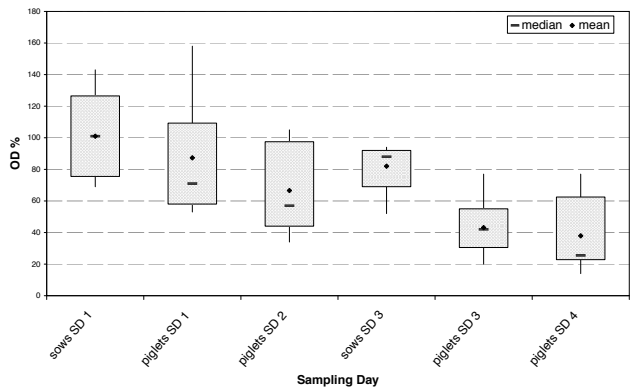


Fig. 1: Serological results of the in-house ELISA for detection of antibodies against *Campylobacter* spp. in the sows and piglets at the four sampling days

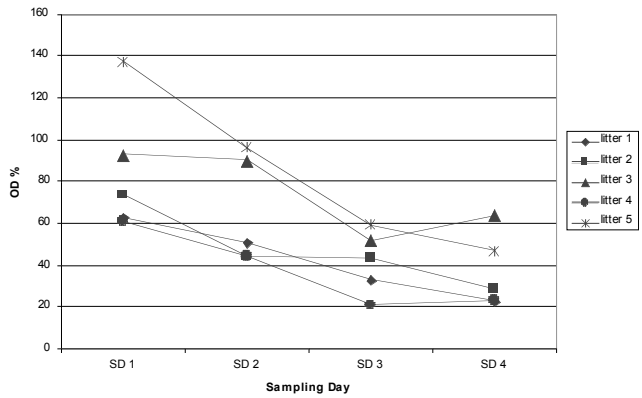


Fig. 2: Mean OD % of *Campylobacter*-antibodies of each investigated litter at the different sampling days

Discussion

While the isolation rate of *Campylobacter* from the faeces of the piglets increased to 100 % up to the time of weaning, serologically the portion of maternal antibodies decreased steadily during the first five weeks of life. While Kley [5] found very low antibody levels in piglets with body weight less than 25 kg in comparison to farrowing sows, the amount of antibodies of suckling pigs during the first days of life did not differ significantly from the sows. However, at the last sampling day an increase of the antibodies against *Campylobacter* could be observed in two litters, which argues for building the first antibodies due to infection between the fifth and seventh week of life. Nevertheless, neither maternal antibodies nor antibody response at the later stage of life seem to limit colonisation of the intestine. Bacteriamia in pigs is not documented. Therefore, circulating antibodies may play a role in limiting invasion as assumed in humans [2]. Regarding the high prevalence of *Campylobacter* in pig herds, constant exposure leads to permanent colonisation of the gut [4], but it will also lead to repeated challenge with *Campylobacter*, which in turn may induce permanent antibody responses.

Conclusion

This is the first investigations on seroconversion of weaners after natural *Campylobacter* infection. The production of antibodies seemed to start between the fifth and the seventh week of life. But neither maternal nor antibody response at a later stage of life seemed to protect the gut from colonisation. Nevertheless, serologically results reflect the high prevalence of *Campylobacter* spp. in pigs according to bacteriological findings, but serological testing is preferable to bacteriological methods on the basis of practicality, time-saving aspects and costs.

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Serological study of leptospiral infection in man in Ahvaz, southwest of Iran

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Summary

This study was carried out to investigate the prevalence of leptospiral infection on men in Ahvaz, southwest of Iran. Blood samples were taken from 180 men that divided to contacted (93 person) and non– contacted (87 person) to domestic animal. Sera were initially screened at serum dilution of 1:100 against six live antigens of *Leptospira interrogans*: *Pomona*, *Canicola*, *Hardjo*, *Ballum*, *Icterohemorrhagiae* and *Grippotyphosa* using the microscopic agglutination test (MAT) and sera with positive results were titrated against reacting antigens in serial twofold dilution from 1:100 to 1:1600. Antibodies against one or more serovars at least in dilution 1:100 were detected in 21(22.58%) and 11(12.64%) sera of the men with direct contact and non – direct contact to animals, respectively. Among the positive sera, antibodies were most frequent to serovar *canicola* (56%) followed by *grippotyphosa* (26%), *pomona* and *hardjo* (each of them 8%) in the men with direct contact to animals and in the men with non – direct contact to animals antibodies were most frequent to serovar *canicola* and *grippotyphosa* (each of them 41.2%) followed by *Pomona* (11.8%) and *hardjo* (5.9%). The majority of titre level was 1:100 for all serovars.

Introduction

Leptospirosis which is caused by infections of *Leptospira interrogans* serovars is an important zoonotic disease and has become a major worldwide human concern[4, 5, 6]. Humans beings get the infection either from rodent hosts or from domestic animals. Enviroment contaminated with the urine of carrier animals is usually the immediate source of infection. Because of the ability of the leptospires to survive in the enviroment and infect human beings, leptospirosis is a potential health hazard of occupational groups exposed to the enviroment [4].

Leptospiral infections cause both acute and chronic disease and the severity of infections is related to the virulence of the organism, the susceptibility of the host, and the affected host species. Although most leptospiral infections are subclinical and infection is more common than clinical disease[6].

This study was carried out to investigate the prevalence of leptospiral infection on men in Ahvaz, southwest of Iran.

Material and Methods

Blood samples were taken from 180 men that divided to contacted (93 person) and non-contacted (87 person) to domestic animal. The serum samples were tested for antibodies to six live antigens of *Leptospira interrogans* (serovars *canicola, grippothyphosa, hardjo, pomona, icterohemorrhagiae, ballum*) using the microscopic agglutination test (MAT). Sera were initially screened at a serum dilution of 1:100 against antigens. At first, serum dilution of 1:50 was performed and a volume of each antigen, equal to the diluted serum volume, was added to each well of the micro titrations plates, making the final serum dilution 1:100. The plates were examined by dark-field microscopy. Results were considered positive when 50% or more of agglutination of leptospires at the test serum dilution of 1:100 or greater were found.

The data were analyzed statistically by using Chi–square and Fisher(s – exact test with significance level at 0.05.

Results

Antibodies against one or more serovars at dilution of1:100 or greater were detected in 21(22.58%) and 11(12.64%) sera of the men with direct contact and non - direct contact to animals, respectively 9 table 1). Although the rate of infection in the group of contacted to animal was more than othe other one, but there was no significant diference between them (P=0.081)

Antibody against more than one serovars were found in 25% positive sera, so that 6 (18.75%) and 296.25%) of sera infected to two and three serovars, respectively.

Among the positive sera, antibodies were most frequent to serovar *canicola* (56%) followed by *grippotyphosa* (26%), *pomona* and *hardjo* (each of them 8%) in the men with direct contacted to animals and in the men with non - direct contacted to animals antibodies were most frequent to serovar *canicola* and *grippotyphosa* (each of them 41.2%) followed by *pomona* (11.8%) and *hardjo* (5.9%).

The majority of titre level was 1:100 for all serovars and the frequency of 1:100, 1:200 and 1:400 were 78.57%, 14.28% and 7.14%, respectively. No sera reacted to these serovars in 1:800 or higher dilution

Table1: Comparision of leptospiral infection in Contacted and Non direct contacted to animal

	No. positive	No. negative	Total
Contacted to animal	21(22.58%)	72(77.42%)	93
Non contacted to animal	11(12.64%)	76(87.36%)	87
Total	32	148	180

Discussion

leptospiral infection in humans and animals based on serological testing has been reported in some country. These reported confirm that prevalence of leptospiral infection is different not only between countries but also between different areas of each country. These differences probably due to goographical factors or traditional life systems [1,2,3,4,5]. For example, in the study of Ebrahimi et al (2003) in tribal area of west central Iran showed 48. 50% of tested people infected to one or more leptospiral serovear at a serum titire of 1:100 [1] and in the study of Natarajaseenivasan et al (2002), 68.3% of rice mill workers was seropositive [4]. The highest prevalence rates

remain in tropical, developing countries where leptospirosis cases are on the rise. This rise is associated with urban population growth, urban decay, and flooding. Outbreakes have been related to heavy rainfall in various parts of world [5].

The prevalence of leptospiral infection in men that contacted to animal was higher than in non-contacted animal, but statistical analysis showed no significant difference between these groups. Humans usually become infected through contact with water or soil contaminated with urine or other body fluids from infected wild or domestic animals. Thus, farmers, sewer workers, miners, fishermen, and meat workers have traditionally been at greatest risk for infection (5).

In previous studies in Ahvaz the predominant serovars were *grippotyphosa* in cattle, horse, and donkey and *canicola* in buffalo, it is probable that these serovars may be adapted to and maintained by these farm animals in Ahvaz and infected to human beings [1,2]. In the study of Ebrahimi *et al* (2003) the majority of positive semple related to *leptospira* serovar *hardjo* (54.12%), the other commonly reacting serovars being *pomon* (20.61%), *icterohemorrhagia* (20.10%), *canicola* (11.85%) and *gripotyphosa* (1.00%).

Conclusion

This study indicated *Leptospira interrogans* infects humans without history of direct contacted to domestic animals, because rat and other rodents are the most important sources for human infection.

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Investigation of circulating strains of Influenza viruses among pig handlers and pigs in Ibadan, Nigeria

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Summary

We investigated the types of influenza viruses currently circulating among pig handlers and pigs in Ibadan, Nigeria. Throat and nasal swabs, respectively, collected from 15 pig handlers who had acute influenza-like illness and 50 apparently healthy Landrace pigs at four locations within Ibadan, from April to June, 2008 were used for influenza virus isolation in 10-12 day old embryonated eggs and HEp-2 cell line. Influenza B/Shanghai/361/2002-like virus was isolated from 2 pig handlers, while Influenza A/Brisbane/59/2007-like (H1N1) and A/Brisbane/10/2007-like (H3N2) viruses were isolated from 5 and 2 pigs, respectively. This study shows that different strains of influenza viruses co-circulate among people who have regular contact with live pigs, and among pigs in Ibadan, Nigeria. Reassortment of these co-circulating strains of human and swine influenza viruses may therefore occur in these pigs to form novel viruses which could be highly virulent when established in human, swine, or avian populations. We recommend that regular surveillance of pigs in Nigeria should be done as an early warning system for detection of future pandemic strains.

Introduction

Influenza A viruses constitute one of the genera in the family *Orthomyxoviridae*and are subdivided into sixteen Haemagglutinin (H1-H16) and nine Neuraminidase (N1-N9) subtypes [4]. Pigs can be naturally or experimentally infected with avian and human influenza viruses because the epithelial cells in pig trachea contain both NeuAc-2,3Gal and NeuAc-2,6Gal receptors [10]. Pigs also appear to have a relatively weak species-specific barrier against infection by avian and human influenza A viruses [7]. These observations support the 'mixing vessel' hypothesis that pigs, when simultaneously infected with avian and human influenza viruses, permit the generation of reassortants capable of causing pandemics [8]. There is paucity of information on the types of influenza viruses circulating among pigs in Nigeria, and among humans who have regular, direct contact with them. This study was therefore designed to investigate the types of influenza viruses currently circulating among pigs and pig handlers in Ibadan, Nigeria. This is important for early detection of reassortant influenza viruses.

Material and Methods

Sampling method and specimen collection. Using stratified random sampling, nasal and throat swabs, respectively, were collected from fifty (50/199) apparently healthy Landrace pigs and fifteen pig handlers (15/15) showing influenza-like symptoms [5] within the previous few (three) days, at four locations within Ibadan, from April to June, 2008.

These were tested at the Department of Virology, University of Ibadan (U.I). The locations are: Commercial Pig Farm Unit, U.I (6 out of 31 available pigs, 5 out of 5 affected pig handlers); University Research Farm, U.I (7/35 pigs, 5/5 pig handlers); Municipal Abattoir, Bodija (37/133 pigs, 0 pig handlers); andPFA Farm, Monatan (0 pigs, 5/5 pig handlers). Pigs included boars (19 out of 76 available boars), sows (16/64), and growers (15/59). Pig handlers tested included 13 males and 2 females whose age range was 16-65 years. Swabs obtained from pigs and humans were inserted into virus transport medium (0% D-MEM) in labelled cryo-vials kept inside different ice-packs.

Virus isolation. Each specimen (0.2ml) was inoculated in duplicate into labeled tubes with confluent monolayer of Human Epithelium-2 (HEp-2) Cell Line. Hep-2 Cell Line was used because of our inability to obtain MDCK Cell Line, which has been found to be quite sensitive. Specimens stored at -80 ^{o}C were first thawed on ice. Inoculated tubes were incubated at 37 ^{o}C and observed daily for cytopathic effects (CPE) for 7 days. Cell cultures were harvested when CPE had attained 3+ to 4+. Supernatant from such tubes were stored as aliquots of 2ml at -80 ^{o}C for subsequent passage. Aliquots for blind passage were also taken from cultures without CPE by day 6 or 7 post-inoculation. Supernatant fluids were passaged twice. Those without CPE during the second passage were regarded as negative. Each specimen was also aseptically inoculated in duplicate into viable 10-12 day old embryonated chicken eggs. The eggs were sealed, labelled, incubated at 37°C, and observed daily for embryonic death. Two viable, uninoculated, 10-12 day old embryonated chicken eggs obtained from the same source as inoculated eggs were included with each batch as negative control. Eggs in which death occurred from 24 hours post-inoculation were chilled at 4°C overnight or for 4 hours before harvesting, while those without embryonic death were incubated for 72-96 hours before chilling and harvesting. The allantoic fluids of eggs were aseptically harvested into 5ml cryo-vials. Aliquots were made in duplicate from HA-positive specimens. One of these was stored at -80°C for further identification, while the other was used for passage in a new batch of eggs. HA-negative specimens were also passed into new batches of eggs and retested before being considered to be negative.

Identification of isolates. Haemagglutination Inhibition Assay (HI) was used for isolate identification. The following CDC reference antisera (and their corresponding reference antigens) were used: Sheep influenza A/Brisbane/59/2007 (H1N1); Sheep influenza A/Brisbane/10/2007 (H3N2); Sheep influenza B/Shanghai/361/2002-like (B/Yamagata/16/88 lineage); and Sheep influenza B/Malaysia/2506/2004-like (B/Victoria/2/87 lineage). Non-specific inhibitors of haemagglutination were removed from Reference Antisera and test sera by receptor destroying enzyme (RDE) treatment [12]. The HI procedure was performed according to WHO recommendations, and an isolate was identified as a particular type if it reacted with only one antiserum or, in cases of cross-reactivity, if it reacted with one antiserum giving HI titre four-fold or more than its titres to other antisera [12].

Results

CPE was characterized by cell rounding, degeneration and aggregation of cells into grape-like clusters. As shown in Tables 1and 2, two (13.3%) of the fifteen human specimens were positive by HI. The isolates, typed as influenza

B/ Shanghai/361/2002-like, were from specimens collected in April (2/10) from two men (2/13) within age groups 16-25 (1/6) and 26-35 years (1/4), at the two farms located within the University of Ibadan. Seven (14.0%) of the fifty swine specimens collected for virus isolation were positive by HI. These consisted of Influenza A/Brisbane/59/2007 (H1N1) and A/Brisbane/10/2007 (H3N2) viruses which were isolated from 5 and 2 pigs, respectively.

Tab. 1: Influenza virus isolates from humans and pigs at different locations

Location	Number Positive (%)	
	Human	Swine
Commercial Pig farm , U.I	2(40.0) [B/S]*	3(50.0) [H1N1(2), H3N2]
University Research Farm, U.I.	0(0.0)	1(14.3) [H1N1]
PFA Farms, Monatan	0(0.0)	Not sampled
Municipal Abattoir, Bodija	Not sampled	3(8.1) [H1N1 (2), H3N2]
Total	2(13.3)	7(14.0)

*B/Shanghai/361/2002-like

Tab. 2: Results of virus isolation in two culture systems

Species	Culture System	Number Positive (%)		
		CPE/Embryonic Death	HA	HI
Human	Embryonated egg	11(73.3)	5(33.3)	2(13.3)
	HEp-2	9(60.0)	1(6.7)	1(6.7)*
Swine	Embryonated egg	46(92.0)	13(26.0)	7(14.0)
	HEp-2	4(8.0)	1(2.0)	0(0.0)

*Also isolated in embryonated eggs.

Discussion

Influenza occurs most often in Nigeria during harmattan (November to January) and early (April to May) to peak raining season [3, 6]. In this study, results obtained using samples collected within one of the two peak periods confirm that while influenza B/Shanghai/361/2002-like circulated among pig handlers, two subtypes of influenza A viruses, A/Brisbane/59/2007 (H1N1) and A/Brisbane/10/2007 (H3N2), co-circulated among pigs in Ibadan. Isolation of swine influenza A viruses with antigenic reactivity similar to human influenza A viruses could indicate transmission of these viruses from pig handlers to pigs. The fact that only influenza B viruses were isolated from these pig handlers does not reduce the strength of this evidence. Rather, consistent with previous findings [9, 12], this indicates that influenza A viruses persisted, though sub-clinically, in these pigs, long after they had disappeared from the human population, as these people had relatively very low (waning) mean titres of influenza A H1N1 and H3N2 antibodies in comparison to those obtained for pigs [1]. This could be a strategy for ensuring co-infection by different strains of influenza viruses in pigs for as long as possible, thereby increasing the chances of genetic reassortment [8, 12]. Furthermore, influenza B viruses circulated in people at levels lower than those of influenza A viruses in Nigeria [6], in Asia, and in Europe, although they predominated in some countries [2]. In this study, isolation of only influenza B viruses from pig farmers may indicate either an increase in the infection rate of influenza B viruses or a higher level of exposure to influenza B viruses among pig handlers than that within the general population in Ibadan, Nigeria.

Conclusion

Different strains of influenza viruses co-circulate among pigs and their handlers in Ibadan, Nigeria. The possibility of genetic reassortment to form novel influenza viruses is therefore high. We recommend periodic surveillance of pigs in Nigeria, as an early warning system for detection of future pandemic strains.

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Short stage-conversion time of *Toxoplasma gondii* isolated from Brazilian ewes

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Summary

Toxoplasma gondii is an obligate parasite protozoan with a worldwide distribution affecting warm-blooded hosts, as production animals and humans. This study aimed to evaluate the tachyzoite-bradyzoite conversion time of *Toxoplasma gondii* isolated from Brazilian ewes. Brain, lung and muscle tissue samples of 66 Brazilian ewes, positive in Modified Agglutination Test (MAT), were bioassayed. Material from 20 ewes was positive on bioassay in mice. 12/20 (60.0%) presented tissue cysts. 2/8 (25.0%) sheep bioassays had mice died during the experiment with formation of 100 tissue cysts in brain, and presence of tachyzoites in peritoneal fluid. One of these (1) presented an average of 24 days post-inoculation (d.p.i.) as mice survival time with brain, another one (2) presented 12 d.p.i. with brain, 16 d.p.i. with muscle, and 24 d.p.i. with lung. Thus, *T. gondii* isolated from Brazil and some countries of South America presented different virulence profiles, which can result in different intensity of clinical manifestations in many warm-blooded animals, with or without tissue cysts formation. This probable the first report of short stage-conversion time in mice of *T. gondii* isolated from naturally exposed Brazilian sheep.

Introduction

Toxoplasma gondii is an obligate parasite protozoan with a worldwide distribution affecting warm-blooded hosts, as production animals and humans [5]. The humans and animals can be infected by the ingestion of oocysts in contaminated vegetables and fruits, cysts in raw or undercooked meat of intermediate hosts, or by the placental transmission of tachyzoites [1,7]. This study aimed to evaluate the tachyzoite-bradyzoite conversion time in mice of *Toxoplasma gondii* isolated from Brazilian ewes.

Material and Methods

This study was performed in a Brazilian slaughterhouse and Núcleo de Pesquisas em Zoonoses (NUPEZO), College of Veterinary Medicine and Animal Science, UNESP. The tissue samples of 66 Brazilian ewes, positive for *Toxoplasma gondii* by Modified Agglutination Test (MAT) [2] were collected in a Brazilian slaughterhouse.

Brain, lung and muscle tissue samples were digested by pepsin action and inoculated 1mL subcutaneous per Webster albino mouse, 30 days-old [4] to isolate the parasite forms, with observation time of 60 days. All died animals were researched for tachyzoites and bradyzoites in brain and peritoneal fluid.

Results and Discussion

20 ewes presented positive isolation on bioassay in mice. Evaluating the bioassay, 12/20 (60.00%) survived until the 60th day, with tissue cysts formation, and 8/20 (40.00%) had some or all mice died with parasite forms detected in brain, peritoneal fluid or only positive serologically.

T. gondii is able to cross biological barriers as placental or intestinal epithelium as tachyzoite form by the paracellular transmigration and invade the blood circulating cells, macrophages and dendritic cells e.g., as "Trojan Horse", crossing the blood-brain barrier inside of this cells, and acquire access to privileged sites as the brain, forming tissue cysts [1,12].

2/8 (25.00%) sheep presented high importance in this study due the bioassay survival time. In both sheep bioassays, mice died during the experiment with the formation of 100 tissue cysts.

One sheep (1) presented mice survival time of 24 days post-inoculation (d.p.i.), with sheep brain sample, and the other one (2) presented 12 d.p.i. (100, 60 and 40 cysts), with sheep brain sample; 16 d.p.i. (60 cysts), with sheep muscle sample; and 24 d.p.i. (60 cysts), with sheep lung sample (Tab. 1). These results of stage-conversion time are short compared to Dubey [3] that detected the parasite in many tissues of mice, 15 and 49 d.p.i., peaking at 14 d.p.i. and then persisted until 90 d.p.i. If we consider the virulence of each infectious form of *T. gondii*, oocysts are more virulent than tachyzoites or bradyzoites to non-felid hosts [5,7].

Ferguson and Hutchinson [8] observed 140 tissue cysts with a minimum of 10 tissue cysts at 11, 21, and 28 days and 3, 6, 12, 18, and 22 months.

Tab. 1: Average of mice survival time and parasite forms detected in each sheep sample bioassayed.

Parasite forms	Sheep #1 bioassay			Sheep #2 bioassay		
	Brain (dpi[a])	Lung (dpi)	Muscle (dpi)	Brain (dpi)	Lung (dpi)	Muscle (dpi)
Tachyzoite	14	-	-	11	-	-
Bradyzoite	24	-	-	12	24	16

[a] dpi: days post-inoculation

Interesting data was observed with sheep 2 brain bioassay. 3/4 mice presented tissue cysts while only 3/4 presented tachyzoites. So, 2/4 presented both parasite forms, bradyzoites and tachyzoites, after 12 d.p.i. (Fig. 1). These findings increase the importance of this parasite to the public health mainly due all parasite detections being after mice had died with clinical signs. So, tissue cysts formation must be occurred few days before, 3 to 6 d.p.i., agreeing with Dubey & Frenkel [6] and Ferreira-da-Silva et al. [10].

Dubey & Frenkel [6] related that the biologic time required for bradyzoite/cyst formation is 3 days after oral infection. Other study, using subcutaneous route, show 11-day delay to the identification of the cyst wall [8]. In other hand, Dubey [2] proposed a developmental cycle of *T. gondii* in tissues of mice fed tissue cysts/bradyzoites considering 4 d.p.i. the lowest time for the invasion in lung, brain and other tissues, and 6 d.p.i. the lowest time for the tissue cyst formation.

Ferreira-da-Silva et al. [10] observed tachyzoites invading skeletal muscle cells 4 hours

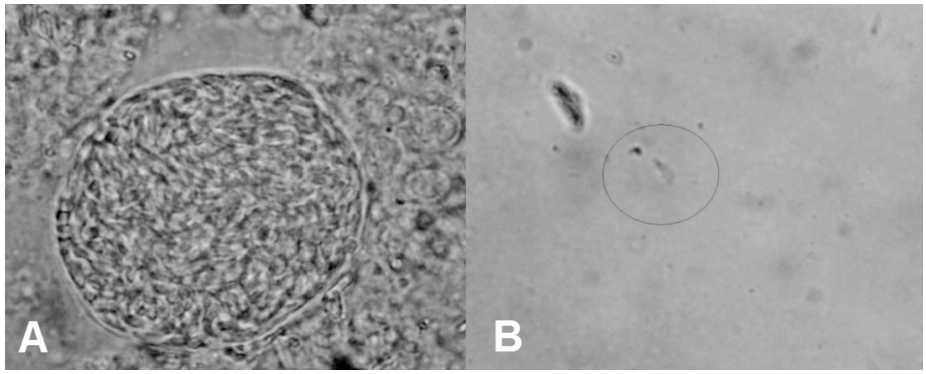


Fig. 1: Parasite forms detected in two mice, concomitantly, from sheep 2. A. Tissue cyst in brain of both mice (100x); B. Tachyzoite in peritoneal fluid of both mice (100x).

post-infection (h.p.i.) and started to replicate within 24 h.p.i., differentiating to bradyzoites until 6 d.p.i. without the need of exogenous stress factors. As detected for this author and in this study, cysts in muscle tissue are the prerequisite for transmission of *T. gondii* by the consumption of raw or undercooked meat products by humans.

The predominant localization of tissue cysts in brain and muscles could, therefore, just reflect the longevity of neurons and muscle cells or the immunological environments in these tissues that would ensure the long term persistence of the latent infection. Conversely, it can also be hypothesized that neurons and muscle cells (or the respective tissues) provide an appropriate cellular environment that readily sustains tissue cyst development [9].

As presented, the chronification of the infection can occur until 12 d.p.i., depending on the route of the infection, parasite load and strain, and the host immunological response [11]. Furthermore, *T. gondii* isolated from Brazil as in some countries of South America presents different virulence profiles, which can result in clinical manifestations of different intensity in many warm-blooded animals, with or without tissue cysts formation.

Conclusion

The chronification occurs until 12 d.p.i., presenting both infectious forms. *T. gondii* isolated from Brazil as in countries of South America presents different virulence profiles, which can result in different intensity of clinical manifestations in many warm-blooded animals, with or without tissue cysts formation. This is probably the first report of short stage-conversion time of *T. gondii*in mice from naturally exposed Brazilian sheep.

Acknowledgement

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Humoral response of ewes naturally infected with *Toxoplasma gondii* in Brazil

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Summary

Toxoplasma gondii produces a disease that generates severe economic losses in the sheep production. The aim of this study was to investigate the immune response of Bergamacia ewes to the natural infection with *Toxoplasma gondii* by the sequential analysis of IgG imunoglobulines for 12 months after the detection of the infection by the Modified Direct Agglutination Test (MAT) and indirect fluorescent antibody test (IFAT) to detect anti-*T. gondii*antibodies. The serological tests showed high degree of concordance, and no significant statistic difference between them.

Introduction

Toxoplasmosis in sheep is a parasitic disease of great veterinary, husbandry and public health importance, causing productive and economic losses [4,6], besides the implication in human health, since the consumption of contaminated meat and milk can facilitate the zoonotic transmission [7]. Naturally *Toxoplasma gondii* infected sheep in lactation were used for the accomplishment. The modified direct agglutination technique (MAT) and indirect fluorescent antibody test (IFAT) were used to determine the toxoplasmic infection. This serological tests constitutes a valuable tool for diagnosis, and it's able to supply a fast result, with low costs, reproducibility and of high sensibility and specificity.

Material and Methods

40 Bergamacia ewes in lactation were used, 20 serologically positive, with no clinical signs of the infection, and 20 serologically negative for toxoplasmosis. The animals were maintained on grazing and "ad libitum" access to water (Figure 1A). The serological monitoring was made fortnightly for 12 months. Blood samples were obtained by jugular punction (Figure 1B), centrifuged at 1600 X g, for 10 minutes. Serum samples were sent to Núcleo de Pesquisas em Zoonoses (NUPEZO), College of Veterinary Medicine and Animal Science, UNESP, and kept at -20 °C until the moment of the serological tests. The IgG antibodies were quantified by the indirect fluorescent antibody test (IFAT), as described by Camargo [2], and the modified direct agglutination test (MAT), following Desmonts and Remington protocols [3]. The serum were serially diluted in the following dilutions,1/16, 1/64,1/256, 1/1024, 1/4096. In both cases, the samples with titers equal or higher than 16 were considered positive. The data were submitted to Minitab Statistic Package®(Version 11) for the Variance Analysis. The values referent to the imunoglobulines were previously transformed to stabilize the variance by the $\log_{10}$ (x+1). The association between the results obtained

on the IFAT and the MAT were calculated by the Pearson's Correlation Coefficient.



Fig. 1. Sheep flock from the Experimental Farm at UNESP (A); Blood collection by jugular venipuncture (B). Botucatu, 2008

Results and Discussion

Until the present moment 24 samples collection were performed, corresponding to 11 months of serological accompaniment. The animals from the naturally infected group, did not present any pertinent toxoplasmic clinical signs. None of the animals from the negative serum group acquired the infection during the 11 monitored months. The highest titer found, by both serologic techniques was 1/4096. The dynamic of the seric antibodies titers, detected by the IFAT and the MAT can be observed in the Figure 2, according to the moment when the samples were collected.

It can be observed that the MAT was more sensitive when compared to the IFAT-IgG. The titers obtained by the MAT were either than those titers obtained by the IFAT-IgG.

In sheep, the use of serological tests for the demonstration of anti-*T. gondii*antibodies has a great value, facing the limitations on parasitological, molecular and clinical diagnosis, aggravated by the almost complete absence of clinical signs [1]. When symptomatic, the disease can assume clinical forms and can be easily confused with a great variety of diseases, difficulting the implantation of specific control measures and treatment [9].

There is a great number of studies about the prevalence of the toxoplasmic infection in sheep, by serological methods, Tenter et al [8] exposed that the frequency of serologically positive animals can be variable, from 3%, in Paquistan and Zimbabwe, to 92% in France. El-Moukad [5], found variable prevalence varying from 0 to 100% sin sheep flocks from Syria.

Conclusion

The persistence of the antibodies titers can be observed in the studied animals, alternating periods of ascension and decreasing, but in a long term evaluation the titers were maintained. The IFAT and the MAT techniques presents high sensitivity and specificity, though there are some favorable aspects related to MAT as simplicity, costs and no need of expensive equipments.

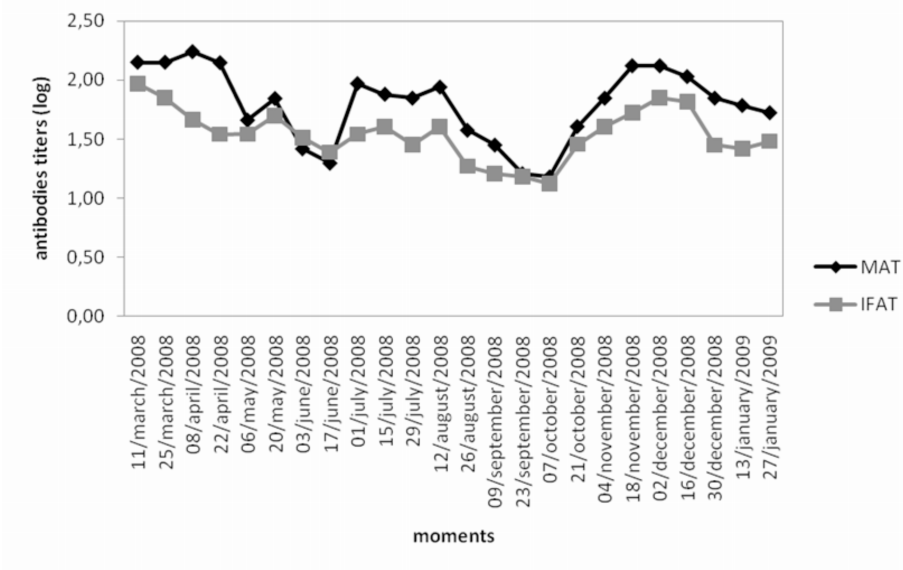


Fig. 2. Seric anti-T. gondii antibodies titers dynamic, detected by the MAT and the IFAT, in naturally infected ewes (G1). Botucatu, 2008.

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Occurrence of *Toxoplasma gondii* and *Neospora caninum* in sheep from Sao Paulo state, Brazil

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Summary

Neoporosis and toxoplasmosis are important infections to ovine production. This study aimed to determine the frequency of antibodies to *Toxoplasma gondii* and *Neospora caninum* in ovine from Sao Paulo state. 382 Brazilian sheep serum samples from eight sheep farms were researched for *Toxoplasma gondii* and *Neospora caninum* antibodies by modified agglutination test (MAT) and indirect fluorescent antibody test (IFAT). 71/382 (18.59%) sera samples reacted to *T. gondii*, with 2/8 (25.00%) of them presenting each one almost 40% positive animals. To *N. caninum*, 49/382 (12.83%) animals reacted, predominantly distributed for 4/8 (50.00%) farms. One farm was negative for both parasites. *T. gondii* and *N. caninum* infections present a concentrate distribution in four studied sheep farms each one where sheep plays an important role for the public health needing the adoption of sanitary measures.

Introduction

Toxoplasmosis and neosporosis are worldwide infections, caused by *Toxoplasma gondii* and *Neospora caninum*, respectively, two parasite protozoan with high importance to ovine production. Toxoplasmosis is the major cause of abortion in sheep causing also foetal malformations, pre-term deliveries and stillbirths and thus generates severe economic losses, and neosporosis is associated with abortion and weak preterm offspring [2,5]. Brazilian seroprevalence of both parasites in naturally exposed sheep is variable depending on the management [9]. This study aimed to determine the frequency of antibodies to *T. gondii* and *N. caninum* in sheep flocks from Sao Paulo state, Brazil.

Material and Methods

382 sheep of eight farms (A to H) from Sao Paulo state, seven located in Botucatu (22*53'09"S, 48*26'42"W) and one in Pardinho (23*04'52"S, 48*22'25"W), upper than two years-old, most of Santa Ines breed and some Bergamacia, were studied. Dog access to the flocks was related by farmers.

Blood samples were obtained by jugular punction, centrifuged at 1600 X g, 10 minutes. Serum samples were sent to Nucleo de Pesquisas em Zoonoses (NUPEZO), College of Veterinary Medicine and Animal Science, UNESP, and kept at -20 °C until the moment of the serological tests.

All serum samples were researched for *T. gondii* by modified agglutination test (MAT) [4], and for and *N. caninum* antibodies by indirect fluorescent antibody test (IFAT) [3], with cut-off titers as 16 (*T. gondii*) and 25 (*N. caninum*). Both tests used homemade antigen.

For MAT, a clear-cut button-shaped deposit of parasite suspension at the bottom of well was interpreted as negative, and a carpet of agglutinated organisms, ranging from 50-100%, was considered to be positive. IFAT was positive when up to 50% of tachyzoite forms, per field, presented complete fluorescence.

Results and Discussion

Tab. 1 shows all serological results for *T. gondii* and *N. caninum* in the farms.

Lower frequency (71/382; 18.6%) of *T. gondii* antibodies was observed in sheep flocks in this study compared to Figliuolo et al. [6] (207/597; 34.7%) in Sao Paulo, Ogawa et al. [7] (185/339; 54.6%) and Romanelli et al. [8] (157/305; 51.5%) in Parana, Soares et al. [9] (85/409; 20.7%) in Rio Grande do Norte, and Ueno et a. [10] (393/1028; 38.2%) in Federal District of Brazil.

To *N. caninum*, 49/382 (12.8%) reacted without any clinical signs, higher than observed for Al-Majali et al. [1] (14/320; 4.3%) in Southern Jordan, Figliuolo et al. [6] (55/597; 9.3%), Romanelli et al. [8] (29/305; 9.5%), Soares et al. [9] (7/409; 1.8%), Ueno et al. [10] (91/1028; 8.8%), Vogel et al. [11] (2/62; 3.2%) in Rio Grande do Sul.

Tab. 1: Frequencies of antibody per farm for T. gondii (MAT) and N. caninum (IFAT) in sheep from Sao Paulo state, Brazil

Farm	Samples	*Toxoplasma gondii*		*Neospora caninum*	
		Positive	Percentage (CI95%)[a]	Positive	Percentage (CI95%)[a]
A	100	11	11.0 (6.3-18.6)	14	14.0 (8.6-22.2)
B	47	18	38.3 (25.8-52.6)	10	21.3 (12.0-35.0)
C	55	4	7.3 (3.0-17.3)	12	21.8 (13.0-34.4)
D	33	3	9.1 (3.3-23.7)	8	24.2 (12.9-41.2)
E	33	2	6.1 (1.9-19.7)	1	3.0 (0.7-15.3)
F	15	0	-	0	-
G	27	11	40.7 (24.5-59.4)	2	7.4 (5.5-48.4)
H	72	22	30.6 (21.1-42.0)	2	2.8 (0.9- 9.6)
Total	382	71	18.6 (15.0-22.8)	49	12.8 (09.8-16.6)

[a] Confidence interval 95%

112/382 (29.31%) positive samples were positive at least to one parasite. Only 8/382 (2.1%) animals presented mixed infection, similar to Figliuolo et al. [6] (21/597, 3.5%), Romanelli et al. [8] (16/305, 5.2%) and Ueno et al. [10] (48/1028, 4.7%), but lower than Bartova et al. [2] (53/547; 10%) in Czech Republic that has different management and breeding. 63/112 (56.2%) only *T. gondii* infection and 41/112 (36.6%) only *N. caninum* infection. High frequency of low *T. gondii* titers was found (Tab. 2), but 27 animals presented high titers as 256 (21; 29.6%) and 1024 (6; 8.4%), showing a probable active infection in most of the studied farms and that sanitary measures must be applied to control both infections [9].

The distribution of both infections is not uniform for all farms in this study. Highest concentrations were observed in four farms for *T. gondii* ("A", "B", "G" and "H") and *N. caninum* ("A", "B", "C" and "D"). Farm "F" was negative for both parasites.

Farm "B" presented high divergence with frequency of antibodies for both parasites from the other farms. 10/18 (55.6%) animals present titers >= 256 for *T. gondii*, and 6/10 (60%) >= 100 for *N. caninum*, indicating active infection and probable disease in these animals. Similar problem was observed only for *N. caninum* in farms "A" (10/14; 71.4%), "C" (2/12; 16.7%), "D" (1/8; 12.5%) and "H" (2/2; 100%), and only for *T. gondii* in farms "C" (1/4; 25%), "D" (1/3; 33.3%), "E" (1/2; 50%), "G" (7/11; 63.6%) and "H" (8/22; 36.4%).

As all farms are dairy farms and most of animals are female, the results show a high economic impact and for public health considering that sheep can abort and shed tachyzoites by milk (*T. gondii*) in acute stage of the infection. Milk is used for human consumption in most of studied farms with positive results for *T. gondii*. This high occurrence in some farms is a problem with the sheep flock management, probably caused on the environmental contamination by oocysts of both parasites shed by cats (*T. gondii*) or dogs (*N. caninum*) with allowed access to the flock [1,2,7,9].

Soares et al. [9] determined that *N. caninum* infection may be not disseminated among dogs, even in farms that allow closed contact of dogs with sheep and grass (40% of the studied farms) or by eating sheep placenta (14.3%).

Tab. 2: Frequencies of antibody titers for T. gondii (MAT) and N. caninum (IFAT) in sheep from Sao Paulo state, Brazil

Titer	*Toxoplasma gondii*		Titer	*Neospora caninum*	
	Positive	Frequency (%)		Positive	Frequency (%)
16	28	39.4	25	17	34.7
64	15	21.1	50	11	22.4
256	21	29.6	100	11	22.4
1024	6	8.4	≥200	10	20.4
Total	71	100.0		49	100.0

Based on this, it is necessary to explain for the rural population about the importance of these two infections and, through some preventive measures adopted in the management, mainly with cats and dogs access to the sheep flock, aiming to reduce the risks of the risk transmission to humans and animals [7].

Conclusion

Both *T. gondii* and *N. caninum* infections are active and disseminated in dairy sheep farms from East of the Sao Paulo state, where sheep plays an important role for the public health, allowing the adoption of sanitary measures.

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Molecular detection of *Toxoplasma gondii* in tissue samples of slaughtered Brazilian sheep

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Summary

Toxoplasmosis is a worldwide zoonosis causing serious damages in humans by the consumption of raw or undercooked meat, mainly from ovine. This study aimed to detect the DNA of *Toxoplasma gondii* in tissue samples of slaughtered Brazilian sheep. 602 sheep slaughtered in a Brazilian slaughterhouse were researched for *T. gondii*. Those serologically positive by modified agglutination test (MAT) were bioassayed in mice and researched for *T. gondii* DNA by polymerase chain reaction (PCR) in tissue samples. The association between the bioassay and PCR was performed using the Fisher's exact test, with EpiInfo-6. 66/602 (11.0%) sera samples presented antibodies to *T. gondii*, with 20/66 (30.3%) positive bioassays and 22/66 (33.3%) positive PCRs for at least one tissue. Significative difference was detected comparing brain PCR and bioassay, lung bioassay:brain PCR and all bioassays with muscle PCR ($P < 0.05$). PCR presented higher sensitivity than bioassay. Thus, PCR was better to detect *T. gondii* being an important to the public health.

Introduction

Toxoplasmosis is a worldwide zoonosis caused by the protozoan *Toxoplasma gondii* that infects all warm-blooded animals including humans, livestock and marine mammals [6,8], causing serious damages in human beings through the consumption of raw or undercooked meat, mainly from sheep [1,7]. This study aimed to detect the DNA of *T. gondii* in tissue samples of slaughtered ovine destined to human consumption in Sao Paulo state.

Material and Methods

602 sheep serum samples from a Sao Paulo slaughterhouse were researched for *T. gondii* antibodies by modified agglutination test (MAT), considering a clear-cut button-shaped deposit of parasite suspension at the bottom of well as negative, and a carpet of agglutinated organisms, ranging from 50-100%, as positive (Fig. 1) [3].

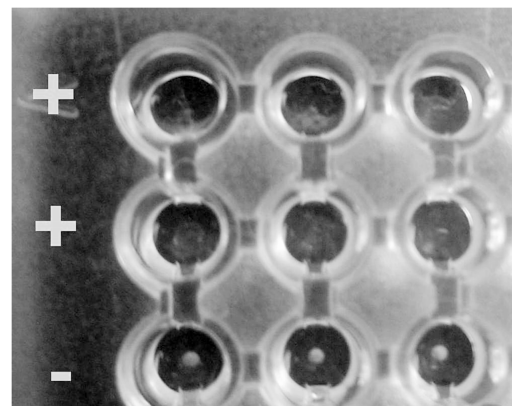


Fig. 1. Positive and negative controls for *T. gondii* using Modified Agglutination Test.

Bioassay and the research for DNA of *T. gondii* by polymerase chain reaction (PCR) were performed with brain, muscle and lung samples of all seropositive animals ($\leqslant 16$). In bioassay, all tissues were digested by pepsin action and inoculated 1mL subcutaneous in Webster albino mouse, 30 days-old, and observed for 60 days [5].

The research for DNA of *T. gondii* was performed by polymerase chain reaction (PCR) with TOX4 and TOX5 primers [11], detecting 10^0 (saline solution and brain) and 10^1 (lung and muscle) parasites in analytical sensitivity. All experiments were performed in the Nucleo de Pesquisas em Zoonoses, DHSVP, FMVZ, UNESP, Campus of Botucatu, SP.

The associations between the serology, bioassay and PCR were performed using the Fisher's exact test with EpiInfo-6, adopting 5% as significance level.

Results and Discussion

66/602 (11.0%; 8.7%>CI95>13.7%) sera samples presented antibodies to *T. gondii*, lower prevalence than Dubey et al. [7] (104/383; 27.1%) in United States, Masala et al. [13] (2048/9639; 28.4%) in Italy, and Ragozo et al. [15] (120/495; 24.2%) in Sao Paulo, but higher than Da Silva and Langoni [2] (40/522; 7.7%) in Sao Paulo.

Only 20/66 (30.3%; 20.6%>CI95>42.3%) resulted positive to bioassay for at least one tissue. Higher sensitivity was verified than Dumetre et al. [9] (26.6%) in France and Ragozo et al. [15] (19.5%), but lower than Da Silva and Langoni [2] (59.0%) and Dubey et al. [7] (77.9%). The same was observed in PCR detecting 22/66 (33.3%; 23.2%>CI95>45.4%) positive PCRs, lower than Aspinall et al. [1] (38.03%), in meat samples from US, and Dubey et al. [7] (77.9%), but higher than Da Silva & Langoni [2] (7.7%), Masala et al. [13] (11.1%) and Masala et al. [14] (14.4%).

Among all bioassayed tissues, significative difference was observed only between brain and lung with four samples detected for both tests, with 13 positive only in brain ($P<0.05$). On the other hand, Tab. 1 shows significative difference in PCRs association between brain:muscle and lung:muscle ($P < 0.05$). Even four positives detected by both brain and muscle PCR, brain PCR detected 13 positive samples alone.

Brain is an important tissue and most parasited by *T. gondii*. Dubey et al. [7] showed heart as another important tissue for *T. gondii* parasitism isolating and detecting the parasite by PCR in 53/68 (77.94%) heart of lambs. The same was observed by Da Silva & Langoni [2] in diaphragm digested by pepsin or trypsin action. Masala et al. [13] with placenta PCR (31.5%), followed by muscle (15.3%) and brain (12.5%), different from Masala et al. [14] detected the high number of positive samles for *T. gondii* in total foetuses PCR (18.1%), followed by muscle (12.4%) and brain (12.0%).

Tab. 1: Association among all tissue PCRs from seropositive sheep samples.

PCR[a]	Brain			Lung			Muscle		
	Positive	Negative	*P*[b]	Positive	Negative	*P*	Positive	Negative	*P*
Brain (+)	-	-	-	0	17	0.5482	4	13	0.0066
Lung (+)	0	2	0.5482	-	-	-	1	6	0.0033
Muscle (+)	4	3	0.0066	1	3	0.0033	-	-	-

[a] PCR: polymerase chain reaction; [b] *P*: significance level (α) = 5%

In Tab. 2, both brain PCR and bioassay detected 12 simultaneously, and five different positive brain samples were detected by each test presenting significative difference between both tests in brain ($P < 0.05$). The same occurs comparing lung bioassay and brain PCR, and when all bioassays were compared to muscle PCR ($P < 0.05$), with 7 (brain:muscle), 3 (lung:muscle) and 3 (muscle:muscle) positives for both tests. In this case PCR presented higher sensitivity than bioassay with a total of nine positive animals ($P < 0.05$).To the lung, no one significative difference was detected between the tests.

Based on these data, PCR presented higher sensitivity than bioassay. In brain most of positives were detected, followed by muscle and lung, respectively. The same as observed by Freyre et al. [10], in rats, and Hugues et al. [12], detecting *T. gondii* in all brains and most of heart and cord of lambs from United Kingdom, specially used to determine co-infection in all studied animals.

Tab. 2: Association among bioassay and PCR results from seropositive sheep samples.

Bioassay	Brain-PCR[a]			Lung-PCR			Muscle-PCR		
	Positive	Negative	*P*[b]	Positive	Negative	*P*	Positive	Negative	*P*
Brain (+)	12	5	0.0000	0	17	0.5482	7	10	0.0006
Brain (-)	5	44		2	47		2	47	
Lung (+)	4	0	0.0033	0	4	0.8815	3	1	0.0068
Lung (-)	13	49		2	60		6	56	
Muscle (+)	2	5	0.5844	0	7	0.7977	3	4	0.0476
Muscle (-)	15	44		2	57		6	53	

[a] PCR: polymerase chain reaction; [b] *P*: significance level (α) = 5%

Brazilian commercial sheep flock is controlled in some areas, but in others the flocks have a closed contact with other animals, mainly cats, allowing the vertical transmission for lambs [4]. Serious problem to public health is marked for Aspinall et al. [1] that consider the majority of the samples studied for food consumption a mixture of meat from several animals (e.g. sausages, pate, etc.), it is expected levels of infection amongst farm animals to be somewhat lower than the measured incidence of parasites in this type of foodstuff. So, the final product has a high concentration of the parasite.

As in Brazil, Aspinall et al. [1] detected a significant proportion of meats commercially available are contaminated with *T. gondii* in United Kingdom. In this study, all animals were destined for human consumption, reinforcing the role of this species as a potential source of infection to man and the importance to have a high sensitivity test to screen all animals before to be slaughtered.

Conclusion

Thus, sheep is an important intermediate host of *T. gondii* and plays an important role for the public health, with some of them detected only with molecular tests, allowing the people to be infected eating the food products of production animals, as sheep meat.

Acknowledgement

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An approach to prevent illegal or aberrant utilisation of animal by-products from slaughter or meat processing for food production

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Summary

The aim of our study was the development of a staining method to mark by-products (category-3-material) from healthy animals slaughtering. Therefore a conclusive, long-term, non-cancellable identification, neutrality in feedstuff, legal innocuousness and a guarantee for the traceability after insertion in food chain were required. For that reason an optic visible marking was intended and investigated for its applicability as marking method on site. On laboratory scale animal by-products were marked with colouring substances, selected from the spectrum of stains registered for use in food and feed production, by an air pressure based spraying device and subsequently evaluated in unprocessed form, after homogenisation as well as after heating and autoclaving. According to the established procedure in meat control, five impartial persons controlled the tissue visually in terms of detectable marking in order to define the required dosage. Meat products and canned pet food were produced from marked material for chromatographic, photometric and fluorescence-spectrometric detection of marking-colorants. Green S (1,875 mg/kg) appeared as useful for positive marking on site, while it showed conclusive marking, no washing effect and it was not visible in processed and heated material. Titanium dioxide (125 mg/kg ABP) was provided for photometric detection. An intermixture of the two pigments as marker solution enables a secure marking of by-products and might help to divide process trails between products intended for food industry and category-3-material for pet food industry. The possibility of detection in processed material promises an improved traceability and a better consumer protection.

Introduction

Misuse of by-products from animal origin (ABP) and false handling of spoiled meat products were key elements of so called meat scandals which recently affected food safety [8,10]. It became evident that the protection of the food chain against aberrant or deliberate utilisation of slaughter by-products, at least up to 14 million tons (giblets, blood, acra) in Europe per year, is deficient [2,7].
Disposal with ABP is specified by Regulation (EC) No 1774/2002 (amendment: No 808/2003) laying down health rules concerning animal by-products not intended for human consumption [12,13]. The named regulations define three categories of ABP, whereat category 3 can be used e.g. in pet food industry, due to the fact that it comes from healthy animals with no or only low risk to animals or humans (fit for human consumption, but not intended for such). Existent uncertainty is caused by unsafe differentiation between commodity flows of animal products for food industry and category-3-material for pet food industry among others [9]. The aim of our project was the development of a marking method for ABP, which might realize a conclusive, long

term-, non-cancellable identification, neutrality in products (feedstuff, further cosmetics and pharmaceutics) and legal innocuousness. For that reason an optic visible marking was intended. The direct visible properties of selected substances might ensure identification in slaughterhouses, meat processing plants and during storage or transport [15]. Chemical analysis of applied markers could guarantee traceability in all grades of food chain.

Material and Methods

Experimental design. ABP were marked with pigments selected from the spectrum of stains registered for use in food and feed production [14]. In addition, substances with fluorescence property and known suitability in medicine were taken into consideration. Available properties of the marking colorants were directly visible qualities, fluorescence or chemical verifiability. On laboratory scale ABP were marked with the selected substances by an air pressure based spraying device and subsequently evaluated in unprocessed form, after homogenisation as well as after cooking or autoclaving. According to the established procedures in meat control five impartial persons controlled the tissue visually in terms of detectable marking in order to define the required dosage. The test discerned between visible marking of the raw material and the non-visible effect in the processed material. Colour scaling was complemented by estimation of RGB-values after scanning and analysis (Image J). Accordingly five persons appreciated fluorescence quality (UV light, wavelengths 256 nm and 366 nm) and possible discolouration in processed ABP (e.g. extracted fat, pet food).

Samples and markers. ABP (tongue, oesophagus, trachea, larynx, lungs, heart, diaphragm, kidneys, belly fat, skin and muscle flesh) were marked with dilutions from several registered food and feed colorants. Amongst others Brilliant Blue FCF (E133), Patent Blue (E131), Indigotin (E 132) and Green S (E 142) were applied for positive marking on site and chemical detection. Uranin, Riboflavin (E 101) and Fluorescein were appointed as fluorescent markers. TiO_2 was chosen as marking substance for chemical detection. Meat products and canned pet food were produced from marked material for photometric, thin layer chromatographic (TLC) and fluorescence spectrometric analysis.

Analytical techniques. Excitation and emission maximum levels of marker dilutions and limpid extracts of processed meat products from unmarked ABP were analysed with fluorescence spectrometer Fluoro Max 2 (S/N 6130, HORIBA). Regarding their fluorescence characteristics, extracts of stained material and of their products were analysed as well. Visualisation was realised with the programme FluorEssence.

Neutrality in processed material from ABP was estimated by visual evaluation of chemically and thermally extracted fat. Chemical extraction of fat was implemented by Soxhlet extraction with petroleum ether (§ 35 LMBG). Thermal extraction was realised by sample storage in a compartment drier (70 °C, 24 h). Detection of TiO_2 in processed meat products followed an adapted method for determination of TiO_2 as inert marker in several digestibility studies [6] by photometric measurement (wavelength: 410 nm).

Detection of Green S (E142) by TLC was designed by the State Office for Food Safety and Consumer Protection, Oldenburg, Germany. Following preliminary studies the modified method ANGKA02 122 (§ 35 LMBG) was used for conditioning of raw and processed marked material: samples were taken from processed meat products (stained or unmarked raw material). Green S was extracted from the homogenised samples with dimethylformamide, applied to a methanol-free

DEAE-cellulose-column and completely eluted from the column. TLC was used for detection of E142. Samples and reference solution were applied to the thin-layer device and dried (mobile phase on silica gel: n-butanol, ethanol, distilled water and glacial acetic acid). The marking pigment was identified by estimation of retention distance and sample colour in comparison to reference solution.

Results

With the demand of colour neutrality in processed meat products the visual control (five persons, quintuplicate) estimated the minimal concentration of Green S for positive marking of ABP with 1,25 mg/kg ABP (25 positive answers for definite marking): In combination with TiO_2 a minimal concentration of 1,875 mg Green S/kg ABP (0,015% Green S solution) was ascertained. 50μg Green S/kg ABP (3% marked material in meat products) were detected by TLC as lower limit. For secure and traceable detection of 4 mg TiO_2 per kg processed meat (10 % marked material in a falsified meat product) a marker dosage of 125 mg TiO_2/kg ABP was assigned by photometrical measurement.
Thermally and chemically extracted fat from so marked ABP was consistently evaluated as unmarked (five persons, quintuplicate test).
Excitation and emission maximum levels from fluorescence spectrometric measurement of marked and unmarked material showed no sustainable differences.

Discussion

Green S (1,875 mg/kg ABP) appeared useful for positive marking on site, while it showed conclusive marking, no washing effect and it was not visible in processed, heated material (canned and dry pet food). Different toxicological studies term the appointed dosage of Green S as uncritical [1,4]. Several methods for determination of synthetic food colorants allow for detection of E142 [3]. Investigations of further pigments showed a positive identification, but discolouring effect in processed feedstuff [11]. The fluorescent markers revealed positive, but low intensive fluorescent properties. Because of animal tissue's own natural fluorescence (e.g. content of riboflavin), the definite fluorescence-spectrometric differentiation of natural and marker fluorescence is hardly assignable [5]. TiO_2 was provided for chemical detection as it is an established and cheap detection method with a simple equipment demand [6]. 10% of TiO_2-marked ABP (125 mg/kg ABP) can be detected in falsified products by photometric measurements. Toxicological investigations could not prove a perioral toxicity of TiO_2. An intermixture of the pigments was made for combination of their properties.

Conclusion

The developed marker solution (Green S, TiO_2) enables a secure marking of ABP and might help to divide process trails between products intended for food industry and category-3-material for pet food industry. The possibility of detection in processed material promises an improved traceability (Green S: DC, TiO2: photometry) and a better consumer protection without disadvantages for pet food products and the consuming pets.

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Chicken Bred under Extensive Conditions as Sentinels for *Toxoplasma gondii* Environmental Contamination: Serology, Isolation and Molecular Characterization

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Summary

Serum samples of 46 in 201 chickens were positive for anti-*Toxoplasma*antibodies. From 24 seropositive chickens with titers ranging from 10 to 2560, *T. gondii* was isolated in 15 samples submitted to the bioassay in mice. Three strains were virulent for mice. Genotypic characterization was carried out using RFLP-PCR analysis of 12 *locci*. Results showed individual genotypes for each of the six strains analyzed, with predominance of markers of type I and type III.

Introduction

During the last 20 years, Brazilian governmental policies have settled families in small rural communities. High prevalence of human toxoplasmosis was reported in one settlement in the state of Mato Grosso do Sul [4]. Due to their feeding habits, poultry bred under extensive conditions may function as sentinels for environmental contamination by *Toxoplasma gondii* oocysts. The present study analyzed infection rates in chickens raised in one settlement in Eldorado, MS, Brazil, as well as the characteristics of the *T. gondii* strains isolated from these birds.

Animals, Material and Methods

Total chicken population consisted of 5,108 birds distributed in 185 farms. Nineteen farms were chosen by a draw, and blood samples were collected from 201 chickens. Serum samples were submitted to direct agglutination for detection of antibodies anti-*T. gondii*. Tissue samples collected from 24 seropositive chickens were used in the isolation of the parasite by means of the bioassay in mice, and in the genotypic characterization of 12 RFLP– PCR molecular markers.

Results

Forty-six samples were positive (22.9%; CI95%: 17.6– 29.2) in 11 (57.9%; CI95%: 36.0– 76.9) positive farms. Positive results in the farms ranged from 8.3% to 100% positive birds. The parasite was isolated from 15 (62.5%) seropositive chickens, with three

strains virulent for mice (Table 1). Rate of isolation was 62.5% (15/24), with a mortality rate in mice of 17.6 % (9/51). Time to death ranged from 14 to 31 days.

Tab. 1: Results of Toxoplasma gondii isolation in the bioassay in mice using tissue samples of 24 seropositive chickens bred under extensive conditions. Eldorado, MS, Brazil, 2008.

Chickens			Bioassay in mice					
Farm #	Sample #	MAT titer	MAT positive / analyzed	Number of dead mice	Days to death	Numbers of cysts	Tachyzoites	Mice positive for *T. gondii*
1	5	640	5/5	0	-	200/0/0/160/0	NO	5
17	1	640	4/4	1	18	40/0/0/40	NO	4
17	3	40	5/5	0	-	160/0/0/0/0	NO	5
17	4	320	1/3	2	19/31	0	YES	3
17	5	160	5/5	0	-	160/0/0/0/0	NO	5
89	1	1280	5/5	0	-	160/120/40/40/0	NO	5
89	2	640	1/5	0	-	0	NO	1
89	3	2560	2/4	1	25	160/80	YES	3
89	4	10	0/5	0	-	-	-	0
89	5	640	1/2	3	16/16/18	40	YES	4
122	5	40	0/5	0	-	-	-	0
133	1	80	1/5	0	-	0	NO	1
133	2	20	1/5	0	-	0	NO	1
133	3	2560	3/3	2	14/16	120/120/0	NO	3
171	3	40	0/5	0	-	-	-	0
171	4	1280	4/5	0	-	120/0/0/0	NO	4
171	5	40	0	0	-	-	-	0
181	1	640	0	0	-	-	-	0
181	2	640	0	0	-	-	-	0
181	3	1280	0	0	-	-	-	0
181	4	320	0	0	-	-	-	0
181	5	80	0	0	-	-	-	0
183	2	20	3/5	0	-	0/0/0	NO	3
183	3	320	4/5	0	-	120/40/0/40	NO	4

Molecular analysis of six of these 15 isolates showed six individual genotypes and absence of mixed infections. Alleles I and III predominated, but allele II was found in two markers of four strains (Table 2).

Tab. 2: Results of RFLP-PCR genotyping of six Toxoplasma gondii strains isolated from tissue samples of seropositive chickens bred under extensive conditions. Eldorado, MS, Brazil, 2008.

Sample	Markers											
	SAG1	SAG2 (3′+5′)	SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico	CS3
F05S1	I	III	III	III	III	III	I	I	III	III	I	u-2
F06S5	I	III	III	III	III	I	I	III	I	III	III	I
F01S1	I	III	III	III	III	III	I	I	I	u-1	I	II
F03S4	I	III	III	III	III	III	I	I	I	III	III	II
F10S3	I	I	I	III	I	II	u-1	I	I	I	I	I
F06S1	I	III	III	III	I	III	I	III	III	III	III	II

Discussion

MAT detected antibodies anti-*T. gondii* in 48% of the samples studied. These results were similar to those obtained in the state of Paraná [2], of 40% of seropositive animals, most of them highly virulent for mice. In the bioassay in mice, nine of the 13 isolates produced infection in 100% of the animals. Mice were killed by three of these isolates, which were classified as belonging to genotype III. Mice that were inoculated with tissues of naturally infected chickens survived for at least ten days.

Population structure and virulence of around a hundred *T. gondii* isolates obtained from several animal species in 11 locations in Brazil [5] involving ten molecular markers and CS3 marker were associated to virulence in mice. Results led to the identification of 48 genotypes. From these, four were isolated in several hosts, and lineages were called types BrI, BrII, BrIII and BrIV. The most virulent type is BrI; BrIII is non-virulent and the others are phenotypes of intermediate virulence. The analysis of CS3 marker substantiated the correlation with virulence in mice.

Brazilian studies using molecular biology show that *T. gondii* strains have important differences in relation to strains isolated in other countries. Ingestion of oocysts may be the main route of human and animal infection in several locations in Brazil, as suggested by some studies [1;3], mainly due to the finding of strains showing recombinant patterns, an indication of the passage through the definitive host.

Conclusion

The presence of chickens seropositive for *T. gondii* demonstrates both the occurrence of environmental contamination with oocysts and the risk of infection for human and animal populations in this rural community. Three genotypes were described for the first time, considering all Brazilian isolates from different hosts and regions studied until now.

Acknowledgement

This work had been funded by Universidade Paranaense (UNIPAR) at Umuarama-PR and by Fundacao Araucaria. Zamprogna TO and Lucas TM had a grant from UNIPAR (PIBIC Program) and Galli S had a grant from Fundacao Araucaria.

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Serology, Virulence Profile and Quantification of *Toxoplasma gondii* in Chicken Bred under Extensive Conditions

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Summary

Serum samples of 167 chickens were tested for the presence of agglutinating antibodies against *Toxoplasma gondii*. Brains and hearts of 26 positive chickens were analyzed using the bioassay in mice and real-time PCR. The parasite was isolated in 21 samples in the bioassay, whereas DNA of the parasite was amplified in 24 of them. Correlation between parasite load in brain samples and serum antibody titers was significant.

Introduction

Chickens, mainly those bred under extensive management conditions, have an important role as indicators of *Toxoplasma gondii* environmental contamination [3]. Although the parasite may be isolated in mice, the procedure is costly, lengthy and may select genotypes that are more virulent for these animals [7], affecting the interpretation and usefulness of the results. The objective of this study was to quantify *T. gondii* in tissue samples of seropositive chickens, as well as to compare results obtained with those of the bioassay in mice.

Animals, Material and Methods

Blood samples were collected from 167 chickens bred under extensive conditions in seven farms in Toledo, PR, Brazil, and were submitted to the modified agglutination test for detection of anti-*T. gondii* antibodies. Brain and heart samples were collected from 26 seropositive chickens to be used in bioassay in mice and DNA extraction for parasite quantification by means of real-time PCR based on SYBER/GREEN detection system directed to a 529 bp repetitive DNA sequence [4]. Inoculated mice were checked daily for clinical disease or death. Weight loss was assessed every week. The rates of DNA amplification in heart and brain samples were compared. Correlation between serum antibody titers and number of parasites in tissue samples was determined by means of Spearman Rank Correlation Test.

Results

Forty-nine (29.3%) serum samples collected in six farms were positive for the presence of antibodies. From 26 samples inoculated in mice, *T. gondii* was isolated in 21 of them (80.8%). Four (19.9%) of these isolates were virulent for mice. From 26 samples analyzed by PCR, *T. gondii* DNA was amplified in 22 (84.6%) brain samples and in 21 (80.8%) heart samples ($p>0.05$) Fig. 1.

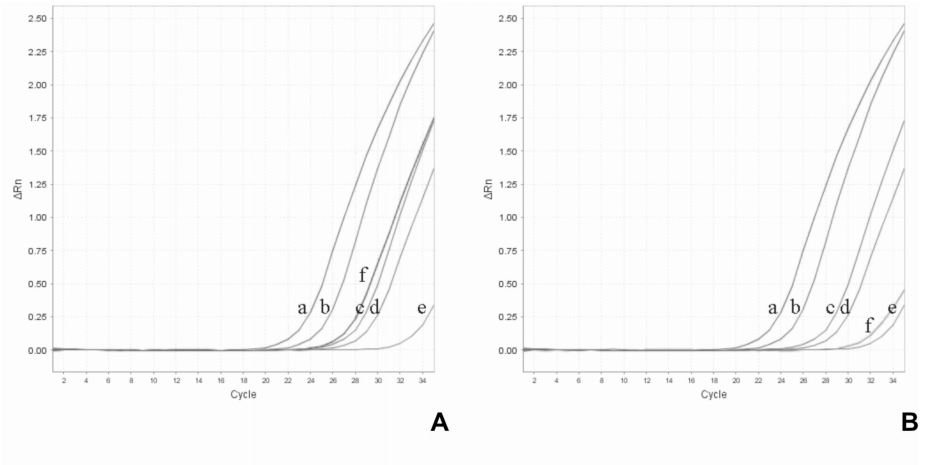


Fig. 1: Real-time PCR targeting a 529 bp repeat element of T. gondii. Amplification plot with 10^4(a), 10^3(b), 10^2(c), 10^1(d), 10^0(e) tachyzoites of RH strain as the initial DNA template mixed with chicken brain suspensions, and a brain sample of a serologically positive outbreed chicken (f) with higher (A) and lower (B) T. gondii DNA content. (Rn, fluorescent signal.

Parasite load in brain samples ranged from 81 to 22,742 parasites/g, with median equal to 437 parasites/g. Parasite load in heart samples ranged from 23 to 982 parasites/g, with median equal to 155 parasites/g. From 0 to 21 days after infection, mice body mass ranged from 44.5g - 5.1g to 45.0g - 6.0g, with 36.4% animals showing weight loss (average = -4.8g). A negative correlation was observed between weight variation and the number of parasites in chicken tissue samples ($r= -0.74$; $p=0.04$), only when the sample showing 22,805 parasites/g was not included in the test. In this case, there was a weight gain of 4.4g - 1.3g. The correlation between antibody titers and the number of parasites in brain (Fig. 2), but not in heart, was significant. A correlation between parasite rates and number of infected mice was also observed.

Discussion

As chickens are biological markers of environmental contamination by *T. gondii* oocysts, the frequency of positive results observed in the rural properties of Toledo is a public health concern. These results demonstrate that other animal populations, as well as men, are at risk for infection. Dubey et al. [3] reported similar results, of 40% of positive animals. On the other hand, Garcia et al.[5] showed only 10.3% of positive results in birds of northern Paran/. The final results may have probably been influenced by the fact that a definitive quantification of the parasite in all brain and heart samples was not possible. However, these

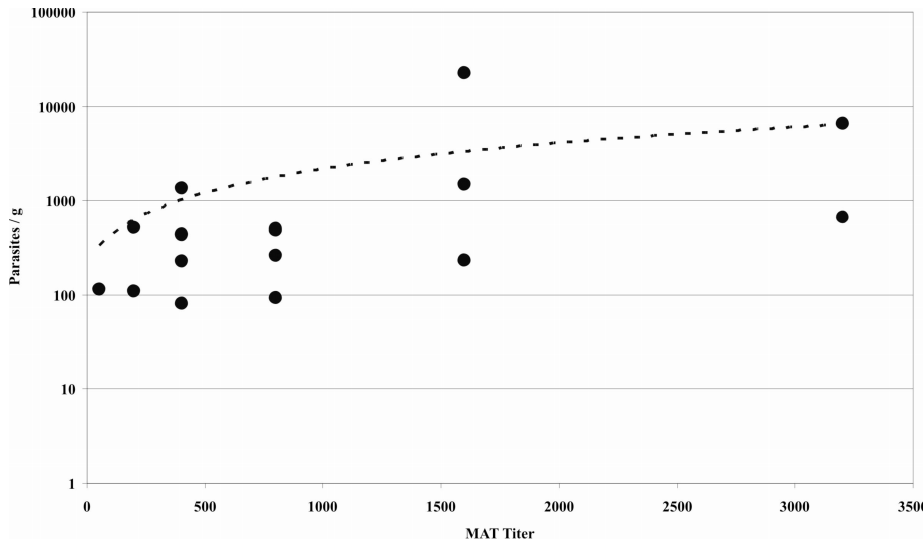


Fig. 2: Correlation between MAT titers and number of parasites / g in brain samples. Toledo, PR, Brazil. 2008.

preliminary results demonstrated that the infective dose may not be the most important factor in determining disease in this experimental model, once weight loss was not observed in animals that received doses of around $2.2x10^4$ parasites, whereas animals that received around 46 parasites lost up to 7g. The influence of parasite genetics is still unknown, and results of future studies on this issue will enable a better evaluation of weight loss as a virulence parameter in mice infected with *T. gondii* strains.

Jauregui et al.[6] demonstrated a correlation between antibody titers and the quantity of parasite DNA in the samples. There were no statistical differences (p=0.6547) between the rates of *T. gondii*detection in the different tissues analyzed, supporting the information that cysts may frequently be found concomitantly in heart and brain, which are the preferred organs for encystation in chickens [6]. On the other hand, the success of the technique is also influenced by the target gene sequence used in the amplification [1].

Conclusion

The presence of chickens seropositive for *T. gondii* demonstrated the occurrence of environmental contamination with oocysts of the parasite. Real-time PCR enabled the quantification of parasites in tissue samples from these birds. A significant correlation between parasite load in tissue samples, mice weight loss and serum titers in chickens was observed.

Acknowledgement

This study was funded by Universidade Paranaense (UNIPAR) at Umuarama-PR, Alvaro Centro de Anilises e Pesquisas Clinicas and Fundacao Araucaria de Apoio Cientifico e Tecnologico do Parana. Aracjo JR and Piassa FR had a grant from UNIPAR (PIT Program); Rosa RC and Mattei RJ had a grant from UNIPAR (PIBIC Program); Silva RC had a grant from Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP); Langoni H had a grant from Conselho Nacional de Pesquisa e Desenvolvimento (CNPq). Piassa FR received young scientist financial support from the Prof. Tielen Fund Foundation for this ISAH 2009 presentation.

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Microarray technique for the detection of zoonotic pathogens in pork (PorkChip)

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Introduction

Zoonotic pathogens are a constant thread to human health and most of the human infections are mediated by food. As pork is one of the most important meat consumed in the world it is necessary to have reliable and fast tools for the detection of food spoiling bacteria. Therefore we developed a new microarray based system for the parallel detection of microbial zoonotic pathogens from swine within the BMBF financed "PorkChip" project. Molecular biological techniques like DNA-microarrays or PCR become increasingly more important for the detection of microbial pathogens in the food chain. Most of the diagnostic DNA-microarrays developed up till now include PCR amplification steps prior to hybridisation. These arrays combine parallel detection of multiple parameters with a fast and precise turnover of results. However the included pre-PCR steps are often based on multiplex PCR, but these-although commonly used-approaches are often problematic because of their high complexity of reaction mixtures. During the exponential phase of amplification the high diversity of reactants (due to the multiplex approach many different primer pairs are present) often leads to the occurrence of non calculable interactions of many different oligonucleotides resulting in false positive signals after hybridisation on the microarray. To overcome these pitfalls, a ligase dependent simplex PCR (LDA-Simplex-PCR) for the parallel detection of seventeen different target sequences of the most common zoonotic swine pathogens, without the use of multiplex primer pairs, was developed. Pathogens included in the project are enteritis infectiosa causative organisms like *Campylobacter*, STEC, *Salmonella* and a broad spectrum of other zoonotic pathogens (*B. melitensis*, *L. monocytogenes*, *M. tuberculosis*, *M. bovis*, *C. botulinum*, *C. perfringens*, *B. anthracis*, *S. suis*, *T. spiralis* and *T. gondii*).

Material and Methods

As shown in the workflow illustration in Fig.1, a two step detection method was applied. Basic principle of the ligase dependent preamplification is the hybridisation of two oligonucleotides in close proximity on the respective target sequence (modified with universal primer sequences), their ligase dependent linking and the following amplification of the LDA generated sequences via a simplex universal PCR and detection with on the DNA microarray.

A. LDA-Simplex PCR. 1. Ligase dependent amplification of target regions with species specific probes
2. Generation of modified preamplicons
3. Simplex-PCR of preamplicons with universal primer pair

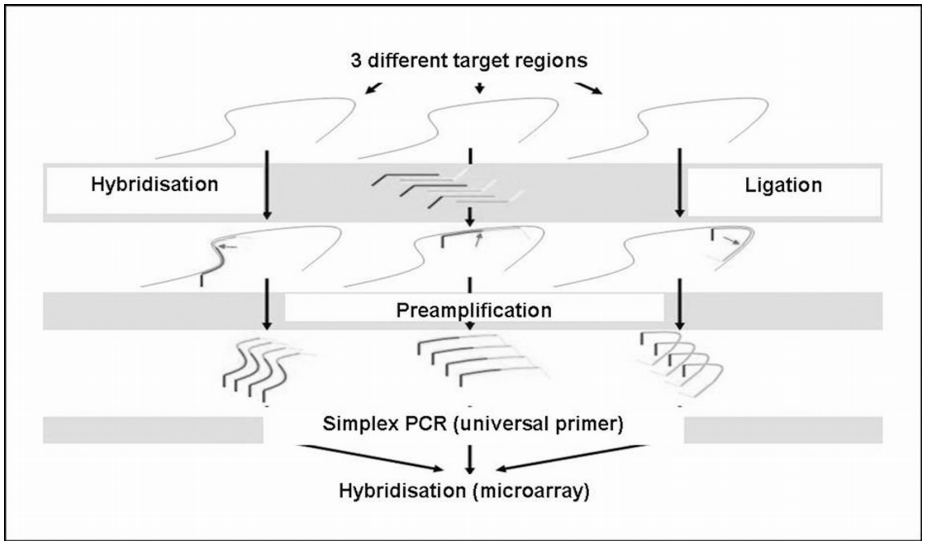


Fig. 1: Workflow of the LDA-Simplex-PCR

B. Hybridisation on DNA microarray. 1. Immobilisation of probes on PorkChip surface
2. Addition of modified preamplicons
3. Specific interaction
4. Detection of specific interaction patterns

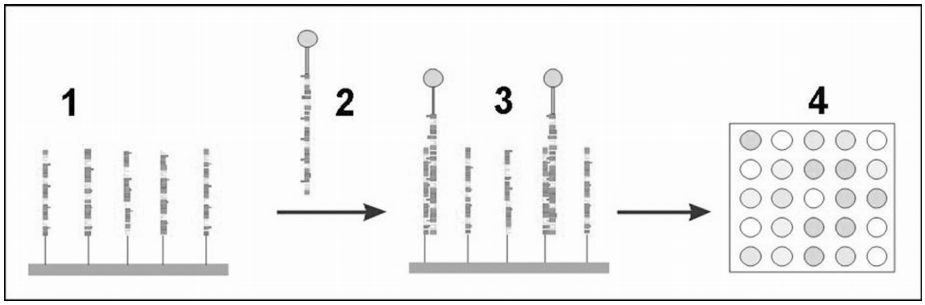


Fig.2: Workflow of the hybridisation on the PorkChip microarray

Results

The proof of principle of the LDA-Simplex-PCR combined with subsequent microarray hybridisation is already accomplished for seven of the selected pathogens.

Conclusion

The first results in the development of a DNA microarray for the parallel detection of microbial zoonotic pathogens from swine as a tool of pathogen monitoring are promising. The next steps include implementation of the other pathogens to achieve to goal of 17 different targets. Furthermore sensitivity and specificity of the Porkchip-Microarray have to be verified.

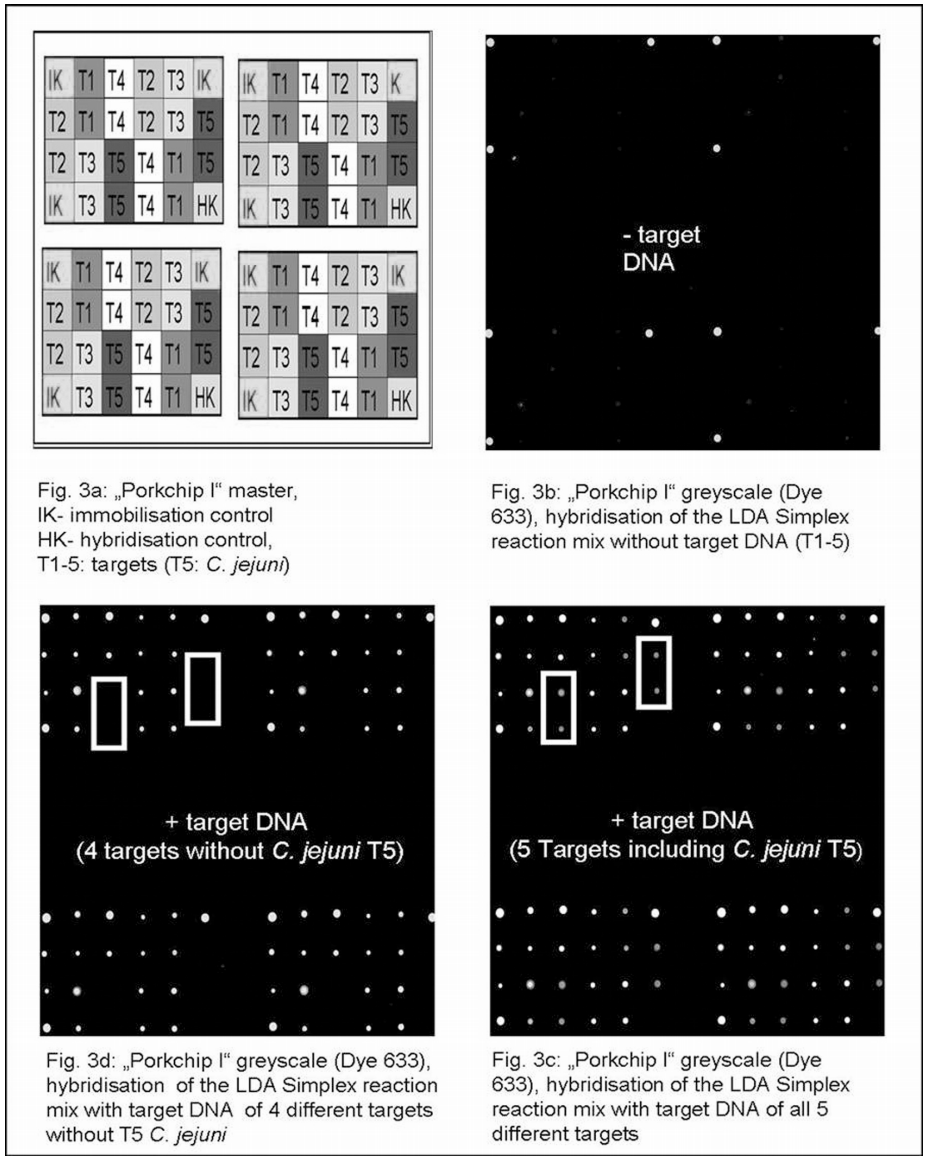


Fig. 3a: „Porkchip I" master, IK- immobilisation control HK- hybridisation control, T1-5: targets (T5: *C. jejuni*)

Fig. 3b: „Porkchip I" greyscale (Dye 633), hybridisation of the LDA Simplex reaction mix without target DNA (T1-5)

Fig. 3d: „Porkchip I" greyscale (Dye 633), hybridisation of the LDA Simplex reaction mix with target DNA of 4 different targets without T5 *C. jejuni*

Fig. 3c: „Porkchip I" greyscale (Dye 633), hybridisation of the LDA Simplex reaction mix with target DNA of all 5 different targets

Fig. 2: Results of hybridisation of 5 targets different after LDA-Simplex-PCR and hybrdisation on the DNA-microarray (Fig. 3d: *C. jejuni* missing)

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Comparative analysis of equine and human MRSA isolates revealed an Extended Host Spectrum Genotype (EHSG)

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Introduction

Methicillin–resistant Staphylococcus aureus (MRSA) are of increasing importance in veterinary medicine, in livestock as well as in companion animals and horses (1, 3, 5, 6, 7). Up to now, knowledge about the epidemiology of these pathogens in horses is still poor, especially regarding a possible species transmission. So far the phylogenetic relationship of strains from human and equine origin has been addressed by utilizing common standard classification methods for MRSA. This work goes beyond this base line of typing procedures in order to comparatively characterize equine and human MRSA strains of similar phylogenetic background.

Animals, Material and Methods

A total of 19 MRSA strains were collected from 2003-2007 and stored at the Institute of Microbiology and Epizootics (IMT), Freie University Berlin, which provides MRSA confirmation and typing for MRSA strains of animal origin. The strains were initially collected from routine microbiological specimens at the IMT, the Synlab-vet laboratories (Augsburg, Bavaria), the Animal Health Services Bavaria (Tiergesund-heits-dienst Bayern e.V), and the Institute of Hygiene and Infectious Diseases of Animals (Justus-Liebig University Giessen, Hesse). On receipt at the IMT, strains were confirmed as MRSA by clinical diagnostic and genotyping methods (6). All strains were typed by Multi locus sequence typing (MSLT), macrorestiction analyses via PFGE, and spa typing. SCCmec typing and PVL detection was done by PCR (6, 7). In addition to this, a microarray analysis of altogether 185 structural, virulence– associated and resistance loci was applied to all strains (2, 4) and various human MRSA strains for comparison.

Results

As a result, the clonal complex (CC) 8 was absolutely predominant (16) in the 19 investigated equine MSRA strains. Of CC8, 13 strains belonged to sequence type (ST) 254, three others to ST8. This genotype has been isolated from different equine patients in various regions over several years, substantiating the apparent predomi-nance of CC8 STs in MRSA

strains of horses worldwide. Beyond that, comparatively investigated human strains of ST254 displayed indistinguishable molecular typing results to those of equine origin. Two further equine strains (ST22, ST1117) showed similarity to ST22 human strains (CC22). The respective STs are typical hospital-asso-cia-ted medical strains, being associated with nosocomial infections in the medical area. One equine strain was of ST398, a genotype recently described to be frequently isolated especially from specimens of pigs and pig farmers as well as veterinarians.

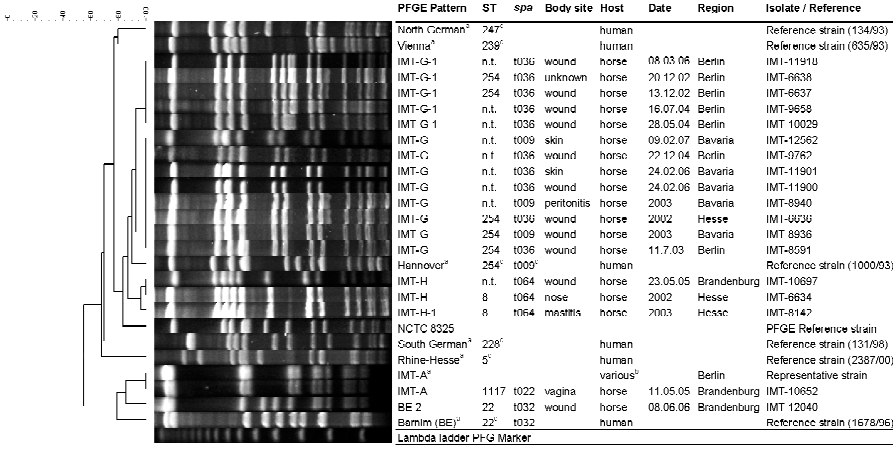


Fig. 1: Dendrogramm (% similarity) showing DNA restriction pattern after digestion with *Sma*I for 18 of 19 equine MRSA PFGE profiles (A, BE-2, H and H-1, G and G-1) of this study in direct comparison to human epidemic strains with GelComparII using UPGMA. Dice coefficient: 1,2% tolerance and 0.5% optimisation). One equine isolate (IMT-12553: ST398) was non-typeable by using endonuclease *sma*I and was therefore excluded for PFGE analysis

Discussion

Accumulation of microbiological characteristics does not replace proper epidemiolo-gical investigation for conclusions regarding (species-) origin of infection or interspecies transferability. Nonetheless, the extensive molecular pathogen profiling of MRSA from human and animal origin presented here may provide evidence for the possible easiness of interspecies transferability of certain MRSA genotypes (here: CC8, CC22, ST398) and may contribute to clarify the question why some genotypes seem to be easily transferable between different hosts: they are obviously adapted to more than one mammalian species, and we propose to call these genotypes "extended host spectrum genotypes (EHSG)". Of particular interest are strains of ST398, as these have recently spread in pigs and are increasingly isolated from farm animals as well as people, having close contact to those animals. Obviously ST398 also is able to infect horses, as strains of this ST have already caused nosocomial infections in horse clinics (1). Thus, ST398 strains seem to be particularly prone to act as EHSG.

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Establishing real-time PCR for detection of *Trichinella spiralis*, *T. britovi* and *T. pseudospiralis* in muscle tissue

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Introduction

Trichinella species cause infections in many wild and domestic animals throughout the world resulting in human trichinellosis. Pigs are of special importance for the infection of men. Domestic pigs in Europe may be predominantly infected by three *Trichinella* species: *T. spiralis*, *T. britovi* and *T. pseudospiralis*. There is a growing number of "imported cases" of trichinellosis [1,5] and an increasing free range farming of swine with possible contact to infectious material as well as a high consumption of game (especially wild boars with prevalence of *Trichinella*: 0.001-0.01%) led to raising numbers of trichinellosis cases in the last years [4,6]. Present methods for detection of *Trichinella* larvae like the compressorium method or artificial digestion often lack sensitivity. Furthermore these techniques are labor-intensive, time consuming and do not differentiate isolates on the species level. Additionally, present available PCRs are used for the identification of isolated larvae on species/genotype level after artificial digestion, but they are not suitable for detection of larvae direct in infectious meat. The aim of this study was therefore to establish a real-time PCR assay for the accurate, rapid and specific detection of the three most common *Trichinella* species in Central Europe in pork without predigestion.

Material and Methods

Detailed protocols are given in Guenther et al. 2008 [3]. Real-time PCR: The real-time PCR detection of *Trichinella* spp. was analyzed with Roche's LightCycler 1.5 instrument, (Roche Applied Science, Basel, Switzerland) including SYBR®-Green amplification detection (SureMASTER dsDYE, Congen, Berlin, Germany). After an initiation step of 10 min at 95 °C we performed 40 cycles of 95 °C denaturation for 15 sec, 58 °C annealing for 5 sec, and 72 °C elongation for 15 sec. A 10 min 72 °C final elongation step followed. Fluorescence was measured at the end of each 72 °C elongation step. After amplification we performed a melting curve analyses from 65-95 °C. Additionally primer pairs were tested against negative controls including eukaryotic DNA from pork, DNA from pig faeces as well as DNA from common European swine parasites (*Ascaris suum*, *Hyostrongylus rubidus*, *Oesophagostomum quadrispinulatum* and *Trichuris suis*).

Table 1. Primers, PCR products with calculated melting temperatures

Species	Primer (5´-3´) [sense/antisense]	Base pairs	Tm (°C)
T. spiralis	ATAATGGTTATTAGGTATTATGT AAACAAAAGAACTACCACCCTTTA	272	82,0
T. pseudospiralis	TAGTAATAATTATGTGGTACAGCTTT GAACCACCACCCTCTTAAATAGAACAG	231	78,3
T. britovi	TAATGATGGTTAAGTATTATG AATAAAAGAATCAACATCCTTTA	271	82,8

Results

Establishment of the real-time PCR conditions with reference strains and negative controls. For each of the three examined *Trichinella* species we were able to establish species specific amplification signals. Fig.1 shows data of one exemplary experiment, illustrating results for *T. britovi*.

Detection limit of *Trichinella*. The specific amplification limit using serial dilutions of pure *Trichinella* DNA in water was found at 10 fg of DNA. With regard to the serial dilutions of *T. spiralis* infected pork tissue, we were able to specifically detect *Trichinella* larvae down to 0.1 larvae per gram pork tissue.

Identification of field isolates. The specificity of the assay was additionally evaluated with field isolates from the BfR strain collection. Nine field strains of *T. spiralis* and 2 strains of *T. britovi* were examined. We assigned all field strains correctly to a respective *Trichinella* species.

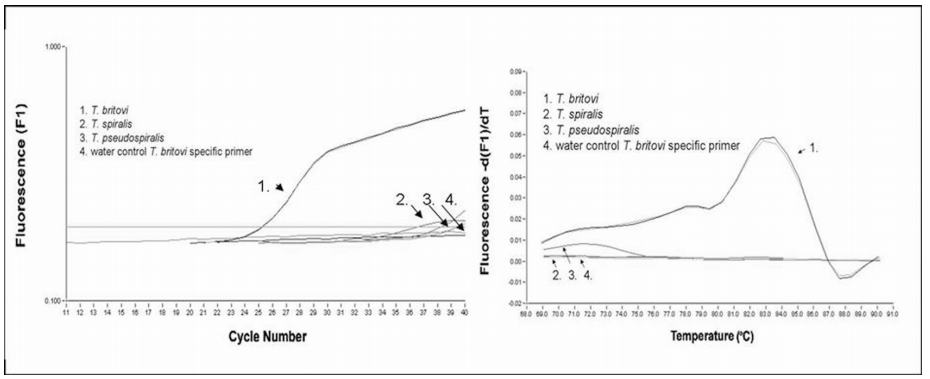


Fig: 1. Left panel: amplification curves of three*Trichinella* reference strains (*T. spiralis*, *T. pseudospiralis* and *T. britovi* and negative controls) with *T. britovi* specific primer. 1. *T. britovi* (ISS002). 2. *T. spiralis* (ISS003). 3. *T. pseudospiralis* (ISS0013). 4. negative control., (concentration of DNA samples. 50ng/l DNA)

Right panel: melting curves of this experiment shown as the -d(f1)/dT derivation. Melting temperature *T. britovi*: 83 °C. Experiments were repeated with at least 3 biological and 2 technical repeats. Shown is one exemplary biological sample.

Discussion

Although rRNA dependent strain identification still presents one of the gold standards in molecular identification of *Trichinella*, our experiments revealed false positive signals with primers targeting the ribosomal ITS region in DNA samples with a high eukaryotic background like pork. Therefore the real-time PCR assay described here utilizes PCR primers targeting the mitochondrial rRNA (*rrnL*) genes of *Trichinella* spp. The mitochondrial rRNA genes have previously been shown to be highly useful to improve the detection of *Trichinella* as well as other nematodes [7] and we found higher sensitivity and specificity utilizing the *rrnL* targeting primers.

The detection limit experiments in this study revealed that the real-time PCR protocol fulfils the requirements for a robust pathogen detection system. After grinding of pork, we were able to detect 0.1 lpg in muscle tissue without digestion of meat, proofing the high discriminatory power of the assay which is needed for sufficient identification of *Trichinella* DNA in high eukaryotic DNA background. This sensitivity is ten times higher then that required for detection of at least 1-3 lpg in muscle tissue in order to prevent clinical trichinellosis in humans [2].

Based on the above described data, we can state that the real-time PCR assay designed in this study is species-specific for the three examined members of the genus *Trichinella* (*T. spiralis*, *T. pseudospiralis* and *T. britovi*) for larvae and spiked tissue samples, as no cross-reactions were observed with *Ascaris suum*, *Hyostrongylus rubidus*, *Oesophagostomum quadrispinulatum* or *Trichuris suis*, common nematodes in swine.

One main advantage of this real-time PCR assay compared to conventional PCR or sequencing methods is thefast performance. By using this approach,it was possible to reliable identify larvae of field strains of *Trichinella* within 2-3 hours, including DNA extraction what may be of diagnostic value for detectionof *Trichinella* in meat samples. The current algorithm for *Trichinella* inspection includes the digestion of meat samples which is more labour-intensiveand lacks the ability to distinguish between the different *Trichinella* species.

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Detection of *Saccharopolyspora rectivirgula* in bioaerosols from animal stables by a species-specific 16S rRNA gene primer system

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Summary

For species specific detection of *Saccharopolyspora rectivirgula* we initially designed a PCR primer system targeting the 16S rRNA-gene of *S. rectivirgula* DSM 43747^T based on current sequence information from primary public databases. Despite the optimization of PCR protocol we could only detect the type strain (43747^T) and strain DSM 43114 whereas five further reference strains remained undetectable. As a consequence, we analysed the 16S rRNA gene sequences of all other strains by cloning, to obtain more detailed sequence information. The analyses revealed sequence differences at the target position, between and within the strains, which can be explained by different 16S rRNA operons. Based on these informations a novel primer system (Act1886F/Srv1432R) for the species specific detection of *S. rectivirgula* was designed for bioaerosol samples. First results revealed a high amplification efficiency of 16S rRNA – genes from all available strains of *S. rectivirgula* using the new designed primer system.

Introduction

Saccharopolyspora rectivirgula is a moderate thermophilic, grampositive species belonging to the phylum *Actinobacteria*. High concentrations of *S. rectivirgula* spores were found in different environments like agricultural production, compost facilities, mushroom cultivation or rooms with technical air moistening. *S. rectivirgula* is described as a causative agent of exogen allergic alveolitis (EAA). Because of this medical relevance, a standardised detection process is urgently needed. Current detection methods for *S. rectivirgula* e.g. at working places are often based on cultivation approaches. But these methods are time consuming and low in specificity. Furthermore, nonviable or dead bacterial cells which can also cause allergic reactions remain undetected. Hence, molecular approaches can be a useful alternative.

The aim of this study was the development of a quantitative realtime-PCR assay for the specific detection of *S. rectivirgula* in bioaerosols.

Material and Methods

For testing the species specific realtime PCR assay, we investigated seven *S. rectivirgula* reference strains: DSM 43747^T, DSM 43113, DSM 43114, DSM 43371, DSM 43755, DSM 43169, DSM 43163 ordered from the DSMZ (Deutsche Sammlung für Mikroorganismen und Zellkulturen). All strains were either grown on the medium M65 (www.dsmz.de)

or TSA (tryptone soy agar) at a temperature of 50°C. For extraction of genomic bacterial DNA one incubation loop of bacterial cells were transferred and mixed with 1 g Zirconia beads (Biospec Produkts, Bartlesville) in a reaction tube. Subsequently the cells were disrupted by shaking this mixture on a vortexer (Vortex-Genie 2, Carl Roth, Germany) for 10 min. DNA extraction steps were performed with the GenElute^{TM} Plant Genomic DNA Miniprep Kit (Sigma) following the instructions of the manufacturer. Finally the DNA was dissolved in 150μl of DNA free water. All amplification reactions were performed in the iQ5 Cycler (Bio-Rad, München) using iQ^{TM} SYBR$^{(R)}$ Green Supermix (Bio-Rad).

Results

Initially, we designed a 16S rRNA-gene PCR primer system, based on current sequence information available from primary public databases, for the type strain *S. rectivirgula* DSM 43747^T. We investigated the seven different *S. rectivirgula* strains obtained from the DSMZ, using this primer system. Despite the optimization of a PCR protocol, an amplicon could only be recognized using DNA extracted from the type strain (43747^T) and strain DSM 43114. As a consequence, the 16S rRNA gene sequences of all deposited strains of *S. rectivirgula* were analyzed. Unexpected "background" sequences visible in the chromatogram led to more detailed investigations (cloning approaches) to obtain additional sequence information. The sequence analyses show differences at the target positions for the strains of *S. rectivirgula*. Based on these new information we designed a further primer system (Act1886F/Srv1432R) for the detection of *S. rectivirgula* in bioaerosol samples by realtime-PCR which allowed the amplification of 16S rRNA gene fragments of all investigated *S. rectivirgula* strains. As standards for quantitative realtime-PCR, 16S rRNA-gene amplicons from the type strain (43747^T) were used (Fig 1A). Amplification efficiencies (85% till 100%) of 16S rRNA – genes from all tested *S. rectivirgula* strains were detected. The corresponding melting curves showed high specificity of amplicons (Fig. 1B). Using a generated calibration curve, we successfully quantified the adjusted genome numbers (10^5) in all PCR assays of tested DSMZ strains.

Discussion

Current detection methods for measuring microbial exposure levels at work places are based mainly on cultivation approaches which are carried out after bioaerosol sampling. With these approaches however, only viable microorganisms which are able to grow under the selected cultivation conditions (medium, pH, temperature, humidity etc.) are detectable. Therefore, these methods are unsuitable for the detection of dead or slow growing microorganisms and also for viable but non-cultivable (VBNC) microbes which can also cause allergic reactions. Molecular methods, like the detection of selected genes by the polymerase chain reaction (PCR) offer the advantage of a more sensitive and specific detection approach. These methods targeting specific gene sequences have already been applied successfully to investigate microorganisms in different environmental samples. Quantitative realtime-PCR (qPCR) seems to be a potential method for the species or genus specific quantification. While this method has rarely been applied to bioaerosols, it has not been standardized for occupational exposure measurements. First results in this study revealed a high amplification efficiency of 16S rRNA – genes from all available strains of *S. rectivirgula* using the new designed primer system. To ensure a valid and reliable quantification method the specificity has to be tested in future with DNA isolated from complex bioaerosols from different working places.

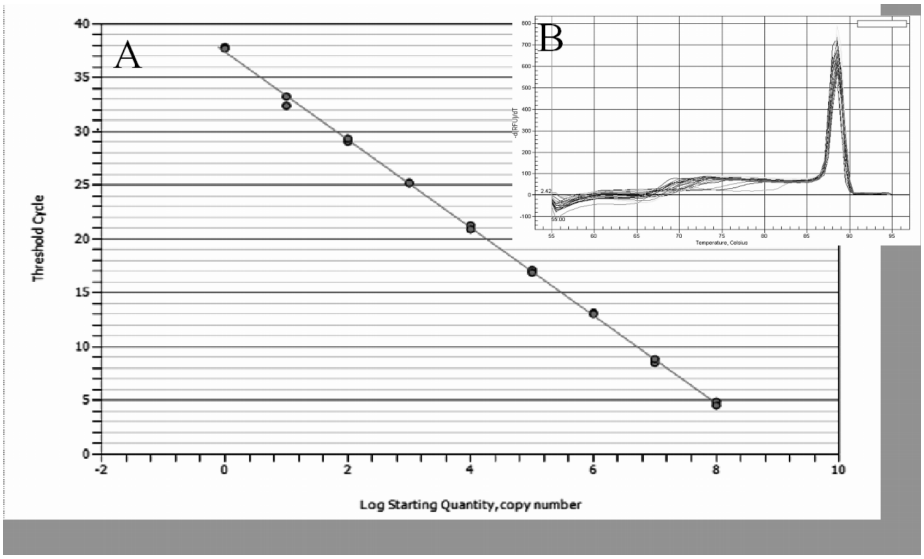


Fig. 1 Quantitative realtime-PCR calibration curve of *S. rectivirgula* specific 16S rRNA-gene primers (Act1886F/Srv1432R) (A), and melting curves of obtained PCR products out of DSMZ strains (B).

Listeria Contamination in Meat processing plants

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Summary

The aim of this study was to examine the environment contamination with *Listeria* in the slaughtering and meat processing facilities, having the same characteristics for handling routine. The test of *Listeria* was used to evaluate the microbiological quality of the slaughtering line and meat processing facilities contamination level. In the slaughtering plants *Listeria innocua, L. monocytogenes* and *L. welshimeri* were found (22%), mostly on different surfaces of chilling chambers and occasionally in stables, but not along the slaughtering lines; however in the processing plants same *Listeria* species were mainly found (57%) in chilling chambers, processing and packing rooms. High levels of potential zoonotic *L. monocytogenes* in a range from 11% from climate conditioners to 100% on the surfaces can be attributed to irregularly sanitation routine, biofilm and a human factor, such the inadequate separation between raw and processing areas.

Introduction

Listeria monocytogenes is the important foodborne pathogen to humans causing illness listeriosis. Although listeriosis is less common than salmonelosis or campylobacteriosis, 92 percent of listeriosis cases require hospitalization. In Slovenia the number of human listeriosis is relatively low, however between 1965 to 1970 the incidence was in average 3,5 cases yearly, in the next eight years decreased to only 1,5 cases yearly [1]. *Listeria monocytogenes* is ubiquitous and widely spread in the environment, thus the contamination of dairy and pig slaughtering likewise processing plants are now widely recognized as important transmission routes of *Listeria*. *L. monocytogenes* can be disseminated by several environment sources like floors, drains, standing water, equipment, employees, animals, food additives, packing material and other [2]. The possible cross-contamination by many reservoirs, especially food processing environment is suggested, thus effective sanitation in slaughtering and processing plants is recommended [1]. On the other hand it should be considered that *L. monocytogenes* is psychrotropic and resistant at a wide range of temperatures and disinfectants, especially when entrapped in a biofilm. *Listeria* is capable to growth in moist and refrigerator environments [3]. It has been suggested that in unsuitable conditions for other bacteria, listerias may grow easier, nevertheless the interactions in biofilms owing to resident microorganisms may restrain unimpeded *Listeria* growth [4]. *Listeria* survives in refrigeration temperature that is why *Listeria* potentially colonizes refrigeration units and air climate conditioners; therefore it could easily colonize various surfaces including raw and ready-to-eat meat products [5]. Furthermore *Listeria* has special ability of growth under conditions which are unpleasant to *Listeria* competitive microorganisms [6]. Understanding the prevalence of *Listeria* contamination of the red meat slaughtering and processing plants and its incidence to contaminate tested

surfaces, mostly described like potential reservoirs of *L. monocytogenes* were the main objectives of this test.

Material and Methods

In order to investigate*Listeria* presence from potentially contaminated surface areas samples, (n=135 in 3 slaughter houses and n=43 in 3 processing plants) were taken in similar slaughtering stables regulated to EU standards, arranged for pork and beef slaughtering and red meat processing on the equipment and areas along slaughtering lines and in alike emptied meat chilling chambers, meat processing and packing rooms, before and during slaughtering. The swabs [20 cm^2] from the surfaces for *Listeria* examination were taken before and after slaughtering and amongst meat products processing on specific locations where *Listeria spp.* might be found and where surface food contact is expecting. Swabs from surfaces were examined on *L. innocua, L. monocytogenes and L. welshimeri*, using standard laboratory methods for *Listeria* isolation using selective broths and selective agars (Fraser broth, Oxford agar, Palcam agar, Aloa agar).

Results

Tab. 1 *Listeria* in stable and chilling chamber from slaughtering plants

Stable				**Chilling chamber**					
N°	**Location**	n	***Li***	N°	**Location**	n	***Li***	***Lm***	***Lw***
1	surface of stable wall	8	***0***	4	floor	10	***5***	***0***	***3***
2	pen crossbar	7	***1***	5	wall	16	***2***	***0***	***2***
3	crossing and pen floor	14	***3***	6	climate conditioner [fan flaps]	9	***1***	***1***	***0***
				7	ceiling traverse	4	***1***	***0***	***0***
					overhead rail	7	***0***	***0***	***0***

Li – L. innocua, Lm – L. monocytogenes, Lw – L. welshimeri, n – number of sampled smears, N°– sample number

Discussion

Regarding on potential reservoirs, stables seems to be contaminate by *L. innocua* most on the floors, boxes pens construction and on crossing pathways as well (Tab. 1). Positive samples (pen crossbar/14,2, floor/21,4%) in the stables suggest to improper sanitation procedures, which provide biofilm and nutrients, suitable for the growth of tenacious bacteria like *Listeria*. The chilling and the storage of meat products is very important, thus the meat concentration, the use of the air conditioners and the people movement leading to the high possibility of the meat contamination. In our study the floors and the walls of chilling chambers in slaughtering plants were contaminated by *L. innocua* (floor/50%, wall/12,5%) and *L. welshimeri* (floor/30%, wall/12,5%), since climate conditioners were contaminated even by *L. monocytogenes* (11%), *L. innocua* (11%) and on the ceiling traverses (25%) as well (Tab. 1). High *Listeria* positive tests in the tested chilling chambers may indicate problems with condensate on the ceilings, floors, walls and floors, thus moist and chill

Tab. 2 *Listeria* along the slaughtering line from slaughtering plants

Slaughtering line

N°	Location	n	*Lspp*	N°	Location	n	*Lspp*
8	wall and floor under stunning box staircase	7	***0***	18	tripe removal stand floor	1	***0***
9	floor under skinning and wall next to skinning	14	***0***	19	drain next to the blood channel	1	***0***
10	wall near entrance to chopping	1	***0***	20	floor under the trimming stand	2	***0***
11	head washing fence	5	***0***	21	floor under the carcass finalization	1	***0***
12	floor of veterinary inspection place	1	***0***	22	floor under veterinary samplig	1	***0***
13	Traverse	1	***0***	23	floor next to the entrails removing channel	1	***0***
14	floor next to the tripe removal	1	***0***	24	wall near entrance to veterinary inspection place	1	***0***
15	floor under the pillar standing	1	***0***	25	carcass washing stand	3	***0***
16	floor under the carcass labeling	1	***0***	26	floor under carcass splitting	4	***0***
17	head washing stand	1	***0***	27	floor under weighing	1	***0***

Lspp–L. innocua, L. monocytogenes, L. welshimeri, n – number of sampeled smears, N° – sample number

Tab. 3*Listeria* in chilling chamber, processing room and packing room from meat processing plants

	Chilling chamber						**Processing room**						**Packing room**				
N°	**Location**	n	***Li***	***Lm***	***Lw***	N°	**Location**	n	***Li***	***Lm***	***Lw***	N°	**Location**	n	***Li***	***Lm***	***Lw***
29	floor	12	***7***	***11***	***1***	33	wall	3	***0***	***1***	***0***	40	wall	1	***0***	***0***	***0***
30	wall	2	***0***	***0***	***0***	34	floor	6	***2***	***1***	***0***	41	floor	2	***1***	***1***	***0***
31	traverse	2	***0***	***0***	***0***	35	cutter	1	***0***	***0***	***0***	42	processing board	1	***1***	***1***	***0***
32	drain	1	***0***	***1***	***0***	36	trolley	2	***1***	***0***	***0***	43	packing engine	2	***1***	***1***	***0***
						37	meat grinder	1	***1***	***0***	***0***						
						38	drain	4	***2***	***3***	***0***						
						39	processing board	1	***1***	***1***	***0***						

Li – L. innocua, Lm – L. monocytogenes, Lw – L. welshimeri, n – number of sampled smears, N° – sample number

environment stimulate *Listeria* growth. Beside this, positive test of *L. monocytogenes* in the climate conditioner has to trigger the alert about potential airborne *Listeria* transmission and necessary sanitation measures against feasible meat contamination. The importance of the effects of proper sanitation routine management in our study was exposed as for the absence of any *Listeria* positive samples on locations along the slaughtering lines in any of the tested slaughtering plants (Tab. 2). According to the present results it has to be emphasized daily cleaning and sanitizing of the all processing equipment, floors and drains, regularly use of acid detergents and weekly use of disinfectants. A strong *Listeria* contamination was ascertain on chilling chambers floors in processing plants where almost 91% samples were positive on *L. monocytogenes*, 58% on *L. innocua* and 8,3% on *L. welshimeri* (Tab. 3). Even single sample on the floor drain was positive on *L. monocytogenes*. This unfavorable situation should be explained by inadequate design and incompetent management, therefore critical *Listeria* introduction and growth is feasible. Sanitation routine in all tested processing plants was critical, employees and meat consignments changing frequently and inadequate. However bio control measures were not considered properly. This was manifested in more than 16% and 75% positive tests on *L. monocytogenes* on the floors and drains and even on the single sample on meat processing board. Moreover tests were positive on *L. innocua* on other tested locations (33% to 100%) and above all we found severe positive single randomly taken samples, what should be even of more concerning, since the

share of positive *Listeria* tests was significant, no matter to the modest number of sampling (Tab. 3). Same situation was manifested in the packing rooms where tests on *L. monocytogenes* and *L. innocua* were positive (50% to 100%) on most of tested surfaces (Tab. 3). Packing rooms should be sanitized, however no matter to visually clean surfaces we found on those areas, they were very likely covered by biofilm which shelter *Listeria* and resistant to usually used sanitation measures. Average gained results of our test were higher (54,5% from equipment, 42% from environment) like the range of results of Pritchard et al [7] who found 7% level of *Listeria* contamination from equipment and 49,7% from the environment. In average we found 57% of positive tests on *Listeria* in processing and 22% positive tests in slaughtering plants. None of gained differences were significant by two-tailed student *t*-test analysis.

Conclusion

1. *Listeria* (*L. innocua*, *L. welshimeri* *L. monocytogenes*) in the slaughtering plants was mainly found on the surfaces of chilling chambers and stables.
2. In the processing plants, no matter to the small number of randomly taken samples, all species of investigated *Listeria* was determined in chilling chambers, processing and packing rooms.
3. Regarding to severe positive findings of *L. monocytogenes*, should be of the great concern to considerate all standards of the proper sanitation routine to prevent food *Listeria* contamination.

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Antibiotic Residues Detection in Poultry Meat Using PABA

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Summary

This study was conducted to determine the presence of antibiotic residues in poultry meat extracts with the use of Para-amino benzoic acid (PABA). Meat samples were acquired from Market A, Market B, Market C and Market D. Macerated poultry meat samples from the breast, thigh and wing were used in this study. Out of four places, Market D showed a positive result while Market A, Market B and Market C yielded negative results.

Data show that inhibitory effect of poultry meat extract containing antibiotic residues on test pathogen was observed at concentrations of 50% to 100% but not on lower concentrations of 25% to 0.1%. Using Para amino benzoic acid (PABA) at a concentration as low as 0.1ug/ml as a screening medium was not effective in detecting antibiotic residue in poultry meat.

Introduction

Antibiotic drugs have long been used in food animals for disease prevention, treatment and growth promotion. Their use, however, may lead to the presence of antibiotic residues in foods of animal origin, produce adverse effects ranging from mild sensitizing responses to fatal anaphylactic shock, and induce resistance or alteration of the intestinal microflora of exposed individuals, suppression of beneficial and desirable organisms, and other toxic effects (WHO, 1997). Hence, there is a need to detect for the presence of antibiotic residues in these foods.

Veterinarians in the poultry industry use antibiotics to enhance growth and feed efficiency and reduce disease in the flock. Although these uses benefit all involved, a common notion exists among consumers that edible poultry tissues are contaminated with harmful concentrations of drug residues. Effects of antibiotic residues include allergic/toxic reactions, disruption of normal human flora in the intestine, anaphylactic reactions, crystalluria, trembling, headache, malaise and death. Ways to detect, neutralize, reduce or eliminate antibiotic residues in meat should be attempted to safeguard the public consumer against some health risks.

Drug residue in meat is a public health concern. As advocate of health in animals and man, a step ahead should be initiated to establish levels of antibiotic residues that may be present in poultry meat. Corollary to this, the use of substances or substrates having similar analog as those of the suspected antibiotic residues can be tested as measure/s or device/s that confirm this detection or neutralization in examined meat samples.

Material and Methods

Bacterial Strains and Culture Conditions. Stock culture of *Bacillus subtilis* maintained at the Microbiology Laboratory was sub cultured in Nutrient broth before the start of the experiment. This bacterial strain was

maintained in Nutrient agar and used for the subsequent experiment.
Poultry meat samples were collected for 3 consecutive weeks from Market A, B, C and D. Three (3) samples (50) grams each collected at a time and this involved meat samples from the breast, thigh and wings. These were stored in the sterile plastic bags, held in the Styrofoam with ice packs and brought to the laboratory for examination

Evaluation of Bacterial Growth in Media Supplemented with PABA. Various concentrations of Para-amino benzoic acid (PABA, Sigma, Germany) included 100ug, 50ug, 25ug, 10ug, 5ug, 1ug and 0.1ug/ml of solution were prepared. A flask of sterile nutrient agar was seeded with 5ml of the test organism after determining its cell density. The nutrient agar was dispensed in 3 replicated plates. Holes were made on the surface of each medium to accommodate 50ul-volume of each of the seven (7) concentrations of PABA previously described.
Plates were incubated at 37 ^{0}C for 24 hours. The ability of the bacteria to survive in the nutrient agar with the presence of PABA was evaluated after incubation. The lowest concentration of PABA favoring growth of bacteria in nutrient agar was noted carefully.

Detection of Antibiotic Residues in Poultry Meat. Poultry meat samples (50 grams) were ground or cut finely by means of scissors. Samples were collected on a sterile aluminum or plastic mesh wire and were autoclaved to ensure sterility of extracts. Meat suspensions were centrifuged to facilitate collection of meat extracts. Meat extract concentrations of 100%, 50%, 20%, 10%, 5%, 1% and 0.1% were prepared in distilled water.
A flask of 75ml sterile nutrient agar was seeded with 5ml of the test organism. The nutrient agar was agitated gently to ensure thorough mixing of the organism. The nutrient agar was dispensed and allowed to set in 3 sterile plates. Holes were made at the surface of each medium to accommodate 50ul volumes of each of the seven (7) concentrations of meat extracts indicated below. Zone of inhibition surrounding the holes were measured in millimeters with the use of a Vernier Caliper. The lowest concentration of meat extract producing significant zones of inhibition or clearing was taken and recorded.

Screening of Antibiotic Residues with the Use of PABA. One (1) flask containing 75ml sterile nutrient agar was supplemented with the lowest concentration of PABA (0.1 ug/ml) that favored bacterial growth in nutrient agar as observed in the previous experiment before it was seeded with the test pathogen.
The Nutrient agar in 3 sterile Petri plates was set as 3 replicates. Holes were made on the surface of the medium to accommodate 50ul volumes of the lowest meat extract concentration that was inhibitory to bacterial growth (50%) and 1 hole for distilled water as a negative control.
Plates were incubated at 37 ^{0}C for 24 hours. The plates were evaluated and the diameters of the zones of inhibition surrounding the wells/holes were measured in millimeters with the use of Vernier Caliper. Measurements of the three replicates were reported as the concentration of meat extract that inhibit growth of bacteria in the presence of a low PABA concentration.
In this experiment, the presence of a zone of inhibition was indicative of the presence of a Sulfonamide residue in meat extract not inactivated by an antimetabolite like PABA present in the medium. Conversely, the absence of a zone of inhibition was indicative for the non-existence of a trace amount of Sulfonamide residue in meat extract failed to bring about bacterial sensitivity to the drug.

Results

The inclusion of PABA at concentrations of 100, 50, 25, 10, 5, 1 and 0.1 ug/ml in nutrient agar did not alter the growth of the test pathogen. The absence of the zone of inhibition in all the wells containing the various concentrations of PABA indicated that the preparation was not inhibitory to the growth of the test pathogen. Experiment results showing non-inhibition of bacterial growth in the presence of PABA reflect normal processes of microbial metabolism that favor bacterial growth.

Table 1 shows the presence of antibiotic residues based on the presence of zone of inhibition produced by poultry meat extracts derived from different supermarkets. Result showed that only in Market D that poultry meat extracts showed zone of inhibition indicating the presence of antibiotic residues. Poultry meat extracts derived from Market A, B and C didn't showed zone of inhibition indicating absence of antibiotic residues. This observation provides proof that poultry farms that supplies Market A, B and C follow specific standards of withdrawal period before slaughter except for the distributors supplying Market D.

It only happened on the 3rd weekly collection when a positive indication of a meat residue was found (Table 1). This finding was noted in breasts, wings and thigh meat samples collected from Market D. Results also showed that the inhibitory effect on test pathogen was observed at higher meat extract concentrations of 50% and 100% but not on lower concentrations (25% to 0.1%). The largest zone of inhibition on *B. subtilis* in 50% was induced by wing samples. Breast meat sample was secondary with a mean zone of inhibition of 13.33mm with the thigh meat samples producing the least size (12.53mm) in terms of zone of inhibition produced.

Tab.1: Measurements of the zones of inhibition (mm) of poultry meat extracts from different sources.

Concentrations of Meat Extract	Market A W	Market A B	Market A T	Market B W	Market B B	Market B T	Market C W	Market C B	Market C T	Market D W	Market D B	Market D T
0.1%	0	0	0	0	0	0	0	0	0	0	0	0
1 %	0	0	0	0	0	0	0	0	0	0	0	0
5 %	0	0	0	0	0	0	0	0	0	0	0	0
10 %	0	0	0	0	0	0	0	0	0	0	0	0
25 %	0	0	0	0	0	0	0	0	0	0	0	0
50 %	0	0	0	0	0	0	0	0	0	14.35(±0.72)	13.33(±1.02)	12.53(±2.19)
100 %	0	0	0	0	0	0	0	0	0	17.41(±1.98)	16.75(±1.49)	16.82(±1.51)
Negative control	0	0	0	0	0	0	0	0	0	0	0	0

Tab. 2: Measurement of the zones of inhibition (mm) of poultry meat extract

Minimum Inhibitory Dilution of meat extract	Wing	Breast	Thigh
50	9.7mm (±2.92)	11.17mm (±1.43)	9.33 (±2.19)
Negative control	0	0	0

From another experiment, 50% concentration of poultry meat extract was considered as the established Mean Inhibitory Concentration (MIC) against *B. subtilis* as a test pathogen. After the established MIC level of meat extract at 50%, the need to screen a specific

antibiotic residue was a challenging task for reasons that PABA as known antimebolite of sulfonamide was available at hand. Results show that with the use of a 50% poultry meat extract, the sensitivity of *B. subtilis* cultivated in a PABA-supplemented Nutrient agar was significant (Table 2). The degree of sensitivity of *B. subtilis* was supported by the sizes of the zones of inhibition that manifested upon exposure to the meat extracts. With wing meat extract, 9.7mm zone of inhibition was recorded; breast samples produced 11.17mm zone of inhibition was noted while thigh meat samples induced 9.33mm size of inhibitory zone.

Data showed that at 50% concentration of poultry meat extract antibiotic residues can be detected to the presence of zone of inhibition. The meat extract may contain the antibiotic residues other than Sulfonamide. Since zone of inhibition was still apparent even in the presence of PABA. The PABA could have inhibited the Sulfonamide action on bacteria, however, the presence of zone of inhibition showed the presence of antibiotic that cannot be inhibited by PABA. And at 0.1ug/ml level of PABA used in this experiment was not enough to counter the antibiotic action of the meat extract component.

Conclusion

Poultry meat samples from meat distributors (Market A, Market B, Market C and Market D) were tested for the detection of possible antibiotic residues. Only meat samples acquired from Market D showed a positive indication of the presence of antibiotic residue. The inclusion of low level of PABA in the screening medium was not possible to provide detection of antibiotic residue in poultry meat sample.

Results showed that the inhibitory effect on test pathogen was observed at higher meat extract concentrations of 50% and 100% but not on lower concentrations (25% to 0.1%). The largest zone of inhibition on *B. subtilis* in 50% was induced by wing samples. Breast meat sample was secondary with a mean zone of inhibition of 13.33mm with the thigh meat samples producing the least size (12.53mm) in terms of zone of inhibition produced. A higher meat extract concentration of 100% produced relatively larger sizes in inhibitory zones on *B. subtilis*. With wing samples, the mean inhibitory measurement was 17.41 mm that of breast samples was 16.75mm while that of thigh samples was 16.82mm.

The use of PABA as a detecting agent with the application of a higher level or concentration can be tested in *in vivo* experiments that employ direct administration of a desired antibiotic. With such experiment, confirmation on the potential of PABA as a detecting agent for antibiotics in meat is not far to be achieved.

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A survey on the consumers utilization of crude milk in a province of Northern Italy

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Summary

In Italy, in the last two years, the demand of crude milk has increased dramatically. Due to the particular hygienic condition of crude milk, a debate on its supposed potential danger for the consumer's health has started. A survey on the consumer's utilization of crude milk was carried out in the province of Bologna, concentrating about 5% of the total crude milk vending machines operating in Italy. Data were collected through 171 interviews conducted at 8 machines. The questionnaire included the family structure, the information on the purchasing of crude milk and its use. Half of the purchasers interviewed is more than 50 years old and the majority of them are retired people. Advertising placed close to the machines were the major source of information about the availability of the product. Among the quality traits found in the crude milk, taste was the most frequently mentioned. With regard to its use, only 58.4% of the interviewed drinks it, 36.6% uses it to prepare food and 5% to make homemade cheese. More than 80% of the persons interviewed heat the crude milk before its use because, due to their prevalent age distribution, they remember how "necessary" this practice is, or they strictly follow the rules listed in the warnings placed on the machines. The results of this survey suggest a generally correct practice adoption when consuming crude milk thus reducing its supposed potential danger.

Introduction

In Italy, the demand of crude milk produced in compliance to the Regulation (EC) No 853/2004 has increased dramatically in the last two years. In this period the number of vending machine increased then fold, reaching now the number of 1,176, mainly located in the Northern Italy. In this area there is 90% of the machines while the rest is divided between the Centre (8%) and the South (2 %). Lombardy region has the highest portion of distributors (33.8%) followed by Veneto (17.2%), Emilia Romagna (14.4%), and Piedmont (13.5%). The province of Bologna is a leader in Emilia Romagna with its 63 distributors (5.4% of the total amount in Italy).
The fast growth of crude milk consumption is due to diffuse information on the positive aspects of this product with respect to the industrial milk, such as the better taste, the lower price and the reduction of transport and wastes impact on the environment. Before January 2009 a warning was placed on the machines suggesting the need of a heat treatment for the milk before its use, in order to avoid a potential transmission of pathogens.
After 9 cases of intoxication, occurred at the end of 2008, which were supposedly related to the crude milk consumption, it is now compulsory to report on the machine the warning of boiling milk before use. Recently, some advices on the risks associated with the consumption of crude milk have been published in Canada and in the U.S.A. [3, 4, 5]. However, the non-pasteurized milk to which these authors refer is not the same which is sold under the EC Regulation No. 853/2004. A confirmation of the different and better hygienic quality

of Italian crude milk came from different surveys conducted in Lombardy [2] and Veneto [1] regions.
Despite the relevance of crude milk consumption, there is not yet available information on the consumer utilization of crude milk. The aim of the present survey was to better understand the consumer's behaviour with respect to the utilisation of this product.

Material and Methods

Data were collected through interviews with 171 buyers at 8 distributors of crude milk, located both in the plain and in the hill areas of the Bologna province. The questionnaire was divided into three parts. The first concerned the description of consumer's families structure and the travel distance from crude milk distributor; the second was focused on its consumption, i.e. frequency and quantity purchased on each visit, and its utilization; the third regarded the use of the vending machine, in particular the type of difficulties encountered.

Results

The sample of crude milk consumers was balanced when gender is considered, with a slight prevalence of males (53.2%). Regarding the age of purchasers (Fig. 1), the most represented groups were the over seventy (28.6%) and the 50 - 60 years old (22.8%). The youngest groups were weakly represented; the purchasers under 30 years old did not reach 6% of the total consumers interviewed. The family contest showed an absolute majority of purchasers who live in the family (87.0%). More than half of the purchasers was retired (53.2%) while the other categories were represented by the employed (27.5%), self-employed (11%), housewives (6.4%) and students (1.8%).

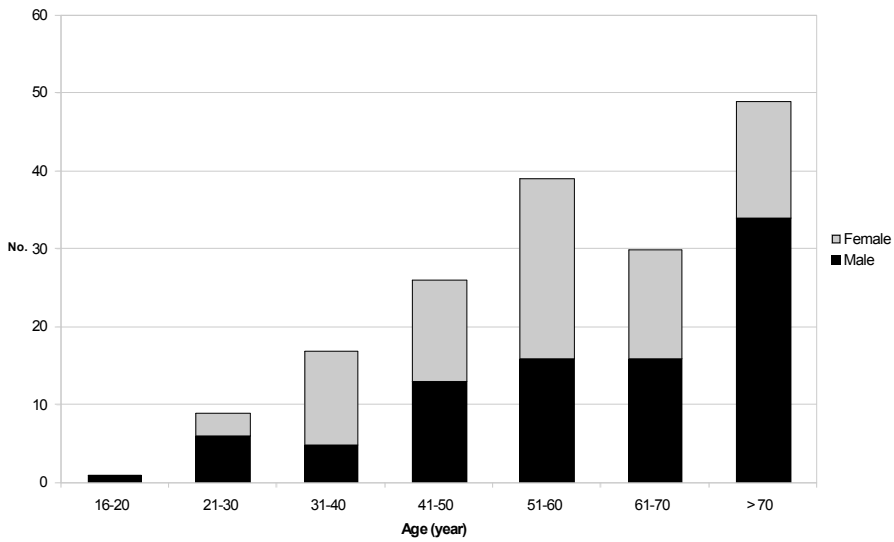


Fig. 1: Consumer's sample.

As shown in Fig. 2, the majority of purchasers use vending machines located at less than 5 km from their homes; this is particularly evident for retired people. However, the distance

from the distributors does not seem to be an obstacle to the purchasing of crude milk, especially for those workers who have the opportunity to stop at the machine on their way back from work. Both groups showed a certain amount of loyalty to the vending machine: even if there are distributors close to home, some purchasers prefer others located further away; possible explanation for their preference are related to the presence of the farmer or to the machine model. The consumer's information on the presence of crude milk machines and their choice of using them where influenced by farmers' advertising posters (60% of the sample), 27.5% by friend's suggestions and 7% by direct contact with the farmers for reasons not related to the crude milk sales. The media (newspapers, television, radio and internet) played a minor role (3.5%). The frequency of purchasing showed that the majority of interviewed carries out two (29.2%) or three visits (24.6%) to the distributor per week, while only 16.4% of consumers purchase crude milk daily. The sample showed that 66.1% and 22.2% of the people interviewed purchase a litre and two litres, respectively. The remaining was distributed in a range of between half litre and 12 litres per visit. Among the positive quality traits perceived in the crude milk, a better taste was indicated by the majority of consumers (77.2%) while other characteristics such as naturalness, healthiness and digestibility showed very low percentages. A non negligible part of purchasers (8.2%) was not able to find any particular quality traits in the crude milk.

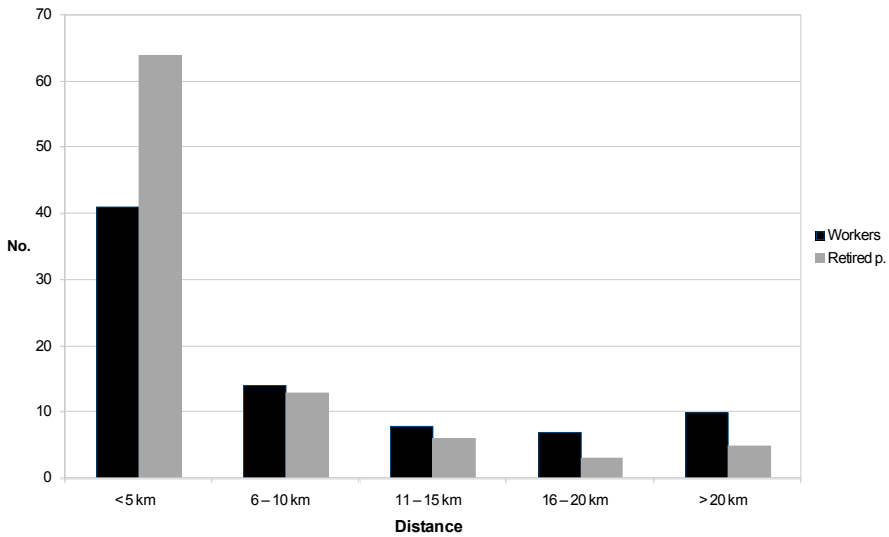


Fig. 2: Distance travelled for purchasing the crude milk.

There is an interest in evaluating the degree of substitution of the industrial milk by the crude milk: 55.0% of the people interviewed consume only crude milk, suggesting that there is room for an increase in the replacement of industrial milk. Surprisingly, only 58.4% of purchasers drinks crude milk, while 36.6% uses it to prepare food and 5% for the production of homemade cheese. Irrespectively to its use, 82.5% of the people interviewed warms the crude milk. Due to the prevalent old age of purchasers, the majority of them remembers how "necessary" this practice is, while the remaining consumers follow the warning instructions placed on the distributors. All purchasers have not encountered any difficulties in the use

of the machines, because of the clear and visible instructions. With regard to the containers used, the data showed that 61% of interviewed uses plastic bottles, 35% glass bottles, 3% both, while 1% utilizes containers of different materials (tetra-pack and aluminium). Except for first timers who purchase the plastic bottles from the dispenser located next to the machine the other consumers either re-use the same bottle or use other containers like bottles for mineral water, wine and fruit juice, aluminium containers, etc.) Unfortunately, a non negligible frequency of bottles brought from home, with a low standard of hygiene, due to the lack of or insufficient washing resulted from the sample analysis. The warning instructions reported on the machines, indicating the need of using cleaned and dried bottles result relevant although partially effective.

Conclusion

In general, the results of the survey showed that crude milk consumption involves mainly mature consumers (over 50s) and their families. This strongly influences the utilization of the products, ensuring a heated treatment before its use. Nevertheless the consumer seem to underestimate the negative effect on the hygienic quality of crude milk due to the poor cleaning condition of the bottles used to collect the product. The organoleptic characteristics of crude milk seem to be by far the most important aspects influencing the consumers preference, a the fact that it is an healthy, natural and digestible product is much less important. The consumers loyalty to the vending machines suggests a positive impact exerted by the presence of the farmer on site; the consumers seem to prefer to associate the product with a person rather than a label.

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Effect of fish oil on fatty acids content in milk of cows

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Summary

This research aimed on an evaluation the influence of fish's oil (mineral-fatty preparation) supplementation added to the nutrition doses of TMR for dairy cows, onto content of fatty acids in milk fat. Fatty acids composition was determined in milk samples by using Agilent Technologies 5973 gas chromatograph (GGMS). Use of the mineral-fatty preparation, which contained the fish oil (2 % DM) as an addition to TMR dose for cows caused an increasing of CLA content, as well as n-3 fatty acids (EPA and DHA) content was determined after supplementing of fish oil preparation.

Introduction

A count of fatty acids such as polyunsaturated ones of n-3 fatty acids and conjugated linoleic acid (CLA) can govern physiological functions in human organism [7, 9]. *Cis*-9, *trans*-11 is the major CLA isomer in ruminant milk fat (abort 75–90% of total CLA) [5, 8]. However, 64-78% of *cis*-9, *trans*-11 CLA present in milk fat is synthesized in mammary gland from vaccenic acid (TVA) by delta-9 desaturase [8]. Content of CLA in milk is determined in the highest extend by nutritional factors. Feeding lipid sources rich in mono- and polyunsaturated fattyacids, either as seeds, free oil, or calcium salts, increasedthe *cis*-9, *trans*-11 CLA and VA content of milk [2, 4, 13]. An application of fish oil appeared to be very effective in that kind of modification [2, 14]. Donovan et al. [6] using fish oil in a dose of 2 % DM obtained an increase in CLA in milk fat of 360 %. The increase in a content of CLA was also noted when the fish oil together with plant oils containing in 18:2 n-6 was given to cows [1, 3, 4]. It was also observed that milk of cows receiving fish oil is characterized by higher concentration of fatty acids of n-3 fatty acids, especially EPA and DHA [6, 14]. However, EPA and DHA transfer to the milk fat is low. This research aimed on an evaluation the influence of the fish oil supplementation added to the nutrition doses of TMR for dairy cows, onto content of fatty acids in milk fat.

Material and Methods

The study was conducted on a farm of dairy cattle on cows of Polish Holstein-Friesian breed (black-white). The cows were fed according to TMR system (Total Mixed Rations). The forty clinically healthy cows, which were divided by analog method into three groups (primiparous and multiparous), were chosen to conduct investigation. Fish oil was given as 2% of DM (525 g/d) in form of mineral-fatty preparation (FO-M) to the experimental groups (n=10). The preparation was composed of: fish oil 33 %, baidelit 25 %, vermiculite 33% and Humokarbowit 9 %.

Milk for analysis was collected before the beginning of the experiment (1 week of lactation), and after 12 weeks of an administration of preparations. The separation was performed using a gas chromatograph (Agilent

Technologies 5973) equipped with mass detector (GC/MS), capillary column (Agilent DB-224 MS) parameters 60m(250(m(0,25(m. The injector temperature was raised from 70 (C to 240 (C at a rate of 4 (C/ min. The flow rate of gaseous carrier (helium) was 2.0 mL/min and the split flow ratio was 1:100 for the entire period of investigation. The peaks were identified and quantified by reference to FA standards (Sigma-Aldrich and Supelco). It was marked 24 fatty acids including.
The values obtained were statistically worked out using statistical program Statistica ver. 8.0 and difference significance was estimated according to Duncan test.

Results

There were no organoleptic and sensoric changes of milk quality, when 2% of FO-M preparation was added. The FO-M preparation caused decrease of short-chain fatty acids (C4:0 - C12:0) concentration in milk fat as well as it caused the increase ($P<0.01$) of long-chain fatty acids (>C16:0) level already after 12 weeks of using. These changes were more intensive in multiparous cows. Increased rumen production of *trans*-11-18:1 resulted in increased synthesis of *cis*-9, *trans*-11 CLA in the mammary gland. The highest increase of trans-11 C18:1 was noted at multiparous. After 12 weeks of feeding preparation, the CLA content increase to 2.63 % at primiparous cows and 3.11 % at multiparous cows. At control groups, the content of DHA in milk was below of detection threshold. The significant increase ($P<0.01$) of EPA and DHA in milk fat was stated to experimental cows (0.68 and 0.44%, respectively).

Tab. 1: Effect of treatment diets on chosen milk fatty acid composition (g/100g of fatty acids)

Fatty acids	Primiparous				Multiparous			
	Control		Eksperimental		Control		Eksperimental	
	Period of FO-M preparation administration							
	0	12thweek	0	12thweek	0	12thweek	0	12thweek
C18:1t11 (TVA)	0.98	0.99	0.93A	4.73A	1.45	1.12	1.44A	5.51A
C18:2c9,c12	2.78	2.79	2.76A	1.24A	3.11	3.09	3.10A	1.80A
C18:2c9,t11(CLA)	0.54	0.63	0.56A	2.63A	0.62	0.60	0.63A	3.11A
C18:3 n-3	0.04	0.05	0.03	0.07	0.03	0.09	0.04A	0.22A
C18:3 n-6	0.16	0.18	0.17A	0.73A	0.17	0.22	0.16A	0.86A
C20:5 n-3 (EPA)	0.03	0.03	0.03	0.58A	0.02	0.02	0.03	0.68A
C22:6 n-3 (DHA)	<0.01	<0.01	<0.01	0.41A	<0.01	<0.01	<0.01	0.44A
Short-chain[1]	11.24	11.57	11.25A	9.39A	10.70	10.07	13.69A	10.55A
Medium-chain [2]	42.54	42.16	42.54	42.49	44.03	42.71	44.06	44.66
Long-chain [3]	34.76	36.55	34.91A	40.17A	35.58	36.61	35.44A	42.14A
Saturated	65.18	64.62	63.59A	58.89A	69.76A	65.97A	67.92A	63.35A
Unsaturated	24.94	25.66	25.11A	33.16A	25.41	24.05	25.27A	34.00A

TVA – vaccenic acid, CLA – conjugated linoleic acid, EPA – eicosapentaenoic acid, DHA - docohexaenoic acid. [1] – short-chain fatty acids (C4:0 - C12:0); [2] – medium-chain fatty acids (C14:0 – C16:1); [3] – long-chain fatty acids (> C17:0)
A – differences significant statistically in a given group between research periods, $P<0.01$

Discussion

A decrease in the content of short-chain fatty acids in milk was noted when the cows were fed fish oil [2]. Donovan et al. [6] using the increasing doses of fish oil (from 1 to 3%) observed an increase in a concentration of long-chain and unsaturated fatty acids in milk fat.

Castañeda-Gutieérrez et al. [4], after an application of Ca salts of fish oil observed higher content of long-chain acids >C:16 (47.35 and 46.48 g/100g of fatty acids) in milk comparing to ruminal infusion of fish oil (44.93 g/100g of fatty acids). In the present research, the similar content of long-chain fatty acids was observed in experimental cows.

An application of fish oil in a form of calcium salts did not cause such a distinct increase in a concentration of TVA in milk (1.54 and 2.22 g/100 g of fatty acids) as ruminal infusion (18.33 g/100 g of fatty acids) [4]. Osborn et al. [11] adding the fish oil to the TMR doses and to the drinking water affirmed the lower concentration of TVA in milk, 3.62 and 3.83%, respectively.

Generally, increased rumen production of TVA resulted in increased synthesis of *cis*-9, *trans*-11 CLA catalyzed by delta-9 desaturase in mammary gland [5, 10, 12]. Shingfield et al. [13] affirmed increase concentration in milk of CLA to 5.37 g/100g of fatty acid after application of fish oil together with sunflower oil (at 5th day), however after 15 days of feeding of these supplements content of CLA decreased to 2.35 g/100g of fatty acids. AbuGazaleh et al. [1] obtained the similar results.

Transfer of EPA and DHA to the milk fat was 21.27% and 18.9% respectively, after application of fish oil infusion to the abomasum, 3.08% and 4.36% after application of calcium salts of fish oil (high doses) and 1.86% and 3.30% after intra-ruminal infusion of fish oil [4]. The concentrations of EPA and DHA in milk fat increased ($P<0.01$) when cows were fed with fish oil and extruded soybeans together [3], similar to the response was observed by Whitlock et al. [14]. During pasture feeding the affirmed content of EPA and DHA was 0.02 and 0.03 g/100g of fatty acids [1], however, EPA on 0.01g/100g of fatty acids level and DHA below detect ability level were observed when TMR doses were fed [3]. In present study introduction of fish oil (mineral-fatty preparation) to the cows' diet caused an increase ($P<0.01$) of polyunsaturated fatty acids of n-3 fatty acids, EPA and DHA in milk fat.

Conclusion

Use of the mineral-fatty preparation, which contained the fish oil (2 % of DM) as an addition to TMR dose for cows caused a decreasing of short-chain fatty acids' content in milk and increase of long-chain content. The significant increase of CLA content, as well as n-3 fatty acids (EPA and DHA) content was determined after supplementing of fish oil preparation.

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Part IX

Emissions

Chapter 26

Block 1C

Dust emissions from keeping laying hens - a comparison of different systems

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Summary

Due to the ban of conventional cages in the EU, alternative systems must be introduced and evaluated. In stables with floor keeping, aviary and large furnished cages all relevant measures were investigated. Environmental concern is elicited by ammonia and dust emissions, whereby PM emissions are in the focus of this paper. Large differences between the particular systems were found. Seasonal variations could be observed. PM10 emission factors at all ranged between 0,3 mg/(h*bird) in the night and 12 mg/(h*bird) for peaks at day time.

Introduction

After banning conventional cages to keep laying hens, other systems must be introduced, which consider the requirements of the birds on their health and welfare in a better way. Nevertheless these systems must be evaluated also with respect to occupational and environmental impact. One of the requirements consists in larger areas for more mobility of the birds. It is well known that higher activity of animals causes higher production of airborne dust. Depending on the ventilation system also emissions of particulates may increase. This paper is focussed to dust emissions from different types of hen keeping. In four different stables emissions of dust, PM10 and PM2.5 were measured at noon as a 1h spot once a month. Additionally 24h monitoring was added every quarter of a year to get the opportunity to indicate how the spots reflect the average of a day.

Material and Methods

Keeping. The evaluation of the influence of the system is the main task of a comprehensive study comparing different keeping systems for laying hens: aviary with integrated litter space (system 1), a floor keeping system with integrated litter space (2), a floor keeping system with outdoor access (3) and a large furnished cage system (4). All stables were forcedly ventilated. The light programme was mainly driven artifically. One stable is part of a research facility, the others are commercial farms.

Measurements. Dust emissions were determined by measuring quasi simultaneously concentrations in and air flow through the exhaust openings of the single stables. One example for a measuring array is given in figure

1 with two isokinetic probes and a measuring fan for direct air flow monitoring.

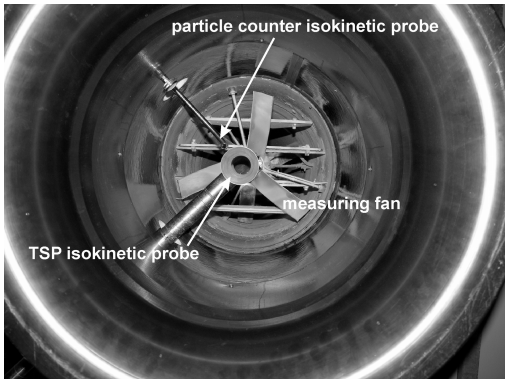


Fig. 1: Look inside an exhaust duct with installed probes

In the given example exhaust air flow was measured by the given device of the ventilation system. In other cases fan wheel anemometers were used to get the velocity profiles in the balancing area, from which the air flow can be calculated. Additionally the condition for the isokinetic probe was obtained. Pre checks showed smooth profiles of velocity and dust in the measuring area. This allowed the one point measurement in the centre of the duct.

Figure 2 shows the scheme for isokinetic probe and the following analyses.

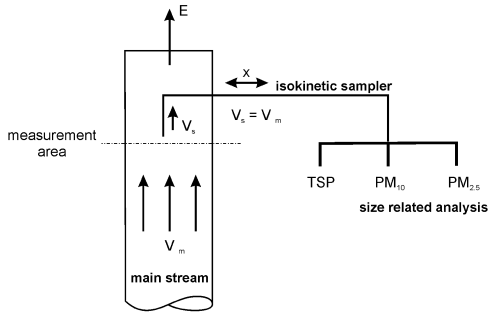


Fig. 2: Scheme for isokinetic probe

With view to the environment, TSP (total suspended particles), PM10 and PM2.5 are of particular interest. For this purpose dust concentration is measured conventionally gravimetrically with a cyclone separator and following particle size analysis.
To get more detailed information about time dependencies an optical particle counter was installed aditionally.
More information about the stables and measuring devices is given by Winter et al. [1].

Results

In the following the results are given for emission factors of TSP, PM10 and PM2.5. Emission factors are used to compensate the different stocks of the stable by relating the emission mass flow to the number of bird places.

For figure 3 the 1h spot measurements are used to demonstrate the differences between the systems and the possible saisonal influence.

With one exception the TSP emission factors are below 100 mg/(h*bird), but mainly below 40 mg/(h*bird).The differences between the systems are obvious with a clear advantage for system 4, the large furnished cages which are favoured in Germany.
TSP was used to compare our results with previous studies found in the literature. In the past PM10 and PM2.5 often were given as ratio of TSP. For using these values the information is needed how TSP is defined or measured. In the still most comprehensive study on emissions in and from animal houses Takai introduced inhalable particles as equivalent to total dust [2]. That means that particles larger than 100 m are neglected. To indicate possible consequences in figure 4 examples of the particle size distributions for the four stables are demonstrated.
The distributions differ up to 300 m geometric particle diameter.

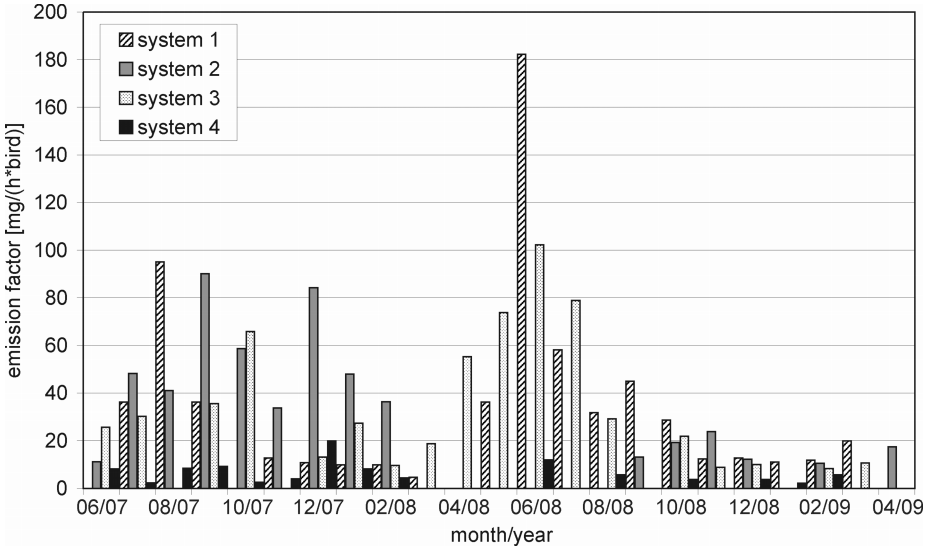


Fig. 3: TSP emission factors of the systems during different seasons

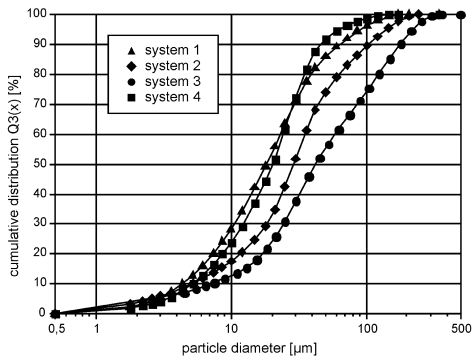


Fig. 4: Cumulative distribution of the dust from the different keeping systems

The advantage of determination of the total size distribution is that all interesting particle fractions can be calculated using their definition functions, the disadvantage is that no time resultion can be obtained. Only the information for an average of the sampling time can be given. But its well known that particle concentration and emissions show distinct diurnal courses [1, 3].

Therefore online measuring particle monitors were used to measure PM10 and PM2.5. Results are shown in figure 5 for the example of system 2.

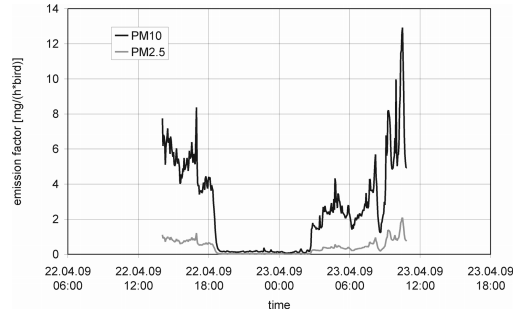


Fig.5: PM10 and PM2.5 emission factors for system 2 during one day

The dust emission factor, which also means dust emissions, follow a strong course of the day with higher value at day time and very very low values at night time.

PM10 ranges in all systems from approx. 0.3 mg/(h*bird) during the night, between 2 and 4 mg/(h*bird) at day time and up to peaks of 12 mg/(h*bird) in connection to high activity inside the stable.

Discussion

Dust emissions show, for all systems investigated, distinct but similar courses of the day. Higher values at day time than in the night are observed with a sharp separation between both caused by the lighting in the stable. Light influences activity of the hens and this activity causes dust production in the stables. The higher emissions in summer than in winter are mainly due to a higher ventilation rate.
Different particle size distributions and by this different ratios of PM10 and PM2.5 are caused by keeping, litter and manure system. Compared with other results given in the literature the emission factors are lower but in the same order of magnitude.

Conclusion

Large differences between the particular systems were found.
Seasonal variations could be observed.
PM10 emission factors range between approx. 0.3 mg/(h*bird) and 12 mg/(h*bird) for peaks at day time. These are first results and must be seen in relationship with the duration of measuring.

Acknowledgement

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Dust and Bioaerosols in the air of a new housing system for laying hens

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Summary

Airborne inhalable dust, endotoxins and mesophilic bacteria concentrations were measured as part of a current study in six laying hen houses equipped with the new German "small colony keeping system" (Kleingruppenhaltung). The median values of inhalable dust (1.83 mg/m^3) and endotoxins (119 ng/m^3) and the mean value of mesophilic bacteria (102,000 cfu/m^3) indicate a relatively low exposure of animals and farmers to these air pollutants. These results need to be confirmed by more investigations in small colony keeping systems over longer periods of time.

Introduction

The air of animal houses often contains high amounts of airborne dust and bioaerosols, which can affect the health of animals and farmers (1, 2(. The concentrations in air and the composition of these airborne pollutants are mainly influenced by animal species, housing type and management (3(. In Germany a new housing system for laying hens was recently developed after the ban of battery cages following a decision of the constitutional court in 1999. This system is called "small colony keeping system" and operates commercially since 2007. Approximately 1,100,000 hens are housed in this system today (4(. The design is similar to furnished cages (EU system) but it offers more space per hen (890 cm), contains two perches in different heights and is about 60 cm high at the feed trough sides. Very little is known about the air hygiene in this system. Hence this paper reports about concentrations of inhalable dust, airborne endotoxins (lipopolysaccharides) and airborne mesophilic bacteria measured in the new German "small colony keeping system" (SCKS).

Animals, Material and Methods

Sampled flocks. Samples were taken in six laying hen herds housed in the new German SCKS. The herds consisted of 50% of groups of Lohmann Brown (LB) hens and of 50% of groups of Lohmann Selected Leghorn (LSL). Table 1 shows the number of houses (herds), the flock size and the number of birds in the colony groups at the beginning of the investigations.

Sampling and laboratory methods. Inhalable dust, endotoxins and mesophilic bacteria were collected at three locations in house 1, 2, 3, and 6 and at two locations in the smaller houses 4 and 5 in 1.5 m height above floor

level using IOM samplers (Institute of Occupational Medicine, Edinburg, UK) and Universal-Deluxe (SKC) pumps (Analyt-MTC, Mühlheim) with a flow rate of 2.5 l/min. Dust and endotoxins were sampled with glass fibre filters and micro-organisms with polycarbonate filters (both filter types from Whatman International Ltd., Maidstone, UK). The sampling time was two to three hours per filter. Between 3 samplings (September) and 6 samplings were carried out per sampling period (see Fig. 2). The filters were transported to the laboratory within 24 h and analysed as soon as possible after arrival according to Saleh [5].

Tab.1: Number of animal houses, flock and group sizes

	flock size	hens per colony group
house 1	1040	40
house 2	1512	33 and 60
house 3	1512	33 and 40
house 4	300	50
house 5	480	40
herd 6	1365	36 and 54

Statistical analysis. For box plots and bar graphs Microsoft Office Excell 2003 was used.

Results

Figure 1 shows the median concentrations of the inhalable dust fraction (particles $< 100\mu$m) and the airborne endotoxin concentrations in the SCKS.

The concentrations of inhalable dust ranged between 0.23 and 2.73 mg/m^3 and the median was 1.18 mg/m^3. The maximum (805 ng/m^3) endotoxin value showed a relative strong deviation from the median (119 ng/m^3), however approximately 75% of all results showed concentrations less than 200 ng/m^3. There was no significant correlation (r = 0.15) between dust and endotoxin concentrations detected simultaneously at the same locations in the different houses.

The average counts of airborne mesophilic bacteria showed partially distinct differences within the same building but also between the different animal houses (Fig. 2). Cfu/m^3 varied from 5 x 10^3 to 3,8 x 10^5. The mean of all measurements was 102,000 cfu/m^3.The highest amounts of bacteria were measured in the cold season (winter and early spring time) but no clear saisonal trend could be observed. In contrast, two houses showed the maximum amount of airborne bacteria in the summer. Interestingly, much lower means of cfu/m^3 (55,000 and 28,000) were found in the houses with smaller flock sizes (4 and 5) compared to the houses with larger flocks (1,2,3,6). In the latter, mean values between 126,000 and 153,000 cfu/m^3 were found. No significant correlation (r = -0,21) was observed between the counts of airborne mesophilic bacteria and inhalable dust concentrations.

Discussion

This paper is the first report about inhalable dust and bioaerosol concentrations in six different animal houses equipped with the German SCKS. Comparable findings to other housing systems for laying hens were obtained by using the same methods as in previous studies. Dust, endotoxin and mesophilic bacteria concentrations were considerable lower as in littered aviaries or floor raised systems [5, 6, 7, 8]. It is evident that litter is a significant

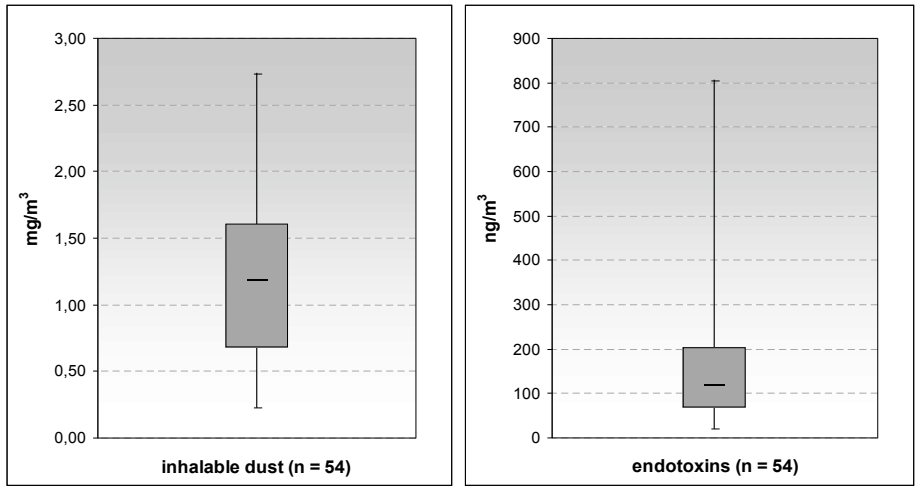


Fig. 1: Concentrations (medians) of inhalable dust and airborne endotoxins in the German small colony keeping system. n = number of samples.

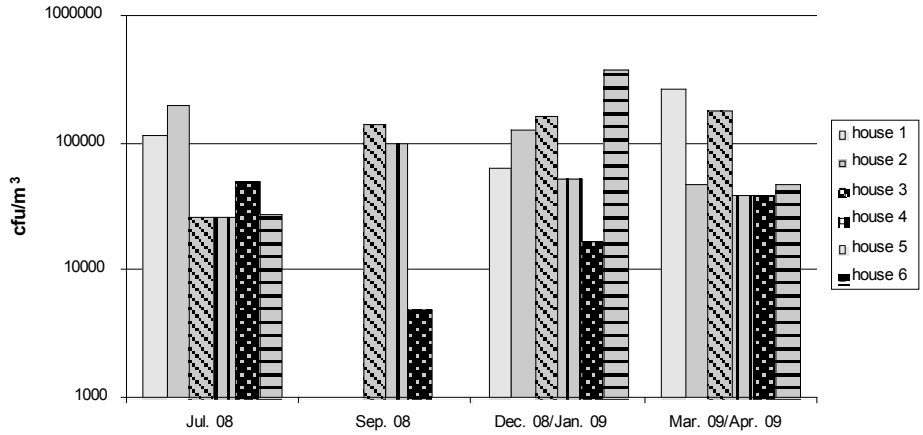


Fig. 2: Concentrations of airborne mesophilc bacteria measured three or four times respectively in six laying hen houses equipped with the German small colony keeping system. cfu = colony forming units.

emission source for dust particles and bioaerosols which are released into the air due to the activity of the hens and the airflow within the animal house. However the average values of this investigations were also below the results of Saleh [5] who measured in a battery cage and an enriched cage system with a comparable management. This indicates relatively low dust and bioarosol exposure of animals and farmers in SCKS but further results are necessary for a validation of these first results. The marked differences of bacteria concentrations between the houses with smaller and bigger flocks observed in this investigations can't be explained presently. In general, bioaerosol concentrations are not associated directly with the number of animals but rather to the live weight of animals which is closely related to the ventilation rates of an animal house [9]. It seems that management and ventilation intensity are more important for the levels of bacteria in these types of laying hen houses than the flock size. Differences in management and ventilation rates should be more intensively taken into account when air quality measurements and assessments of housing systems are carried out.

Conclusion

The results of this study show that the pollution of the air by inhalable dust, endotoxins and mesophilic bacteria in the new German small colony keeping system for laying hens is relatively low compared to other systems such as aviaries. Consequently, the exposure of the animals and farmers with inhalable dust and bioaerosols is also lower and there are lower emissions from these buildings. It seems that the new system has a potential to reduce the occupational and environmental health risks associated with laying hen farming. These findings should be substantiated by further research on the health of animal and man inside and outside these laying hen housings.

Acknowledgement

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Concentration of airborne particles and emission of gaseous compounds (carbon dioxide and ammonia) in large, cold, loose housing cowsheds in Estonia in the winter period

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Summary

Over the period from September 2008 to March 2009, measurements of inhalable and respirable airborne particles, carbon dioxide and ammonia concentrations, air temperature, and relative humidity were taken at 8 to 13 locations within nine large cold loose housing cowsheds in Estonia. The mean recorded concentrations of inhalable and respirable airborne particles were $261\pm247\mu g/m^3$and $24\pm18\mu g/m^3$, respectively. The overall mean inside air concentration of carbon dioxide was 676±248 ppm and that of ammonia 1.0±1.1 ppm. The mean air temperature was 4.8±4.4'C, and relative humidity 92.0±10.4%. The microclimate parameters, including the concentrations of respirable and inhalable airborne dust in the cold loose housing cowsheds, were in accord with approved norms throughout the measurement period.

Introduction

Regardless of the typically harsh winter conditions, over one hundred large, uninsulated, dairy buildings have been constructed in Estonia in the past decade. Microclimate parameters such as air temperature, relative humidity, inside air exchange rate, gas concentration as well as interactions between these factors in livestock buildings have been thoroughly studied [1,4,5]. Furthermore, the impact of microclimatic conditions on thermal comfort and health parameters of dairy cows has also been established [2]. However, there is little experimental data about indoor inhalable airborne particle concentrations, particularly respirable airborne particles ($< 2.5\mu m$), in loose housing large scale farms. The fine respirable airborne dust is considered to be one of the potential risk factors for animal and human health, as dust may penetrate into the alveoli through the respiratory tract. Dust in livestock buildings is comprised of feed and other plant-derived fractions, epithelial cells detached from animal body, urine, faeces, micro-organisms and other particles. Likewise, ammonia and odours in cow housing can be adsorbed onto dust particles. Bacteria and viruses attached to dust particles may spread and cause infectious and allergic diseases in farm animals and humans, even in neighbouring areas. The microclimate of uninsulated large scale farms is strictly related to environmental factors [1], thus it can be assumed that this also affects the concentration of dust particles.

Material and Methods

Microclimate parameters (temperature, 'C; relative humidity, %; carbon dioxide and ammonia, ppm) as well as inhalable and respirable dust concentrations ($\mu g/m^3$) were measured in nine large cold loose housing cowsheds on five

dairy farms in Estonia over the period from September to March. The number of animals in the buildings varied between 300 and 600. The concentrations of inhalable and respirable dust, carbon dioxide and ammonia were measured at one meter height from the floor, at 8 to 13 locations, depending on the size of the building, for 10 minutes per measuring site (Fig.1). The concentrations of inhalable dust and its fine fractions were recorded at the same time. Measurements were performed in the daytime while the cows were most active, and different work routines (TMR feeding, manure removal) were being carried out. Inside air concentration of carbon dioxide provided the basis for indirect assessment of the effectiveness of the ventilation in the dairy buildings, i.e. lower carbon dioxide emissions indicated a higher air velocity (ventilation rate) within the cowhouse.

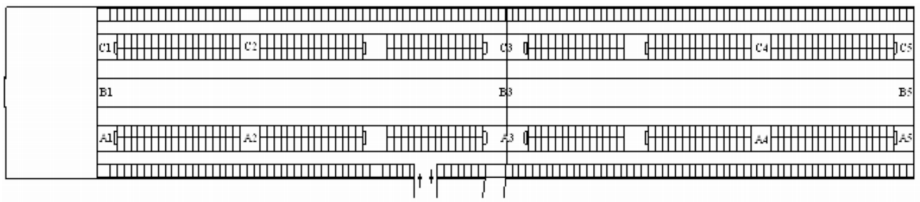


Fig.1: Barn with 13 measuring points

A Grimm 1.108 portable aerosol spectrometer (Grimm Technologies Inc., Aerosol Technik GmbH and Co) was used for the measurements of the concentrations of airborne dust particles, and a Dräger X-am 7000 multigas detector (Dräger Safety GmbH) for recording gas emissions. In all the buildings the temperature and relative humidity were constantly determined at intervals of 15 min. The measurements were performed with a Rotronic HygroLog data logger (Rotronic AG). Statistical variation between the air quality data was analysed by using the procedure of SAS.

Results and Discussion

The mean inhalable airborne particle concentration (Fig. 2a) on loose housing dairy farms during the experimental period was $261\pm247\mu g/m^3$, whereas it varied monthly in a wide range, from 141 to $398\mu g/m^3$. The mean respirable airborne particle concentration (Fig. 2b) was $24\pm18\mu g/m^3$, with a monthly range between 8 and $37\mu g/m^3$.

Under winter conditions, loose housing large scale farms showed lower inhalable and respirable dust concentrations compared with insulated livestock farms where tethered keeping of cows was practiced in accordance with other findings [3,6]. This might presumably be associated with the amount of litter used on the farm. In contrast to cattle buildings in which cows are kept tethered and substantial volumes of bedding material are used and renewed on a daily basis, loose housing large scale dairy farms operate with minimal litter, or even without any bedding. Also, in uninsulated loose housing large scale farms, the distribution of litter is carried out according to need, usually once a week. The carbon dioxide concentration in the indoor air of dairy buildings was directly affected by outside environmental conditions. The more the curtain walls, and other ventilation outlets, were opened, the greater was the volume of air that passed through the building, and the lower the concentration of carbon dioxide. The mean carbon dioxide concentration in the indoor air of dairy buildings (Fig. 2c) was 676±248 ppm, ranging monthly from 452 to 861 ppm. The concentration of ammonia in indoor air

was affected primarily by indoor temperature and ventilation rate. In the current study, the mean ammonia concentration (Fig. 2d) of the indoor air of the cattle buildings was 1.0±1.1 ppm, with a monthly range between 0.3 and 1.9 ppm. The indoor air temperature (Fig. 2e) of an uninsulated cattle building is directly determined by the outside temperature [2]. The overall mean temperature was 4.8±4.4'C, with a monthly range from 0.8 to 11.4'C. The overall mean relative humidity (Fig. 2f) was 92.0±10.4%, with a monthly range between 72 and 100%.

Statistical analysis of the results (Tab.1) showed that the concentration of respirable dust was positively correlated with the concentration of inhalable dust in the air inside a cattle shed. There was also a moderate correlation between the concentration of respirable dust and carbon dioxide – the higher the ventilation rate, the lower the concentration of respirable particles. No statistically significant relationship was found between the concentration of respirable dust and the emissions of ammonia, indoor temperature and relative humidity of the cattle sheds. Moreover, there was no statistically significant correlation between the concentration of inhalable dust and the rest of the measured parameters, except for the concentration of respirable dust. This means that the concentration of inhalable dust in the air inside a cattle shed is considerably more affected by all the different activities carried out inside a cattle building, e.g. the activity of cows and work routine, rather than ventilation rates. A negative correlation was observed between the carbon dioxide content in the air and the temperature inside the cattle building, which can be explained by the fact, that in case of lower outside temperatures, the total area of inlet ventilation outlets is smaller (curtain walls were not widely opened), which leads to a lower ventilation rate, and results in a higher concentration of carbon dioxide.

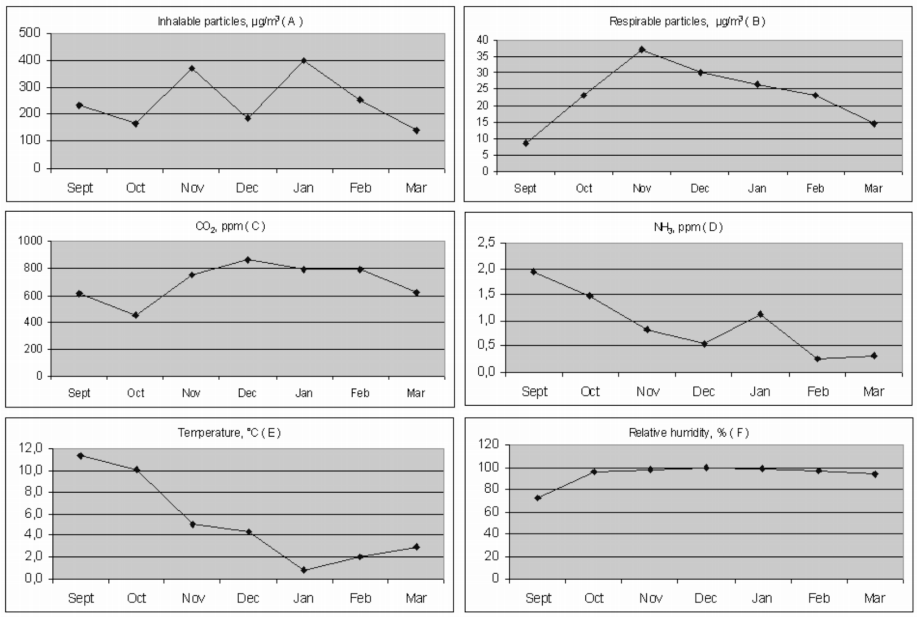


Fig. 2: Mean concentration of inhalable (a) and respirable airborne particles (b), carbon dioxide (c), ammonia (d), air temperature (e) and relative humidity (f) in cattle buildings

Ammonia emission in livestock buildings depended primarily on the indoor temperature and relative humidity of the air. A positive

correlation was due to the increased ureolytic activity of bacteria at higher temperature.

Tab. 1: Correlations between measured variables

Variable	Inhalable particles, $\mu g/m^3$	Respirable particles, $\mu g/m^3$	CO_2, ppm	NH_3, ppm	Temperature, °C	Relative humidity, %
Inhalable particles, $\mu g/m^3$		R=0.364 P=0.005**	R=0.183 P=0.208	R=0.132 P=0.367	R=-0.133 P=0.391	R=-0.365 P=0.095
Respirable particles, $\mu g/m^3$	R=0.364 P=0.005**		R=0.304 P=0.034*	R=0.228 P=0.115	R=-0.163 P=0.289	R=-0.211 P=0.346
CO_2, ppm	R=0.183 P=0.208	R=0.304 P=0.034*		R=0.092 P=0.523	R=-0.397 P=0.006**	R=0.290 P=0.169
NH_3, ppm	R=0.132 P=0.367	R=0.228 P=0.115	R=0.092 P=0.523		R=0.420 P=0.004**	R=-0.406 P=0.049**
Temperature, °C	R=-0.133 P=0.391	R=-0.163 P=0.289	R=-0.397 P=0.006**	R=0.420 P=0.004**		R=-0.621 P=0.001**
Relative humidity, %	R=-0.365 P=0.095	R=-0.211 P=0.346	R=0.290 P=0.169	R=-0.406 P=0.049**	R=-0.621 P=0.001**	

Conclusion

Over the period from September 2008 to March 2009, the mean recorded concentrations of inhalable and respirable airborne particles in large cold loose housing cowsheds in Estonia were moderate ($261\pm247\mu g/m^3$ and $24\pm18\mu g/m^3$). The concentration of inhalable dust in the air inside a cattle shed was primarily affected by the activities taking place inside the cattle building, e.g. the activity of the cows and the work routine (TMR feeding, manure removal). The concentration of respirable dust depended mostly on the concentration of inhalable dust, as well as on the air ventilation rate. Ammonia concentration, air temperature and relative humidity inside a cattle shed had no effect on the generation of inhalable and respirable airborne particles.

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Ammonia Concentrations in Dairy Cattle Houses with Tie Stalls in Transylvania

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Summary

The aim of this study was the quantification of ammonia concentrations in the air of dairy cattle tie-stall barns in Transylvania (Romania) and its comparison with the threshold limit value recommended for our country. The significance of the interactions between ammonia concentrations, temperature and relative humidity was also determined. We investigated 24 dairy shelters with tie-stalls in Transylvania, between December 2007 and January 2008. The statistically processed results showed a mean ammonia concentration of 30.68 ppm, a mean air temperature of 8.25 C and a relative humidity of 82%. The ammonia concentrations varied between 8 - 10 ppm in 8.33 % of the investigated shelters, between 11 – 26 ppm in 20.83% of the barns and in 70,83% of the barns it exceeded 26 ppm. A significant positive correlation between relative humidity and ammonia concentrations was found in all shelters. Assuming an ammonia threshold limit value of 26 ppm, 70.83 % of all dairy cattle houses in this study exceeded this recommended limit, indicating a need for improved housing conditions in the future.

Introduction

Airborne pollutants in livestock buildings increase susceptibility to common and important respiratory diseases and have to be considered in terms of environmental risk assessments due to emissions [9]. Ammonia has been considered the most significant air pollutant in dairy cattle barns; concentrations of less than 100 ppm ammonia appear to affect pulmonary function in cattle [7]. For this reason ammonia concentrations in animal shelters are set as threshold limit values. The most commonly recommended ammonia threshold limit value is 20 ppm, although other recommendations indicate 15 ppm or a threshold limit value extrapolated from humans of 7 ppm, considering the cumulative effect of other noxious elements [9]. In Romania the maximal admitted ammonia threshold limit value in cattle houses is 26 ppm [2]. The airborne ammonia concentration depends on several factors: housing type, breeding management, stock density, ventilation, temperature, relative humidity etc. [6,9]. This study's aim was to determine airborne ammonia concentrations in tie-stall system cattle shelters in Transylvania (Romania) and to compare the obtained results with the threshold limit values recommended in our country for dairy cows. The significance of interactions between ammonia concentrations, temperature and relative humidity was also determined.

Material and Methods

The study was conducted in 24 Transylvanian dairy cow shelters (15 – 68 cows/shelter) with tie stall system, in the period December 2007 – January 2008. All of the shelters used natural ventilation systems. The ammonia concentration was determined with Dräger indicator

tubes, early in the morning, before milking, at the cows' respiration level. We made 4 measurements in each shelter, in different sampling points and after we calculated the mean value of ammonia concentrations. In the same time we measured the inside air's temperature and relative humidity in the shelters, using the electronic thermo-hygrometer. The obtained data was processed with the GraphPad InStat, v.3 statistic software. The descriptive statistical indicators (mean, standard deviation, median, minimum, maximum) for the determined parameters, and Pearson's correlation coefficient (r) between ammonia concentration, relative humidity and air temperature were calculated. The relative frequency of the distribution of ammonia concentration, depending on the optimal values and the ammonia's maximal threshold limit values admitted for air in the cattle houses in our country was also determined.

Results

The ammonia concentration had a mean value of 30.68 ppm, ranging between 8 – 50.5 ppm; the mean temperature was 8.25'C, with limits of 6 – 11'C; and relative humidity's average was 82%, with a domain between 72.4% and 94.6% (Tab.1). Considering the values cited in scientific literature and the maximal admitted threshold limit for ammonia in the air of cattle shelters in our country, a relative frequency distribution was generated, showing that in 8.33% of the investigated shelters ammonia concentrations ranged between 8 – 10 ppm, in 20.83% of the shelters ammonia levels were between 11 – 26 ppm, and in 70.83% of the cattle houses ammonia levels exceeded 26 ppm (Fig.1). A significant positive correlation ($r = 0.9011$, $p<0.0001$) between relative humidity of the air and ammonia concentrations was found for all cattle houses, but not between the inside air temperature and ammonia concentration (Tab.2).

Tab.1: Descriptive statistic analysis for ammonia concentration, relative humidity and air temperature in the investigated 24 dairy cattle houses

Parameter	n	Mean	Standard deviation	Median	Minimum	Maximum	95% C I	
							From	To
Ammonia (ppm)	24	30.688	10.742	32.625	8.000	50.500	26.151	35.224
Temperature ('C)	24	8.258	1.325	8.100	6.000	11.000	7.699	8.818
Relative humidity (%)	24	82.004	7.056	83.150	72.400	94.600	79.024	84.984

Discussion

Ammonia concentration varied within the investigated shelters, ranging between 8 – 50.5 ppm, with a mean value of 30.68 ppm. This value is higher than the results of other researches, determining usual ammonia levels between 8 – 10 ppm in dairy cattle houses [1,3,6]. In our study only 8.33% of the assessed shelters showed ammonia concentrations between 8 – 10 ppm. The airborne ammonia quantities depend on flooring type, bedding, ventilation, the animal's age, the microclimatic factors, the dejection evacuating systems, the frequency of mechanical cleaning, the food intake [6]. The shelters with higher

Tab. 2: The correlation between airborne ammonia concentration, relative humidity and temperature of the air. *P < 0.0001, considered extremely significant, ^{P} = 0.6270, considered not significant.

Correlation	Number of points	Correlation coefficient (r)	Coefficient of determination (r squared)	95% C I	
				From	To
Ammonia concentration and relative humidity	24	*0.9011	0.8120	0.7819	0.9567
Ammonia concentration and temperature	24	**0.1045	0.01092	0.3121	0.4874

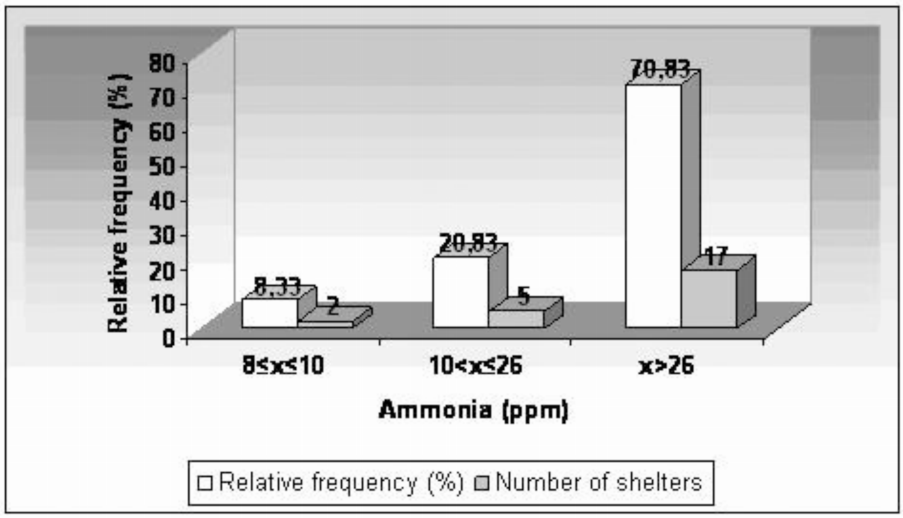


Fig.1 : Relative frequency distribution of ammonia concentration in Transylvanian dairy cattle houses with tie-stall system

airborne ammonia levels were poorly ventilated and dirty. In pursuance of an adequacy criterion of ammonia concentration of 26 ppm, 70.83% of the shelters were inappropriate, having more than 26 ppm ammonia concentration in the air. The results of one German study showed that in 72% of the cattle houses ammonia level was below 7 ppm and none exceeded 15 ppm [9]. Ammonia production and emission are usually influenced by air temperature and humidity. In our investigation the only correlation found was between relative humidity and ammonia ($r = 0.9011$, $p<0.0001$). In a study of Seedorf and Hartung [9], these significant interactions were not observed, but the positive correlation between relative humidity and ammonia was demonstrated for pig sheters. Ammonia is considered the major pollutant of the cattle houses' air, due to its irritant effect on respiratory epithelium [8]. Even in low concentrations, bellow 100 ppm, ammonia seems to affect the bovine pulmonary function [7]. Ammonia also contributed to creating an indoors pollutant factor for the outside environment [5]. In conformity with the obtained results, it is possible that bovine species might represent the largest source of airborne ammonia, as it is affirmed by several researchers [4,5].

Conclusion

Assuming an ammonia threshold limit value of 26 ppm, 70.83% of the investigated dairy cattle houses in this study exceeded this recommended limit, indicating a need for improved housing

conditions in the future. The air's relative humidity represents a factor influencing airborne ammonia concentrations in dairy cattle houses with tie-stall system.

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Composition and variability of airborne fungi in a closed rabbit house in China

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Summary

To investigate the composition and variability of fungal aerosols in the environment of a closed rabbit house, air sampling was performed for a period of 1 year from June–May, 4 days per month, using 6-staged Andersen sampler. The monthly mean concentration ranged from 2.7910^3 to 5.4610^3 CFU/m^3 air (CFU, Colony Forming Unit); variations in monthly concentrations of fungi were statistically significant (P(0.01). In the same day, it had the maximum level at 09:00 a.m., and its concentration changes were characterized as 9:00>17:00>13:00. Through analysis and identification, 6,523 fungal colonies were obtained; they belonged to 17 genera and 43 species, of which the predominant genera included *aspergillus*, *cladosporium*, *penicillium*, and *alternaria*, making up 62.21% of the total colony count. The obtained fungi were mainly centralized in the Grade D of the sampler (2.0-3.0μm), accounting for 37.8% of the total colony count.

Introduction

Fungal aerosols persistently produced from the environment of a livestock and poultry house may threaten not only the health of caretakers, as well as of livestock and poultry, but also contribute to pollution of the external environments [1]. With intensive development of the breeding industry, more and more livestock and poultry houses apply the totally enclosed breeding mode; under such breeding environment of high-density animals, fungal aerosols are more easily accumulated to result in harms.

An enclosed rabbit house with high breeding density is favorable to growth and reproduction of various fungal aerosols; however, little is known about airborne fungi in the environment of an enclosed rabbit house until now. This study was intended to detect composition, level, and particle size distribution features of airborne fungi in the environment of an enclosed rabbit house.

Material and Methods

Sampling Site and Time. The sampling site was the rabbit breeding farm of Shandong Qingdao Kangda Food Co., Ltd., 15 km away from the downtown area. This study was conducted in a period of one year from June 2007 to May 2008. Samples were collected on 4 randomly selected work days each month, at 3 sampling time points selected each working day, i.e. 09:00, 13:00, and 17:00, respectively.

Collection of Samples. The 6-staged ANDERSEN air sampler of international standard was placed in the center of the rabbit house, 1.5 M above the ground; using Sabouraud culture medium as sampling medium, the air flow velocity was 28.3 L/min during sampling, and the drive time was 2 min [2].

Treatment of Samples. The air samples collected were placed in an incubator at 28 'C, and incubated for 3 days, then the colony count of each culture dish was recorded; the colony count correction was conducted once in 7 days, and then fungal aerosol concentration (CFU/ m^3) was calculated. Subsequently, Morphological identification of fungal colonies was carried out.

Statistical Analysis. The experimental data obtained were analyzed and processed by Excel 2003 and Spss 11.5.

Results

Changes of Airborne Fungi (Table. 1). The monthly mean concentration of airborne fungi in the environment of the rabbit house was from 2.7910^3 to 5.4610^3 cfu/ m^3, with the maximum level in Oct. (5.4610^3cfu/ m^3), and the minimum level in Jan. (2.7910^3cfu/ m^3). The variations in monthly concentrations of fungi were statistically significant ($P<0.01$). Of the three time-points in the same day, the concentration of fungi at 09:00 was the maximum level, and it was greater compared to those at the other two time-points ($P<0.05$); however, there was no significant difference between the concentrations at the other two time-points, i.e. 13:00 and 17:00 ($P>0.05$).

Tab. 1: Concentrations of total fungi in air of the rabbit house during the year(June 2007-May 2008)(unit:cfu/m^3). n-number of days in which samples were collected.

	Jun (n=4)	Jul (n=4)	Aug (n=4)	Sep (n=4)	Oct (n=4)	Nov (n=4)	Dec (n=4)	Jan (n=4)	Feb (n=4)	Mar (n=4)	Apr (n=4)	May (n=4)
9:00 Median	4123	4528	4396	4608	6213	4096	3498	3003	3249	3907	4652	4308
13:00 Mean	3390	3533	3378	3840	4716	3097	2988	2471	2652	2985	3170	3907
13:00 Median	3207	3651	3421	3789	4651	3196	2685	2208	2799	2688	3011	4009
17:00 Mean	3877	4002	3901	4209	5377	3381	3201	2790	2970	3391	4007	4233
17:00 Median	3809	3925	4000	4220	5209	3290	3217	2788	2899	3286	3971	4228
Monthly mean concentrations	3853	4014	3918	4260	5458	3481	3219	2786	2963	3454	3948	4246

Composition of Airborne Fungi. Of the airborne fungi collected during the 12 months, 17 genera or flora, and 43 species of air fungi were identified. The predominant genera included: *aspergillus*, *penicillium*, *cladosporium* and *alternaria*, making up 22.25%, 16.65%, 14.64% and 8.67% of the colony count respectively. Other common genera included *fusarium*. and some sterile mycelia, making up 37.79% of the colony count (Table. 2).

Features of Fungal Particle Size Distribution. The percentages at the six grades of the ANDERSEN sampler were 14.2% (>8.2μm), 17.2% (6.0-8.2μm), 19.3% (3.0-6.0μm), 37.8% (2.0-3.0μm), 10.4% (1.0-2.0μm), and 1.10% (<0.65μm), respectively.

Tab. 2: Fungal species and quantity relations

	Number of colonies	Percent of total fungi (%)
Absidia sp.	12	0.18
Alternaria alternaria	396	6.07
Alternaria tenuissina	169	2.60
Aspergillus carbonarius	419	6.42
Aspergillus flavus	90	1.38
Aspergillus fumigatus	12	0.18
Aspergillus niger	136	2.08
Aspergillus oryzae veffusus	159	2.44
Aspergillus oryzae var oryzae	93	1.43
Aspergillus sydow ii	69	1.06
Aspergillus tamarii	18	0.28
Aspergillus versicolor	32	0.49
Aspergillus sp.	293	4.49
Aureobasidium pullulans	11	0.17
Bipolaris sp.	4	0.06
Bipsora betulina	97	1.49
Botryotrichum piluliferum	78	1.19
Cladosporium herbarum	694	10.63
Cladosporium macrocarpum	393	6.02
Cladosporium sp.	596	9.14
Emericella nidulans	59	0.89
Epicoccum nigrum	266	4.08
Fusarium sp.	147	2.25
Gliocladium roseum	225	3.45
Mucor plumbeus	17	0.26
Mucor sp.	9	0.14
Paecilonyces lilacinus	263	4.03
Penicillium canescens	151	2.31
Penicillium daleae	90	1.38
Penicillium expansum	9	0.14
Penicillium funiculosum	43	0.66
Penicillium griseofulvum	184	2.82
Penicillium nigricans	121	1.85
Penicillium purpurogenum	81	1.24
Penicillium restrictum	24	0.36
Penicillium rugulosum	96	1.47
Penicillium tardum	157	2.41
Scolecobasidium sp.	10	0.15
Trichodema harzianum	121	1.85
Trichodema pilulifem	65	0.99
Trichodema polysporum	101	1.55
Trichodema viride	22	0.34
Trichodema sp.	39	0.60
No identification	452	6.93
Total	6,523	100

Discussion

The determined concentrations of fungal aerosols were much higher than air fungi concentrations in the indoor and outdoor environments [3] suggesting that, the breeding environment was one of the major pollution sources of atmospheric fungi.

In this study, 6,523 fungal colonies were isolated. Different fungal species may have different pathogenicities. Therefore, it needs in-depth investigation of aerosol fungal pathogenicity and body immunity to determine harms of fungal aerosols to human beings and animals.

Grouped by seasons, the concentrations in summer (Jun.-Aug.) and autumn (Sep.-Nov.)

are higher than those in winter (Dec.-Feb.) and spring (Mar.-May), consistent with the research results about the environment of hen houses conducted by Wang, et al. [3] The results showed that 31.4% of fungal aerosol particles are larger than 6.0μm. Due to gravity action, such particles float in air for a shorter time. They may enter bronchiole or alveoli directly; aerosol particles of such size do Brown motion in air, and float for a longer time, thus leading to increased opportunities to be absorbed by human beings or animals [4]. Therefore, such absorbable fungal aerosols may pose greater threats to health of human beings and animals.

Conclusion

1. *aspergillus*, *penicillium*, *cladosporium* and *alternaria* in the environment of a rabbit house are major pathogens.
2. The concentration of airborne fungi change obviously in the same day and different months. With the maximum level in Oct. and the minimum level in Jan, the levels in the same day are characterized as 09:00>17:00>13:00.
3. The particle size distribution trend of airborne in the environment of the rabbit house is characterized with the maximum value at Grade D, the minimum value at Grade F, and the air fungi were mainly distributed in the first four Grades.

Acknowledgement

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Chapter 27

Block 2C

Air Leakage and Ammonia Emissions from Covered Slurry Storage Tanks

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Summary

By measuring the decay in concentration of an injected trace gas at different occasions, the air flow leakage was determined in three covered slurry storage tanks with the following coverings, wooden roof, plastic tent and concrete roof.
Air from five different locations in the air space inside the tanks was sucked to an infrared spectrophotometer.
The studies demonstrated that trace gas technique can be a reliable method of determining the air leakage from covered slurry storage tanks. It was not possible to observe any form of layering/accumulation of the gas in any specific areas of the tanks. Individual measuring locations showed very small differences in the decay process both with and without mixing of the air volume above the slurry.
Measurements indicated that wind may have a considerable effect on the air leakage from the tanks.
The mean values of the air exchanges for the tanks varied between 0.33 and 0.86 m3/ m2, h.
The plastic tent had the lowest air exchange in relation to the bottom area.

Introduction

The objective of this investigation was to study whether it is possible to determine the amount of ammonia released from covered slurry storage tanks using trace gas technique by injection of sulphur hexafluoride (SF_6) and measurements of the decay in gas concentrations (infrared spectrophotometer) in the air space above the slurry for determination of air leakages.

Sulphur hexafluoride has the advantage of being detectable at very low concentrations that are far below the hygienic threshold limit value for the gas. It is not present in a normal building atmosphere and is also chemically stable. A disadvantage is the high density of the gas. The gas is 5 times heavier than normal air.

Material and Methods

By measuring the concentration of the trace gas at different occasions, the air flow rate was calculated out of:

$$q = -\frac{V}{t} \cdot \ln\left(\frac{C(t)}{C(0)}\right)$$

Figure: Equation

where
q = air exchange rate, m^3 /h
V = air volume in the tank, m^3
t = time, h
C(t) = trace gas concentration as function of time, ppm
C(0) = initial trace gas concentration, ppm

The study was conducted in two phases. The first consisted of studying whether the air above the surface of the slurry was mixed, and if this was not the case to find a way of achieving mixing of the air. The second phase consisted of using the technique to determine the air flows and the ammonia emissions from three manure tanks with different types of coverings, namely, wooden roof, plastic tent and concrete roof.

An injection hose with small holes (one hole per meter) was put down into the air space in the middle of the tank with the wooden roof in order to determine whether the trace gas inside the tank was mixed or not. Air from five different locations in the air space was sucked to an infrared spectrophotometer (Miran 104, Foxboro Analytical Invensys, Foxboro, Massachusetts, USA) for measurements of the trace gas concentrations in the different locations. The decay of the trace gas was then measured.

Two cases were investigated with this tank:
– No mixing of the air inside the tank
– Mixing of the air with a fan with an air flow rate of 300 m3/h.

The tank with concrete roof had a circular opening (diameter 0.85 m) in the roof for a manure pump. This tank was investigated with and without cover of the opening in order to evaluate the influence of large openings in the roof construction. These investigations were carried out at different wind speeds.

In order to make it possible to compare the tightness of different constructions, the air flows were related to the bottom areas of the tanks.

Results

The studies demonstrated that trace gas technique can be a reliable method of determining the air leakage from covered slurry storage tanks. It was not possible to observe any form of layering/accumulation of the gas in any specific areas of the tanks, see Fig.1. Individual measuring locations showed very small differences in the decay process both with and without mixing of the air volume above the slurry. The high density of the gas is, thus, not a limiting factor for its use in this type of study.

Air exchanges at different locations in the air space in the tank with wooden roof showed small variations within individual treatments.

The measurements indicated that the external climate (influence of wind) may have a considerable effect on the air leakage from the tanks (Fig. 2). The explanation is most probably that the wind pressure affects the air flow through the leaks present in the roofing constructions. The influence of wind speed on air exchange was investigated in the tank with concrete roof in two cases; when the opening for the manure pump was open or covered with a plywood sheet respectively. The measurements clearly showed that the wind speed in both cases affected the air exchange through the tank. In order to be able to characterise the leaks in different constructions in a comparable manner, the external climatic conditions should be standardized when measurements are made. It is proposed that measurements of tightness are made under wind-free conditions.

Measurements also proved that the area of openings in the storage tank influences the air exchange in the air volume inside the tank (Fig. 2).

Results from measurements from the three tanks are presented in Tab.1. The mean values of the air exchanges for the tanks varied between 0.33 and 0.86 m3/ m2, h. The plastic tent had the lowest air exchange in relation to the bottom area.

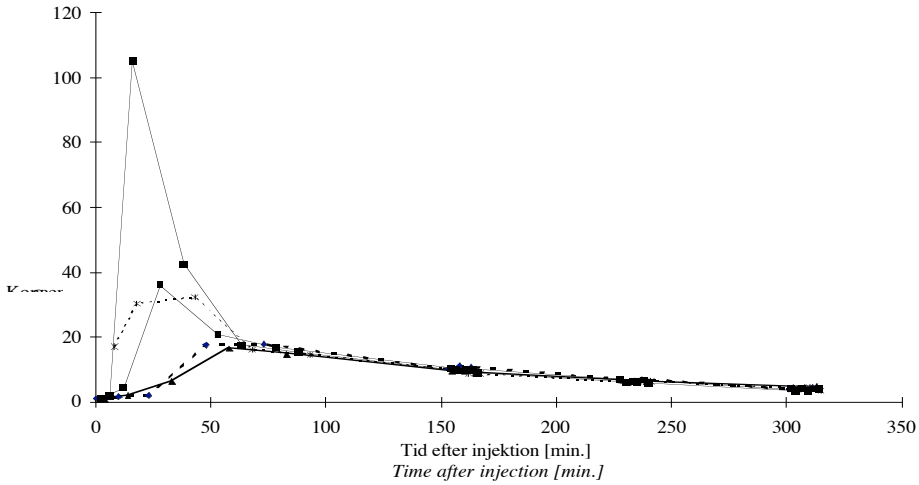


Fig. 1. Decay of trace gas concentrations at different measuring locations when no air mixing occurred in a storage with wooden roof.

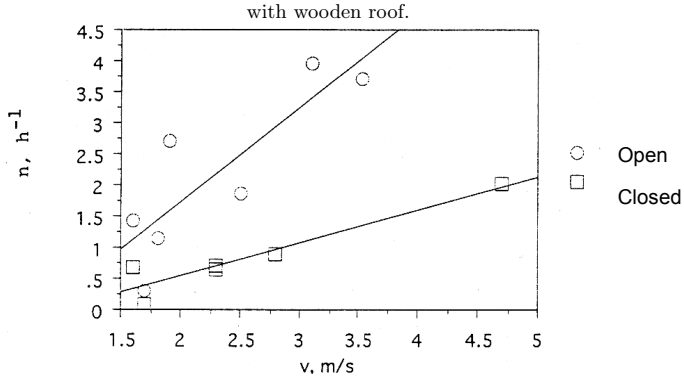


Fig. 2. Air exchanges (n) in a tank with concrete roof at different wind speeds when the opening for the manure pump was open or covered respectively.

Tab. 1. The air flows and the ammonia emissions from the tanks

Exp. treatm.	Replication	Air flow (m^3/m^2, h)	Air exchange (h^{-1})	NH_3-emission (mg/m^2, h)
Wooden	1	0.60	0,19	-
roof	2	1.12	0,36	-
	x	0.86	0,28	-
Plastic tent	1	0.30	0,15	6.7
	2	0.36	0,18	12.8
	x	0.33	0,17	9.8
Concrete	1	0.73	0,92	-
roof	2	1.61	2,02	-
(covered	3	0.08	0,11	-
opening)	4	0.56	0,71	-
	5	0.53	0,67	-
	6	0.56	0,70	-
	x	0.68	0,85	-

x = average.

Conclusion

The studies demonstrated that trace gas technique can be a reliable method of determining air leakages from covered slurry storage tanks. The trace gas used in these studies was sulphur hexafluoride, which has a much higher density than normal air. It was not possible to observe any form of layering/accumulation of the gas in any specific areas of the tanks. Individual measuring locations showed very small differences in the decay process both with and without mixing of the air volume above the slurry. The high density of the gas is, thus, not a limiting factor for its use in this type of study. Air exchange at different locations in the air space in the tank with wooden roof showed small variations within individual treatments.

Measurements indicated that wind may have a considerable effect on the air leakage from the tanks. It is proposed that measurements of tightness are made under wind-free conditions.

Measurements also proved that the area of openings in a storage tank influences the air exchange in the air volume inside the tank.

A plastic tent had the lowest air exchange in relation to the bottom area.

Application of mineral sorbents for reduction of odorous volatile compounds emitted from cattle manure

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Summary

The aim of the research was to estimate the influence of selected aluminosilicates added to the cattle manure on the reduction of organic gaseous compounds emission to the air. The fresh cattle manure was placed in cone-flasks which were divided into 4 groups and investigated sorbents were added: group I – no sorbents addition (control), group II – expanded vermiculite (5 % of the manure mass), group III – raw bentonite (20 % of the manure mass), and group IV – raw halloysite (20 % of the manure mass). Headspace samples from the flasks were collected with solid phase microextraction (SPME) and analysis were performed by using a GC/MS method. In the air samples of all experimental groups 17 odorous compounds, including 4 sulphoorganic compounds, were determined. It was stated, that all investigated sorbents added to the manure reduced the emission of identified odorants. The highest removal efficiency was determined for vermiculite, after 4 days of experiment odorous compounds concentration was about 80% lower in comparison to control group. The potential of removal for identified odorous compounds on cattle manure matrix using investigated sorbents was in order of vermiculite>halloysite>bentonite.

Introduction

The presence of the odorous compounds at animal farms was stated. Moreover, odorants may present a health hazard for both livestock and staff of animal farms [4]. Odours are specific kind of air pollutants mainly due to the fact that humans can perceive it at a very low amount even below the concentration detectable by gas chromatography. It is estimated that the limit of detection of human nose is 10^8 or 10^9 molecules of odorant vapour while 1μg of ethanethiol in air constitutes approximately 10^{16} molecules, 10^7 or 10^8 times the amount necessary for detection [6, 7]. The main odorous compounds generated by animal production are ammonia, sulphur compounds (hydrogen sulphide, mercaptans), VFA and phenolic compounds [3, 5, 8, 10].

One of the potential ways of odorants limitation in air of livestock buildings is application of aluminosilicates sorbents.

The aim of the research was to estimate in laboratory scale the influence of vermiculite, bentonite and halloysite, added to the cattle manure, on the reduction of odorous compounds emission.

Material and Methods

The fresh cattle manure (50g) and wheat straws were placed in 12 cone-flasks (500 ml). The flasks were divided into 4 groups and investigated sorbents were added: group I – no sorbents addition (control), group II – expanded vermiculite (5% of the manure mass), group III – raw bentonite (20% of the manure mass), and group IV – raw halloysite

(20% of the manure mass). Odour compounds were extracted (sampling time=30 min.) from headspace of each flask after 1, 4 and 8 days in the temperature of 30 ‘C. The extraction of analyzed substances was carried out by the SPME technique, a manual SPME holder with StableFlex 50/30μm divinylbenzene/Carboxen/polydimethylsiloxane fibre was used (Supelco, USA). The injection port operated at 240 ‘C for SPME desorption (time of desorption - 7 min.). Before each sampling fibres were conditioned for 1 hour in the injection port at 270 ‘C. Chromatographic separation were performed using Restec column (5% diphenyl; 30m x 250ym x 0,25μm) in Thermo Electron, Finnigan Focus Polaris Q type gas chromatograph coupled with mass spectrometer (GC/MS method). The identification of odorous compounds was based on comparative analysis of determined mass spectrum and MS from spectrum commercial library NIST.

Results

In the air samples collected from headspace of all experimental groups 17 odorous compounds, including 4 sulphoorganic compounds, were determined (fig. 1). The investigation showed that odorous organic compounds emission was the highest in the control group, no sorbents addition. It was stated, that all investigated sorbents added to the manure reduced the emission of identified odorants. The determined levels of reduction are summarized in table 1. The highest removal efficiency was determined for vermiculite, after 4 days of experiment odorants concentration was about 80% lower in comparison to control group. In case of bentonite and halloysite the highest removal efficiency was determined after first day of experiment. The level of reduction was 68,4% for bentonite and 68,5 % for halloysite.

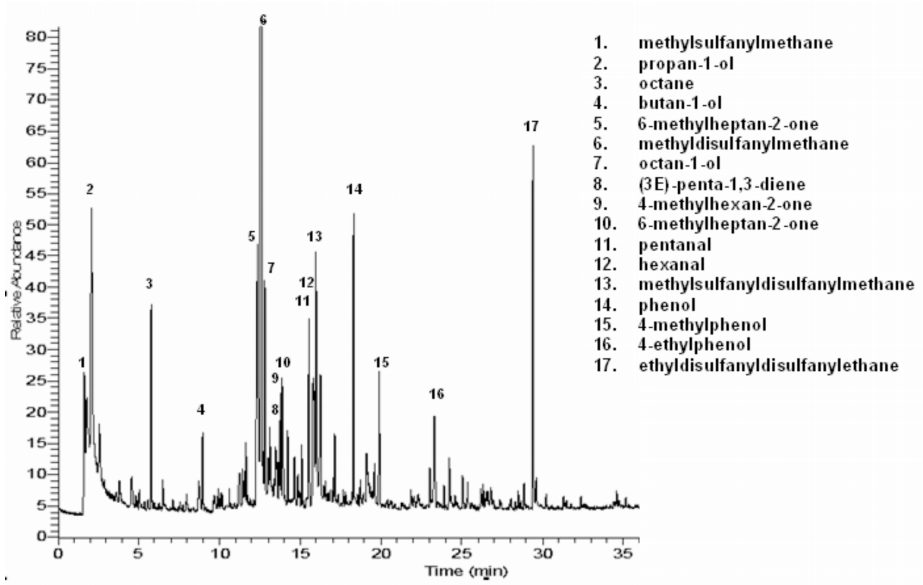


Fig. 1: Chromatogram of gas sample collected from control group

Tab. 1: The level of reduction [%] determined for identified odorous compounds after application of investigated mineral sorbents in comparison to control group

Name	Time of retention (min)	Vermiculite			Bentonite			Halloisyte		
		I	II	III	I	II	III	I	II	III
methylsulfanylmethane	2,07	-29,88	-82,88	-71,14	-22,39	+83,69	-3,40	-71,02	-22,44	-44.40
propan-1-ol	2,41	-21,49	-71,13	-23,20	-100,00	-37,13	-38,74	-100	-70,15	+7,40
octane	5,80	-61,87	-69,14	-9,35	-39,03	-39,03	+115,93	-84,13	-87,50	+193,42
butan-1-ol	8,95	-64,02	-69,39	-71,46	-59,04	-13,34	+0,95	-77,36	-75,02	+27,67
6-methylheptan-2-one	12,40	-60,18	-21,40	+183,96	-49,26	-38,33	-3,24	-13,66	+92,87	+74,36
methyldisulfanylmethane	12,57	-75,40	-86,12	-68,71	-57,12	+24.97	+29.98	-81,93	-78,25	+30,26
octan-1-ol	12,75	-58,70	-84,11	-35,91	-34,95	-49,67	+93,42	-75,39	-76,13	+165,35
(3E)-penta-1,3-diene	13,35	-36,59	-100,00	-74,04	-18,25	-53,20	-39,15	-100	-60,55	-57,50
4-methylhexan-2-one	13,76	-100,00	-100,00	-52,39	-48,49	-44,49	+31,95	-82,58	-69,12	+17,20
6-methylheptan-2-one	13,91	-94,87	-95,03	-100	+274,23	-80,61	+224	-100	-88,96	+10,71
pentanal	15,41	-65,49	-68,13	-43,77	-100,00	-9,40	-100	-66,65	-64,40	-52,16
hexanal	15,77	-72,72	-71,70	+89,41	-100,00	-45,32	-75,73	+69,46	-67,26	-64,09
methylsulfanyldisulfanyl methane	15,98	+116,00	-100,00	-100,00	-83,00	-31,61	-83,81	-68,06	-34,65	-46,41
phenol	18,31	-71,84	-93,96	-57,88	-61,31	-58,21	+94,25	-84,34	-84,35	+88,50
4-methylphenol	19,88	-71,21	-27,75	-62,54	-85,17	+31,93	-51,49	-77,55	+33,00	-33,09
4-ethylphenol	23,31	-59,01	-11,66	-4,13	-73,88	+53,24	-13,47	-61,47	+48,45	+13,91
ethyldisulfanyldisulfanylethane	29,43	-85,67	-88,61	-88,89	-96,41	-88,86	-97,65	-79,67	-35,90	-93,99
Total volatile sulphoorganic compounds		**-34,90**	**-89,94**	**-84,19**	**-68,49**	**-8,34**	**-48,08**	**-76,74**	**-50,55**	**-42,22**
Total volatile organic compounds		**-51,65**	**-80,00**	**-42,22**	**-68,36**	**-34,14**	**-17,85**	**-68,54**	**-36,93**	**-10,13**

Time of collection: **I** – after 1 day, **II** – after 4 days, **III** – after 8 days,

Discussion

The most of odorous compounds determined during investigation were also identified at dairy farm by Filipy et al. [2]. Moreover, Beck et al. [1] determined VOC's like alcohols or hexanal in builidings for cattle however sulphoorganic compounds were not determined. Turan et al. [9] were investigating the potential of natural minerals (also expanded vermiculite) to reduce volatile organic compounds produced during composting of poultry litter. The removal efficiency for expanded vermiculite was about 60% proving that aluminosilicates have potential to reduce concentration of gaseous pollutants and the application of investigated sorbents in livestock buildings look promising and require further research in this field.

Conclusion

It was found that potential of removal for identified odorous compounds on cattle manure matrix using investigated sorbents was in order of vermiculite>halloysite>bentonite.

Acknowledgement

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Dietary influences on ammonia emissions from rabbit excreta

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Summary

The topic of ammonia, which is widely investigated in husbandry of food producing animals, is discussed from the point of view of small animal keeping here. The objective was to evaluate a method to quantify odour caused by ammonia emitted from rabbit excreta. In addition dietary influences on ammonia emissions were investigated. Six female adult dwarf rabbits were kept individually in metabolic cages without litter and the total faeces and urine excreted between 17 and 9 o'clock was collected. Three diets differing inter alia in their crude protein, crude fibre and macro element content were fed to the rabbits and their effects on pH-values and urea content of excreta as well as ammonia emissions were analysed. Especially for this purpose, a new method has been developed. It provides a standardised and quantifiable measurement of ammonia emissions from rabbit excreta and withal stimulates the conditions in a typical small animal cage. A diet rich in crude fibre and low in macro elements and protein (hay) resulted in lower pH-values in urine and excreta mixture and caused marked reductions in ammonia emissions.

Introduction

In contrast to former times, small animals like rabbits are often kept in close habitation with people today. Thus, unpleasant odour, released of faeces and urine in wet dirty litter can cause a nuisance and interfere with the pet owner's well-being. Therefore, in a first step these investigations focus on ammonia (NH_3), which is one of the principal classes of odour compounds released of faeces and urine [5]. The aim of this study was to quantify faecal odours, to establish a standardised measuring technique and especially to investigate dietary influences on NH_3 emissions from rabbit excreta. The most important measure for reductions of NH_3 emissions can be made in the field of nutrition. Particularly, a crude protein supply avoiding protein oversupply leads to reduced nitrogen excretions [2]. Experiments with fattening pigs showed, that a comparatively low crude protein content of diets reduces odour compounds like indole, phenol and sulphur compounds in manure as well as ammonia emissions [4]. Another attempt to lower NH_3 emissions can be the supply of a diet rich in crude fibre. Thereby, the growth of microorganisms in the large intestine is stimulated, which leads to an increased microbial nitrogen fixation and excretion via faeces in the form of bacterial protein [1]. As the pH-value of manure is of great importance for the regulation of ammonia volatilization [6], modelling ammonia losses via the acidification of the excreta is another important attempt. Therefore, the main buffering components in rabbit urine, macro elements like calcium (Ca) magnesium (Mg) and sodium (Na) were varied in the diets used for this study.

Animals, Material and Methods

Animals. Six adult female dwarf rabbits were kept individually in metabolic cages without litter. These cages allow the collection of faeces and urine as well as an exact documentation of feed and water intake of each animal.

Diets. At first, three trials were conducted, in which the rabbits were fed different diets. Due to a cross-over design, each rabbit was fed each diet. The first diet represented a pelleted commercial complete feed (ccf) for rabbits (ration: 40 g diet/kg bw/rabbit/day), the second one was composed of a reduced amount of ccf (20g diet/kg bw/day) in addition to hay (ad libitum) and the third diet consisted of hay exclusively (ad libitum). Water was offered continuously ad libitum. As intended, the three diets varied significantly in their crude protein, crude fibre and macro element content. High pH-values in excreta reinforce the development and volatilisation of ammonia. Thus low contents of buffering macro elements in the diet and therefore in urine should result in reduced ammonia concentrations due to the acidification of excreta. Offering hay in this case corresponds with a protein supply meeting the requirement for maintenance, as it is practised in commercial husbandry. With its low crude protein and high crude fibre content, feeding hay only is supposed to reduce NH_3 emissions by decreasing urea excretion in urine as well as by intensifying the microbial nitrogen fixation in the large intestine. It also should act as an indicator for evaluating the method. The following table shows the three different diets and the allotted ration.

Tab.1: Offered amounts of the diets (g diet/kg bw/day)

	ccf (g/kg bw)	hay
diet 1	40	-
diet 2	20	ad libitum
diet 3	-	ad libitum

To characterize the diets and to point out how far they differ, especially in their crude protein (CP), crude fibre (CF) and macro element content, the next table shows their chemical composition.

Tab 2: Chemical composition of the diets (ccf and hay)

g/kg FM	DM	CP	CF	CFI	CA	NfE	Ca	P	Mg	Na	Cl
ccf	879	160	19.0	144	77.7	608	11.8	5.04	2.23	2.20	13.8
hay	920	101	9.16	301	45.5	463	4.1	3.21	1.64	1.40	16.6

Experimental design. Five days of adaptation were followed by three experimental days. During these three days, the total faeces and urine excreted by each rabbit between 17 and 9 o'clock were collected and quantified. The pH-values in urine, faeces as well as in the diluted excreta mixture (em) were analysed. To prepare the diluted em, the total of faeces and urine excreted by each rabbit excreted between 17 and 9 o'clock was mixed and homogenised. To this mixture distilled water was added until a ratio of 1:5 (faeces: urine/water) was reached. By this means, the moisture content of each diluted em was the same and the homogenisation has been enabled. After that, 20 g of the diluted em of each rabbit were weighed into a bowl together with 10 g straw and put into an exsiccator, which represents a head space with a standardised volume. The straw and the diluted em should simulate the conditions in a rabbit cage and were thereupon incubated for 6 hours

at 37‘C. The incubation at 37‘C simulates the temperature of faeces and urine at the time of excretion and provides good conditions for the microorganisms and therefore for the conversion of urea into ammonia. Hereafter the NH3 concentration in the head space of the exsiccator was detected with a Draeger tube and a gas detection pump. To quantify the urea content in the diluted em, an enzymatic in vitro test was used. Therefore an aliquot of the sample was centrifuged and the urea content of the supernatant of the diluted em was analysed photometrically.

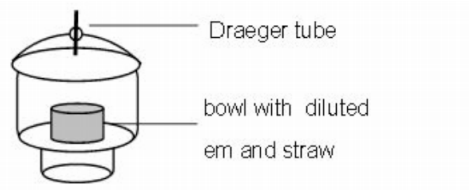


Fig 1: Scheme of experimental-setup

Results

The following results show the average pH-values in faeces, urine and the diluted em as well as the average ammonia concentrations detected in the head space above the sample (20 g diluted em and 10 g straw) after six hours of incubation (37‘C). The values of the ammonia concentration [ppm] are each related to 1 g of diluted em. The results of the urea quantification are related to one kg supernatant of the diluted em after centrifugation.

Tab. 3: Results of pH-, urea- and ammonia measurement after feeding different diets (1-3)

diet	ammonia [ppm]	pH_{faeces}	pH_{urine}	$pH_{diluted\ em}$	urea [g/kg]
1	33.2+-10.0	8.66+-0.28	9.14+-0.33	8.80+-0.26	10.5+-3.77
2	30.9+-8.84	8.77+-0.22	9.08+-0.28	8.79+-0.28	8.21+-2.22
3	7.99+-2.97	8.86+-0.08	8.31+-0.30	8.51+-0.16	5.23+-2.33

The results show that feeding hay exclusively (diet 3) - when compared to diet 1 and 2 - led to lower pH-values in urine and diluted em and reduced urea contents of the supernatant (coming from urine). Furthermore, it caused obvious reductions in ammonia concentrations in the head space above the sample. Offering a reduced amount of ccf and hay ad libitum additionally (diet 2) resulted in slightly lower ammonia concentrations compared to diet 1. Furthermore the pH-values in urine and diluted em slightly decreased as well and the urea content in the supernatant of the diluted em also decreased.

Discussion

As small animals are often kept in close habitation with people today, the results of this study indicate that it is possible to prevent odour nuisance and to improve the pet owner’s comfort. An important aspect is that a protein supply meeting the requirements of animals (well known from commercial husbandry) should also be practised in small animal nutrition. As small animals mostly don’t accomplish high performances, this would also be an approach for prophylaxis of obesity, which is a prevalent problem in pets. The reduced pH-value of urine after the application of hay exclusively on the one hand lowers ammonia

volatilisation from the diluted em. On the other hand, it is an important attempt to prevent uropathy e. g. the precipitation of urinary gravel (sludge) and uroliths. The high urinary Ca concentration, combined with the alkaline pH-value of rabbit urine, increases the risk of precipitation [3]. As commercial feed for rabbits often shows considerable Ca contents uropathy is a severe problem.

Conclusion

1. A standardised method for measuring ammonia emissions from rabbit excreta simulating the typical conditions of keeping dwarf rabbits as pets has been investigated.
2. Ammonia emissions can be reduced by feeding a low-protein diet rich in crude fibre.
3. A diet low in buffering macro elements like Ca and Mg lowers the pH-values in urine and diluted em of rabbits, but only to a small extent.

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Gaseous emissions associated to a high-fibre diet fed to gestating sows group-housed on deep litter

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Introduction

Restricted diet for gestating sows prevents excessive body weight gain and fat deposition but reveals stereotypic behaviour and welfare impairments. On the other side, providing ad libitum high-fibre diet (HFD) may prevent occurrence of stereotypes by reducing feeding motivation without negative impacts on body weight and performance. Sugar beet pulp (SBP) based diets are more effective on satiety and on reduction of voluntary feed intake than other fibres sources, probably in relation with their great water retention capacity. However, there is no data about the environmental impact of this feeding system, particularly about gaseous emissions.

Gaseous emissions from agriculture contribute to a number of environmental effects. Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are greenhouse gases taking part to the global problem of climate change. Ammonia (NH_3) emissions are responsible of soil and water acidification and eutrophication, and contribute also to indirect emissions of N_2O. Therefore, the aim of this study was to investigate the effects of a HFD offered ad libitum to gestating sows on gaseous emissions (NH_3, N_2O, CH_4, CO_2 and water vapour (H_2O)).

Animals, Material and Methods

Four successive batches of 10 gestating sows were divided into 2 homogeneous groups of 5 animals according to the parity, the body weight and the backfat thickness. Each group was randomly allocated to a treatment: restricted (R) or ad libitum (A) feeding regimes. The R sows received a conventional gestation diet (8876 kJ net energy/kg) based on cereals (66% of wheat, wheat bran, barley and corn). They were fed once a day and blocked in individual feeding stalls during the feeding time (1 h). The A sows had unrestricted access to a HFD (8034 kJ net energy/kg) based on SBP (42%).

The animals were group-housed on straw-bedded pen of 12.6 m^2. The sows were housed in identical experimental rooms (1 room per group) from 4 weeks after service until 15 days prior to giving birth. In both rooms, the ventilation with an exhaust fan was automatically adapted to maintain a constant ambient temperature. The ventilation rates were recorded using an Exavent apparatus The gas concentrations in the experimental rooms and in the corridor supplying fresh air were measured by infrared photoacoustic detection (Multi-gas

Monitor - INNOVA 1312) during 6 consecutive days at the 6th, 9th and 12th weeks of gestation. For each gas, the emissions (Egas) were calculated on an hourly basis and expressed in mg/h using the formula: Egas = D (Ci – Ce) with D, the hourly mass flow (kg air/h); Ci and Ce, the concentrations of gas in the air of the room and corridor respectively (mg/kg air). The CO_2 equivalents (CO_2eq) were calculated by using the equation: CO_2eq (kg/d per sow) = 21 E_{CH4} + 310 $E_{N2O}$with E_{CH4} and E_{N2O} being the emissions of CH_4 and N_2O (kg/d per sow) respectively, and taking into account that the warming potentials of CH_4 and N_2O over a 100-year period are, respectively, 21 and 310 times that of CO_2. For E_{N2O}, indirect emissions from atmospheric deposition of N from NH_3 on soils and water surfaces have been added to the direct emissions and were calculated considering an emission of 0.01 kg N_2O-N/kg emitted NH_3-N.

Results and Discussion

While feed intakes were 2.99 and 3.28 kg/d per sow for R and A sows respectively ($P<0.05$), the net energy intakes were similar for both groups with on average 26.45 MJ/d per sow. The non-starch polysaccharides intakes were 782 and 1566 g/d per sow for R and A sows respectively ($P<0.001$). For body conditions and reproductive performance, there were no differences between groups. The mean initial and final body weights were 202 kg and 256 kg respectively with an average daily gain of 727 g/d. The mean initial and final backfat thicknesses were respectively 14.6 mm and 21.0 mm. On average, each sow gave birth to 13.2 piglets of which 11.8 were alive. Thus, sows offered ad libitum HFD can regulate their consumption to recommended levels without negative impact on performance.

About gas emissions (Table 1), the room with A sows produced more NH_3 (+80%, $P<0.001$), less N_2O (-61%, $P<0.001$), more CH_4 (+13%, $P<0.01$), less CO_2eq (-43%, $P<0.001$), more CO_2 (+24%, $P<0.001$) and more H_2O (+28%, $P<0.001$) than room with R sows.

Some authors observed a decrease in NH_3 emissions from slurry of growing-finishing pigs offered SBP based diet [2,3]. This decrease was attributed to a lower slurry pH and the shift of a part of excreted N from urine (as urea, a very volatile form of N) to faeces (as protein form, a more stable form of N). But in the experiments of Canh et al. [2,3], the NH_3 emissions were measured in laboratory conditions after mixing faeces and urine, singly collected in metabolic cages. Thus, experimental conditions differ highly from current essay performed under field conditions with grouped sows kept on straw deep litter. Moreover, straw bedding may constitute a potential source of dietary fibre and consequently may reduce diet difference between groups. Besides, deep litters seem to emit more NH_3 than slurry, as observed by Philippe et al. [5] and Cabaraux et al. [1] with fattening and weaned pigs respectively.

In the present trial, more N_2O and, according to the N-balance, more N_2 were emitted from room with R sows. Thus it seems that nitrification/denitrification process was in progress in the litter of R sows but failed to achieve full conversion of all the substrate into N_2 with more N_2O emitted as by-product. Therefore, favouring emissions of non-pollutant N_2 may be unproductive if suboptimal conditions lead to higher N_2O emissions with large warming potential.

Methane originates both from digestive tract of animal and from manure. In the present experiment, CH_4 emissions from A sows were 2 g/d greater than these ones from R sows and could be explained by a greater enteric production of the sows offered HFD.

Carbon dioxide from piggeries has also two origins: mainly animal respiration but also manure. CO_2 exhalation is estimated to about 2.8 kg/d per sow [4]. This production is also function of metabolism and activity. In this study, video recording of animal activity showed that A sows could be considered as more active because of higher time feeding (twice as much

than R sows). Therefore, greater CO_2 productions from room with A sows could be expected and was observed. With R sows, production from manure seems to be neglected while with A sows, production could be estimated to about 0.2 kg.

Due to the higher warming potential over a 100-year period of N_2O compared to CH_4, the CO_2eq emissions were significantly lower with the A sows (-43%) than with the R sows. Indeed, with the A sows, even if the CH_4 and NH_3 (used for the calculation of the indirect emissions of N_2O) emissions were higher (+13% and +80% respectively), the differences in N_2O emissions were more relevant (-61%).

In the current experiment, the room with A sows emitted about 1 kg H_2O more than the room with R sows what could be explained by the water intakes of A sows who drank about 1 l water more than the R sows.

Tab. 1: Gas emissions per day and per sow from deep litter pens with gestating sows offered a restricted conventional diet (R) or ad libitum diet (A); [a] Mean standard deviation between mean values of the 3 periods of measurements; [b] Mean standard deviation between the 4 batches; *: P<0.001; : P<0.01; *: P<0.05; NS: not significant

	R	A	Significance
Batch 1 [a]			
NH_3 (g N)	5.96 ± 0.75	15.04 ± 3.47	**
N_2O (g N)	1.54 ± 0.80	1.26 ± 0.73	*
CH_4 (g)	18.04 ± 18.17	20.78 ± 12.62	NS
CO_2eq (kg)	1.16 ± 0.70	1.12 ± 0.63	NS
CO_2 (kg)	2.28 ± 0.15	3.20 ± 0.51	**
H_2O (kg)	3.60 ± 0.11	5.43 ± 0.94	**
Batch 2 [a]			
NH_3 (g N)	4.82 ± 0.66	6.64 ± 1.21	*
N_2O (g N)	4.04 ± 3.20	0.81 ± 0.38	**
CH_4 (g)	8.88 ± 4.89	10.64 ± 1.80	*
CO_2eq (kg)	2.18 ± 1.61	0.65 ±0.23	**
CO_2 (kg)	2.11 ± 0.26	2.63 ± 0.40	**
H_2O (kg)	3.07 ± 0.64	3.24 ± 0.61	NS
Batch 3 [a]			
NH_3 (g N)	6.64 ± 2.24	8.77 ± 4.10	NS
N_2O (g N)	2.52 ± 1.26	1.00 ± 0.75	**
CH_4 (g)	25.38 ± 20.55	21.39 ± 9.62	NS
CO_2eq (kg)	1.79 ± 0.77	0.98 ± 0.56	**
CO_2 (kg)	2.62 ± 0.36	3.05 ± 0.41	*
H_2O (kg)	4.30 ± 0.15	4.70 ± 0.96	NS
Batch 4 [a]			
NH_3 (g N)	4.05 ± 0.83	8.09 ± 4.21	*
N_2O (g N)	1.82 ± 2.00	0.79 ± 0.48	*
CH_4 (g)	8.56 ± 1.43	15.98 ± 2.65	**
CO_2eq (kg)	1.09 ± 1.01	0.76 ± 0.31	*
CO_2 (kg)	2.62 ± 0.50	3.12 ± 0.50	*
H_2O (kg)	3.76 ± 0.94	5.47 ± 1.48	*
Batches 1 to 4 [b]			
NH_3 (g N)	5.37 ± 1.15	9.64 ± 3.71	***
N_2O (g N)	2.48 ± 1.12	0.96 ± 0.22	***
CH_4 (g)	15.21 ± 8.08	17.20 ± 5.00	**
CO_2eq (kg)	1.55 ± 0.52	0.88 ± 0.21	***
CO_2 (kg)	2.41 ± 0.26	3.00 ± 0.26	***
H_2O (kg)	3.68 ± 0.51	4.71 ± 1.04	***

Conclusion

Feeding an ad libitum fibrous diet (42% SBP) to group-housed gestating sows kept on straw deep litter did not modify performance at short term. However, environmental impacts of this system seem to be ambiguous with an increase of NH_3 emissions but a decrease of N_2O and CO_2 equivalents emissions.

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The improvement of the composting process of poultry carcasses by adding effective microorganism

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Summary

Sawdust, hay, plastic tubes, plastic covers and poultry carcases were used to make four equal composting piles. Tow of these piles were sprayed with Effective Microorganisms (EM). Cultured petri dishes with *Salmonella* and *E. coli* were put inside the composted piles and regular observations on the temperature, moisture and content development of the composting stages were noted. After eight days the compost content were tested physically for color, odors, pH estimation and anti bacterial effectiveness. Results of this study shown that there is a clear differences between the two methods used in the production of temperature, acidity (pH) and the composting period. The addition of Effective microorganism to the composting substances led to efficient and fast composting process through higher temperature, acidity.

Introduction

Composting is a reduction process in aerobic conditions where organic materials convert to soil like substances by different stages, each stage include different types of microorganisms and different intermediate outputs.

Microorganisms play an important role in this process through the using of nitrogen, fats and carbohydrates included in animal carcases to deteriorate it to simple ingredients through the high temperature produced by this process (60–65 °C)[1, 2].

Composting pass through three stages, the mesophilic stage which continue 2-3 days, the thermophilic stage which continue for several days to several weeks and cooling and maturation stage, which continue for months [1, 3, 4, 5]. Numbers of microorganisms which found during different composting stages play an important role in this process, including bacteria, Actinomycetes, fungus, protozoa and Rotifers [1, 2, 6, 7, 8, 9]. Composting occur depending on many physical and chemical factors where temperature and acidity represent the key of composting success, moisture, particle size, aeration, nutrients and basic structure are important factors to insure raped and ideal compost [1, 5, 10]. The advantages of this method is cheap, biosecure and environmental hygienic but it take a long time [11].

The aims of this study was to compare between two different composting technologies, one of them include the use of Effective Microorganisms, to find the optimal conditions of complete composting processes in shorter time, with the best effectiveness for the environmental issues.

Material and Methods

Sawdust, hay, plastic tubes, plastic covers, water and poultry carcases are used to make four equal composting piles. Tow of these composted piles where sprayed with effective microorganisms (EM) which contains Lactic acid bacilli (*L. plantarum; L. casei; Strept. lactis*),Photosynthetic bacteria (*Rhodopseudomonas palustris; Rhodobacter sphaeroides*),Yeast (*Saccharomyces cerevisiae, Candida utilis Toula, Pichia Jadinii*) Actinomycetes (*Streptomyces albus; S. griseus*),Fermenting fungi (*Aspergillus oryzae; Mucor hiemalis*). Each pile consist of 20 Cm of sawdust in the base, hay above it in 10 Cm, then 10 Cm of poultry carcases with plastic tubes for ventilation, then 20 Cm sawdust, then sprayed with water [12].
Petri dish cultured with *Salmonella* and *E. coli* were put inside the composted piles, then covered by plastic cover to keep it from heat loss, rain, odors, and prevent attract flies, rodents, or dogs. Temperature was taken daily, moisture checking, biological testes to bacterial culture and pH estimation for each composted pile.
Regular observation of the composting stages were registered including temperature, and moisture. After eight days the compost content tested physically for color, odors, pH estimation, and bacteriological examination (re-culturing) of Petri dishes that was put inside the composted piles. After three weeks the composted piles were flipping to increase porosity, then supervision of the experiment for both methods were continued until ending.

Results

The results shown that there is a clear differences between the two methods (normal composting and using Effective Microorganisms (EM). Composting process by using EM were more raped , with higher temperature and more stabilized in second stage of composting where it ranged between 66 – 68 °C in compares with normal composting processes, which were ranged between 52 – 64 °C.

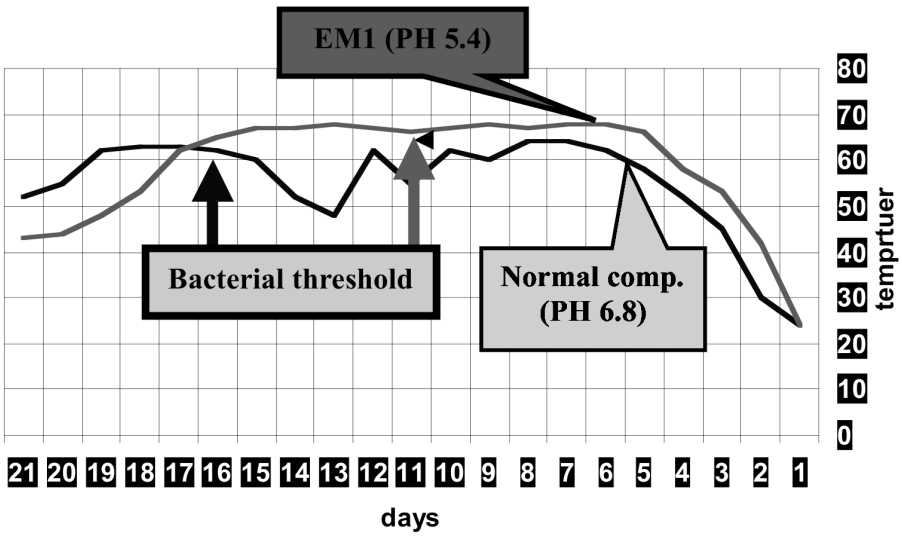


Fig. 1: Represent the differences between the two type of composting

There were no obvious odors in the EM composting piles, the acidity (pH) was 5.4, and the bacteria couldn't grow after ten days of experiment (bacterial threshold) in comparison with normal composting piles were PH was 6.8 and bacteria can not grow after seventeen days of the experiment (figure 1). Composting process by using Effective Microorganisms were more raped in comparison with normal composting processes, where we got about 95% digestion of poultry caecases through 25 days in comparison with normal composting piles which take 50 days for complete process.

Discussion

To complete the composting process temperature should reach 55 – 65 C in the first three days, and must be stable at 60 – 65 °C for the first and second week of composting process to have the necessary disintegration and disengaging [5]. The use of Effective Microorganisms (EM) show higher growth patterns than natural organisms exists in poultry carcasses.

The growth of microorganisms in the compost was according to the needs of nutrient substances and ability to use nitrogen in skin and feathers of the poultry carcasses as a result of protein degradation, which act as a bio-filter. This Bio-filter adjust the microbial activities to keep proper conditions in the compost (humidity, pH, nutrients, temperature, gases), and ensure odors removal and prevent diseases carried by arriving poultry carcasses. This activities can decrease diseases transmition inside farms and from farm to farm.[5, 12]. The analytic enzymes produced from EM destroy keratin and other proteins in feather and skin of the carcasses, which produce Carbon, Sulphur and energy, which bacteria use for its growth and perpetuation[13, 14, 15, 16]. Bacilli pattern bacteria found in all place and grow on natural medias without any special needs. This properties used for feather degradation which found in compost in large amounts. Bacilli are thermophilic microorganisms, this properties used to control raped and efficient disintegration of feather, skin and tissues[17]. Keratin analytic enzymes within the more enzymes largely used in chemical, medical and animal silage industries [16, 18]. The temperature of more than 65 °C reached in the compost with EM led to accelerate tissues disintegration and kill pathogens, insects and fly larvae[2]. The PH of the composting piles were very ideal for the growth of microorganisms, which digest organic materials, producing organic acid in early stages of the composting process, and encouraging fungus to grow and digest complicated substances that bacteria can not destroy. [2, 3, 5].

The addition of EM has a major role in reducing cooling process and maturation time of the composting process in very good form and shorter time. Addition of EM can produce organic analytical enzymes, which considered the use of EM in composting process as a safe disposal method. This results open the floor to use EM for treatment of other types of scraps to protect the environment from different pollution problems.

Conclusion

Appling of EM to composted materials of poultry carcasses led to fast and efficient composting process free from odor and higher in temperature ,acidity and more raped in disintegration providing more power of biosecurity, which could be used in hygienic bioremediation of pathogen inactivation.

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Reduction of livestock-related airborne bacteria by means of a plasma physical abatement technique (PPAT) – a self-critical review of a study

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Summary

A plasma physical abatement technique (PPAT) was checked for its usefulness to reduce considerably bioaerosols in the streaming exhaust air of an experimental pig barn. Depending on the used sampling methods and the set performance of the plasma reactor (e.g. power supply) the reduction efficiency ranged between 53 and 97 % for mesophilic total bacteria, for example. However, it is not fully clear, if reductions were significantly caused by the plasma or by heat or ozone generated by the plasma. Physical mechanisms like impactions and interceptions may also contribute to the reduction of particulates within the streaming crude gas, which was passed through the pipe system of the experimental set-up. Optimization of the sampling procedure is therefore an important point to fulfill the requirements of a good experimental practice.

Introduction

Among the released particulates from animal houses, airborne microorganisms are particularily of great concern due to their potential to carry infectivity, allergenicity, toxicity or genes coding for antibiotic resistance. Biological waste gas purification systems (BWGPS) are currently used in livestock operations to reduce odourous and gaseous emissions at all, but their role as self-emitting devices (primary and secondary bioaerosol releases) is still under investigation, because BWGPS may pose a risk for the environment. To overcome such a risk, the application of the plasma technology is an option to inactivate microorganisms at all [1]. Therefore, reduction principles such as a plasma physical treatment of airborne mircorganisms or in combination with BWGPS are discussed as a possible alternative [2]. The presented results are part of a research report [3] and gives a critical overview of the used measurement procedures and the reduction efficiencies of a non-thermal plasma reactor prototype, based on the principle of dielectric barrier discharge operated with 1 to 10 kV.

Material and Methods

The study were carried out in an experimental animal house with 10-12 pigs (80-110 kg body weight). A part of the exhaust air (approx. 40-80 m/h) from the small pig barn has been directed through the plasma physical treatment reactor, which was operated with different power adjustments. Air samples has been taken before and after the bacteria enriched livestock air have passed the reactor and its catalytic converter (crude gas and clean gas, respectively; see Fig. 1) to reduce gaseous plasma by-products such as ozone. A cooling unit behind the PPAT was used to minimize plasma caused heat, although the PPAT was

originally designed as low temperature PPAT. Mesophilic total bacteria, staphylococci and streptococci were sampled by impingement (AGI-30, sampling time: 30 min) or filtration (IOM samplers, sampling time: 90-120 min) to consider potential differences in sampling efficiencies. The sample size ranged from 4 to 22. After sampling cultivation on appropiate agar plates took place. The concentrations of endotoxins were additionally detected by the Limulus-Amebocyte-Lysat Test (LAL-Test). Finally, the reduction efficiencies (RE) were calculated. The experimental circumstances were further characterized by additional measurements of temperature and ozone concentrations.

Results

The operation of the PPAT with a power between 1200 and 1400 W have shown that mesophilic total bacteria and staphylococci were reduced by nearly 53% and 78%, respectively, in the clean gas when impingers were used. Long-term samplings via filtration and 560 W power supply of the PPAT caused a signifikant reduction of 84% ($p<0.043$) in case of mesophilic total bacteria, while 97% of the cultivable streptococci were reduced in the abatement device. In a continuing trial the power of the PPAT was then increased up to 1200 W and sampling via filtration was conducted again. The RE for mesophilic bacteria could be further increased up to 91% ($p<0.025$). For staphylococci and streptococci a RE of 93% and 87%, respectively, was calculated. When comparing the endotoxin concentrations between the crude and clean gas a RE of 73% ($p<0.046$) was found.

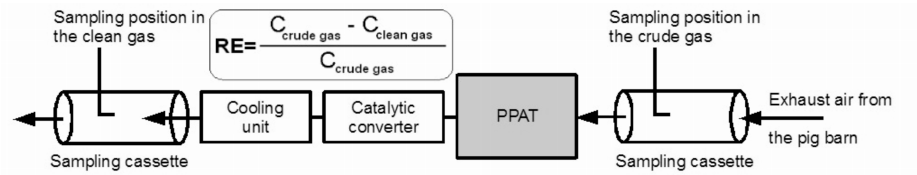


Fig. 1: Scheme of the experimental setup in the pig barn and the equation to caluculate the reduction efficiency RE (C: concentration). Sampling positions were equipped with impingers or filtration devices.

Due to the complex and long pipe system, in which the exhaust air was transported from the barn to the PPAT, there was the question whether the pipes and any other internal barriers (eg. pumps) may cause reductions by mechanical forces and not by the plasma itself. Therefore, the RE was compared between the operated (1200 W) and non-operated (0 W) PPAT. As assumed, non-plasma causing reductions were found. Taking this observation into account, the 'real' plasma causing RE were only 40% for mesophilic total bacteria, 18% for staphylococci and 19% for streptococci. No plasma based reductions were found for endotoxins.

Discussion

The working principle of the PPAT. The investigations have generally confirmed that an active plasma is able to reduce bacteria in the waste gas of a pig barn. However, it could not unequivocally be excluded, whether the plasma itself or other mechanisms were responsible for the main microbiocide effects. Measurements in the air behind the PPAT

revealed temperatures up to 80 °C. Such temperatures are capable to inactivate culturable microorganisms, when exposure times are long enough. It is also well known, that the plasma technology generates ozone, a strong microbiocide. During the measurement campaign ozone concentrations of nearly 300 ppm were temporarily detected in the clean gas, although the catalytic converter should prevent exessive ozone releases. From a microbicide point of view a strong synergistic effect between temperature and ozone cannot be excluded. Therefore, it is doubtful, whether the plasma corona itself in the reactor have caused any considerable declines of bioaerosol concentrations in the clean gas.

Virtual reduction efficiencies. The exhaust air from the pig barn streamed through a pump and long pipes, which were bended several times before the air entered the PPAT. Due to impaction and interception, the lost of culturable bacteria was considerable, because bacterial concentrations of nearly 4×10^4 colony forming units (CFU) per m^3 air in the pig barn were reduced to approximately 5×10^3 CFU/m^3 in the crude gas, just before the waste air has entered the PPAT device. As consequence, the plasma reactor was only flushed with a relatively small amount of bioaerosols and it remains unclear, what maximum concentration could successfully be reduced by the PPAT, when the reactor is continously flushed with typical livestock-related concentrations up to 10^6 CFU/m^3 and more. Another point is related to the construction of the reactor chamber and the catalytic converter, because both units contain an internal structure with large surfaces (e.g. electrodes) which have the potential to cause impaction and interception of particulates. So, it is not surprising that the whole system tested here has shown clear reduction with a PPAT, which was switched-off (0 W).

Sampling methods. Impingement is a typical sampling routine to collect airborne microorganisms suspended in an aqueous medium, which supports the revitalization of sublethal damaged microorganisms. Therefore, cultivation of stressed microbes is possible later in the laboratory; an advantage in opposite to filtration, where lethal desiccation of the microorganisms occurs during sampling. Nevertheless, as long as the PPAT generates ozone, impingement is not suitable, because the toxic ozone is soluble in the impinger fluid and causes a secondary inactivation of already sampled microorganisms. The magnitude of such an ozone-related impact can hardly be estimated and will cause an unpredictable bias of the results. Therefore, it was decided to use filtration with a long sampling time to ensure cumulations big enough to get suitable yields of cultivable bacteria and endotoxins. Another weak point is related to the measurements of particulates in streaming gases. By the means of measurement cassettes it was tried to fulfill the requirements of isokinetic sampling via self-designed probes mounted on impingers and filtration devices. However, it remains finally unclear, if these technical modifications together with the specific flow pattern within the cassettes (laminar or turbulent ?) have guaranteed a good practice of sampling procedures. If not, over- or undersampling of particulates occurs if anisokinetic sampling is applied.

Conclusion

- The non-thermal plasma technology is an interesting technique to treat and to reduce biotic particulates in waste air of animal houses.
- Further investigations must show if usual air exchange rates in livestock operations can be fully and sufficiently treated by a PPAT. Here, only very low air flow rates werde used, uncommon for livestock buildings.

- A comprehensive characterization of the PPAT in future needs specific sampling and measurements strategies to reveal the reduction effects of the plasma and to exclude any other confounding reduction mechanisms.
- The experimental set-up should be optimized in a way, that virtual reduction effects can be neglected as much as possible.
- In contrats to BWGPS it has to be seriously checked, if the application of the PPAT is an appropriate method; ecologically (ozone release), economically (costs) and from a practical point of view.

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Chapter 28

Poster

Air quality and welfare of cage housed laying hens

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Summary

The bioaerosol concentration and main microclimate parameters were analyzed in laying hens dwelling. Pollutants present in the air of animal dwellings, could influence on animal and human health. Received results about concentrations of bioaerosols indicate the need for making standards on air quality in the animals dwellings and the development of reliable systems for monitoring the above factors.

Introduction

Physical and mentaly health of animals define quality of living or level of animal welfare [11]. Animal environment include air quality, feeding, health control, type of housing, number of animals, etc. Considering EU Directive 1999/74 accomodation of laying hens into classical cages will be forbiden since January 2012. year. Air quality in those closed production systems represent potential risk factors of pathological phenomena [6, 8], because of different amounts and composition of bioaerosol particles. Pollutants present in the air of animal dwellings, which are capable to endanger the health and affect the productivity of animals and their amount are still object of many researches with aim for establishing air quality standards [5, 9, 10, 13, 16].

So, aim of this research was to maintain air quality in the poultry house during three last months of exploation of cage housed laying hens. It was determined amounts of airborne dust, endotoxins, bacteria and fungi as well as microclimate parameters. Results were investigate in context of zoohygiene measures considering their influence on health and welfare of laying hens, and as starting data collection for makeing air quality standards.

Material and Methods

In object were 17200 of laying hens hybride Shaver. They were placed in conventional cages, in proportion 90x45x45 cm. Cages were connected into 5 three floor bateria, in each cage were 7-10 hens. Feeding, *ad libitum*, watering, ventilation, lighting, and manure removal were regulated automaticaly. Light was provided for 16 h per day, from 05:00 h to 21:00 h. Eggs were collected manually.

It was determined concentration of bacteria and fungi, amount of dust and endotoxins, ammonia and carbon dioxide. It was also measured microclimate parameters: air temperature, relative humidity, air velocity and lighting.

Marked factors were measured from December

2007 till Febraury 2008, twice a month. Measurements were performed along two central corridor, in the level of second cage floor, on five points. Air was sampled by Merck MAS 100 device (Merck KgaA, Darmstadt, Deutschland). At the same time, air temperature (t 'C), relative humidity (RH %) and air flow rate (w m/s) were measured with Testo 400 (Testo Inc. Lenzkirch, Deutschland). Ammonia and carbon dioxide levels were measured with Dräger-Multiwarn II unit (Dräger). Dust samples were collected from filters (Whatman International Ltd., Maidstone, UK) by means of SKC pump (SKC Ltd., Blandford Forum, UK). The air was sampled on nutrient media for isolation of aerobic mesophilic bacteria and fungi and their respective counts were determined by standard methods after incubation. All measurements were performed from 8 a.m. to 12 a.m. Afterwars the number of microorganisms was counted and filter were weight, and proccesed by LAL method. Program Statistica 7 was used for statistical processing of measured values.

Results

Table 1. Descriptive statistical analysis of airborne bacteria and fungi levels (CFU/m^3), dust and endotoxin concentration in the air of laying hens dwelling

Sampling time	bioaerosol	n	X	Min.	Max.	sd	s
December	dust mg/m**3	4	2.66	2.01	3.61	0.39	0.43
	endotoxin EU/m**3	4	613	468	716	0.67	0.71
	bacteria (CFU/m**3)	10	6.89 x 10**4	1.01 x 10**4	7.12 x 10**4	0,97	0,43
	fungi (CFU/m**3)	10	3.12 x 10**3	1.12 x 10**3	5.67 x 10**4	0.69	0.28
January	dust mg/m**3	4	2.45	2.12	3.45	0.45	0.22
	endotoxin EU/m**3	4	625	534	756	0.57	0.63
	bacteria (CFU/m^3)	10	4,12 x 10**4	0.90 x 10**4	6.18 x 10**4	1.23	0.35
	fungi (CFU/m**3)	10	3.15 x 10**3	2.77 x 10**3	4.23 x 10**3	0.68	0.31
February	dust mg/m^3	4	2.89	2.12	4.11	0.23	0.44
	endotoxin EU/m**3	4	622	310	716	0.45	0.22
	bacteria (CFU/m**3)	10	5.89 x 10**4	4.89 x 10**4	7.25 x 10**4	1,45	0,59
	fungi (CFU/m**3)	10	3.89 x 10**3	3,01 x 10**4	4.56 x 10**4	0.58	0.53

Most dust was measured in February, 2.89 mg/m^3 of air; endotoxin, in January 625 EU/m^3; bacteria in December, 6.89x10^4 CFU/m^3; and fungi in February, 3.89 x 10^3 CFU/m^3 (Tab 1). Measured concentrations of bioaerosol were in accordance with findings from literature [12, 14, 15]. From same sources could be seen need for forming standards about air quality, which can ensure objective interpretation of received results. Established values of air temperature, relative humidity and air velocity were, generally, in accordance with the technology predicted ranges. Mean concentration of ammonia in the air was 2 ppm in December, while in February was recorded 6 ppm. Mean concentrations of carbon dioxide was during the entire period of research below 0.10% vol. Correlation coefficient rank indicates that relationship between microclimate parameters and concentrations of bioaersols was significant ($P<0.05$). Tukey HSD test indicate that during three measured, winter months, was not significant difference in the amount of bioaerosols.

Discussion

Still, in Europe, cage housing system for laying hens is the most common. Reasons for that are different, such as: higher price of eggs in alternative systems, alternative cages and object are expensive, maintenance, hygene etc. Many researches point that mortality is higher from modern systems, laying hens are oftener sick, higher possibility for canibalism and feder pecking. Considering air quality, it is very difficult to measure, exactly, how many microorganisms are present in the air. On their concentration affect sedimentation, aggregation, ventilation, dehydration, radiation and other factors responsible for their viability [17], such as activities of the animals and the implementation of specific work procedures [1]. Furthermore, number of microorganisms will depend on the way of sampling, whereby is important to mention, that obtained number refers to live microorganisms. Sustainability of microorganisms is influenced by microclimate conditions, mostly relative humidity. If relative humidity is between 55% up to 75%, then the majority of microorganisms can survive a short time in the environment, and not perish [3]. Of the surveyed characteristics, statistical significant relationship ($P<0.05$) showed, in most cases, air temperature, relative humidity, air flow rate, concentration of ammonia with the number of fungi and bacteria as well as dust. At least cases showed a relationship between the number of microorganisms and the concentration of carbon dioxide and lightning. Determined amounts of bioaerosols in the air of researched object, throughout three winter months, were on the lower limits of ranges described in the literature [2, 4, 12, 13]. Once again, we must state that the measurements were maintained in the winter months when ventilation is reduced. Ventilation system is real indicatior of air exchange. So,it is for expect, that in other parts of the year amount of biaerosol will be even lower because of higher ventilation volume.

Conclusion

Comparation of received results with citation of Hartung [7] show that accomodation of laying hens into cages is not proper from welfare aspect because impossibility of moving, poking, dust bathing, flaunt and other ethological needs, but is in harmony with fact that are much better hygienic standards, easier control of hens behaviour, less content of damaged and dirty eggs. Received results indicate the need for making standards on air quality in the animals dwellings and the development of reliable systems for monitoring the above factors.

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Potential application of selected aluminosilicates as filtration bed to ammonia reduction

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Summary

The aim of the investigation was to estimate the potential capacity of raw halloysite (RH), raw bentonite (RB) and expanded vermiculite (EV) for removal of ammonia from contaminated air during filtration process. The fresh cow's manure was placed in 3 identical containers and the filtrating device ODOR1, filled with investigated aluminosilicates, was in the middle of each container. The samples of air were collected from the containers before and after 24 hours of filtration process. The reduction of ammonia content in air samples collected from experimental containers, in comparison to control container, was on the level of 37,8, 28,1 and 22,7 % for RH, EV and RB respectively. The results presented in this paper demonstrate that bentonite, vermiculite and especially halloysite have potential capacity for removal of ammonia from air of livestock buildings.

Introduction

Taking into consideration the ammonia emission estimates it is very clear that about 95 % of total ammonia emission in Poland (310,2 Gg in 2005) originates from agriculture practices and more than 70 % of that emission is related to animal farming [6]. Ammonia is responsible for the acidification of water and soil, may cause forest damage and it also contributes to the eutrophication of terrestrial ecosystems and surface waters [1, 3, 8]. Large numbers of methods of reduction of gaseous pollutants from livestock buildings with intensive production have been examined in the last decade [2, 5]. Some classes of clay minerals which have very big sorptive capacity and which are both inexpensive and easy available, can find application as a filtration bed to reduction of gaseous pollution from livestock farms. The aim of the investigation was to estimate the potential capacity of selected aluminosilicates for removal of ammonia from contaminated air during filtration process.

Material and Methods

The investigation was conducted in 3 identical containers where fresh cow's manure were placed (the surface of emission about 8 m^2).The filtrating device (fig.1) ODOR1 (Klimatex, Poland) was in the middle of each container. The filtration time was 24 hours. During the experiment the filtrating device contained filter packs filled with investigated aluminosilicates except the first container (control) where filter packs were empty. The following aluminosilicates were investigated as filtration bed: raw halloysite (RH), raw bentonite (RB), expanded vermiculite (EV). The samples of air were collected before and after 24 hours of filtration process with the use of AMZ-1 aspirator (Rotametr, Poland). The concentrations of ammonia in sampled air were determined with the use of spectrophotometer UV-VIS according to polish standards.

There were three replicates for each of the investigated aluminosilicates and results given are the averages with standard deviations. Data were analyzed by analysis of significant differences assessed by Duncan test.

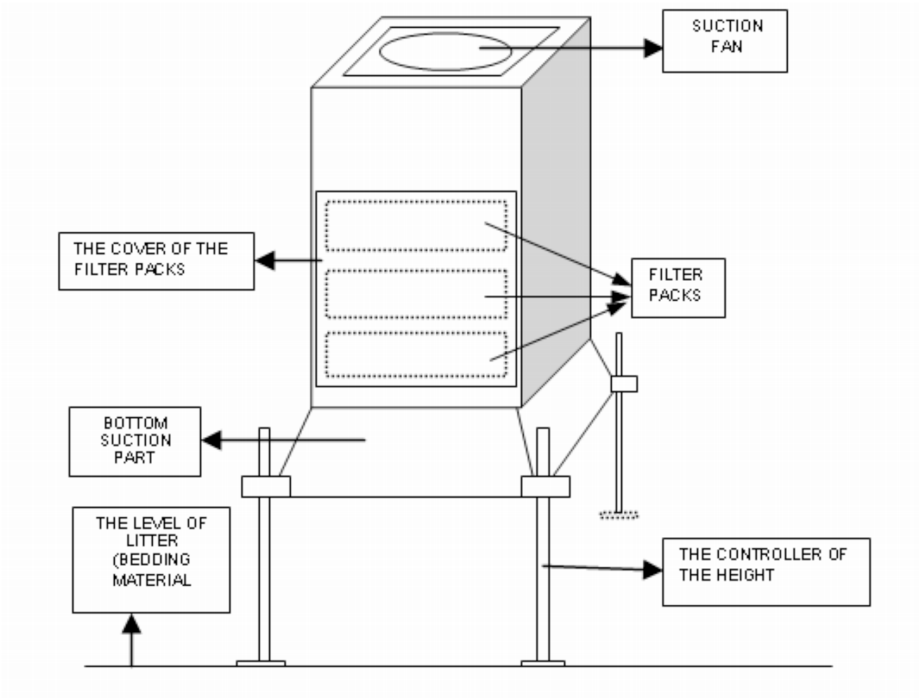


Fig. 1: The pictorial diagram of ODOR1 device

Results

The results of investigation are summarized in table 1. The concentration of ammonia in air samples collected from all containers was on the average level of 48,82 ppm on the beginning of experiment. It was found that the content of ammonia in air samples from experimental containers (filtrating device with investigated sorbents) was lower after 24 hours of filtration than in the control container and the differences were statistically confirmed. The ammonia concentration in air samples from control container after one day of experiment was on the average level of 67,63 ppm while in samples collected from containers, where aluminosilicates where used as filtration beds, was on the level of 42,07, 48,60, 52,30 for RH, EV and RB respectively. The reduction of ammonia content in air samples collected from experimental containers, in comparison to control container, was on the level of 37,8 % for raw halloysite, 28,1 % for expanded vermiculite and 22,7 % for raw bentonite.

Discussion

The potential for removal of NH_3 in case of bentonite was found by Seredych et al. [7]. To evaluate ammonia adsorption from gas stream a laboratory designed dynamic test was made. The glass column was packed with exfoliated graphite/bentonite composites. This unique

Tab. 1: The mean concentrations of ammonia (ppm) in air samples collected after 24 hours of filtration

	Control	RH	EV	RB
Ammonia concentration	$67{,}63^{A} \pm 5{,}35$	$42{,}07^{Ba} \pm 4{,}42$	$48{,}60^{B} \pm 4{,}03$	$52{,}30^{Bb} \pm 3{,}76$
Ammonia reduction [%]	-	37,79	28,14	22,67

A, B... - $p \leq 0{,}01$ – significance of differences between groups
a, b...- $p \leq 0{,}05$ – significance of differences between groups

material, being not only physical mixtures of the components, had enhanced capacity for removal of ammonia from air and adsorption capacity was much greater than that realized for the individual components. Turan et al. [9] were investigating the potential of natural minerals (also expanded vermiculite) to reduce volatile organic compounds produced during composting of poultry litter. Composting was performed in laboratory scale for 100 days. At the end of the processes, removal efficiency for expanded vermiculite was 61,53 %. The ability of vermiculite to reduce ammonia emission was reported also by Liao et al. [4] proving that this mineral is able to reduce concentration of gaseous pollutants in the air.

Conclusion

The results presented in this paper demonstrate that bentonite, vermiculite and especially halloysite have potential capacity for removal of ammonia from air of livestock buildings.

Acknowledgement

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Emission of bacterial bioaerosol and air contamination level at the sewage treatment plant

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Summary

The results of the study of number of selected groups of bacteria in the atmospheric air at the Municipal Sewage Treatment Plant in Toru and in its vicinity were presented. Air samples were taken with the compaction method in the spring and summer period in 2008. The highest emission of bacteria occurred at sand catchers and piles of maturing compost. The level of the total number of bacteria at these points indicated the moderate and heavy air pollution at several times. The concentration of the indicator bacteria *Pseudomonas fluorescens* reached up to 22 CFU m^3 and mostly contributed to the moderate air pollution. In the case of faecal streptococci of D-type, the highest number was found also at the sand catchers in the spring period. At the point situated beyond the sewage treatment plant, streptococci were not detected in any months tested, and the concentration level of the other tested bacteria was usually lower than in the air at the treatment plant.

Introduction

Sewage treatment plants can be the emission source of many chemical compounds and bioaerosols and consequently, pose a potential hazard for the environment as well as people and animal health. The number and type of microorganisms in bioaerosols depend on the applied technology of sewage treatment, exploitation method, aeration of chambers etc [1,7]. Sewage evaporation in the area of sewage treatment devices, pumping station grids, sand catchers and pre-sedimentation tanks plays an important role in the mechanism of penetrating of microorganisms to the atmosphere [10]. The emission of microbiological pollutions depends also on the current climatic and natural conditions. Some microorganisms die of drying, too high or too low temperature, or a lack of nutrients in the air [6]. Some of them retain vitality in bioaerosol for a long time. The best adapted forms are cocci, due to their relatively small cell area, and bacteria producing dyes and mucuses, which are additional protective factors for the bacteria [1,6]. Gram-positive bacteria such as staphylococci and streptococci and Gram-negative bacilli are predominant among the bacteria detected in bioaerosols [8].

The aim of the study was to determine the concentration level of the selected bacteria in the atmospheric air at points located at the sewage treatment plant and off its premises.

Material and Methods

The monitoring study of the atmospheric air was conducted at the Municipal Sewage Treatment Plant in Toru in three measurement points: at the sand catcher (stand 1), aeration chambers (stand 2) and around compost piles (stand 3). The fourth stand was at a distance

of 100 m beyond the sewage treatment plant at the leeward side (stand 4). Analyses were made from March to August 2008. Air samples of defined volumes were taken with the compaction method using the automatic specialist aeroscope Mas-100 Eco by Merck. In microbiological analyses, the total number of bacteria on standard nutritive agar (incubation 37 °C, 48-72 h.), the bacteria *Pseudomonas fluorescens* on Kinga B medium (incubation 26 °C for 48 h.), and fecal streptococci D-type on agar with kanamicine, esculine and azide (incubation at 37 °C, 24 h) were determined. The final identification of streptococci involved using the serological Phadebact-test

All air measurements for each group of bacteria were made in three replications at all the measurement points. The concentration of the tested bacterial aerosol was expressed in colony forming units in 1 m^3 air (CFU m^3). The estimation of atmospheric air pollution level was performed based on recommendations given in the Polish Standards PN-89/Z-04111/02 [11].

Results and Discussion

The results of bacteria number in the atmospheric air at the tested facility was presented in Fig.1 and Tables 1-2. The total number of bacteria in the air at the sewage treatment plant ranged from 32 to 6300 CFU m^3 (Fig. 1). The highest total bacterial emission took place at the composting facility. A strong air pollution occurred here in June and July. At stand 1 (at the sand catchers) a strong air pollution was found only in August. The concentration of bacteria at stands 2 and 4 according the recommendations of the Polish Standards, indicated moderately polluted and unpolluted air, since their number did not exceed 1715 CFU m^3. It is worth noting that adopting the total number of microorganisms as the indicator of air pollution excludes the possibility of finding the pathogenic risk. Periods of fluctuation in the total number of microorganisms are also greatly depended on the field conditions, the intensity of microbial growth in soil, water, on different waste, plant and animal remains [7,10].

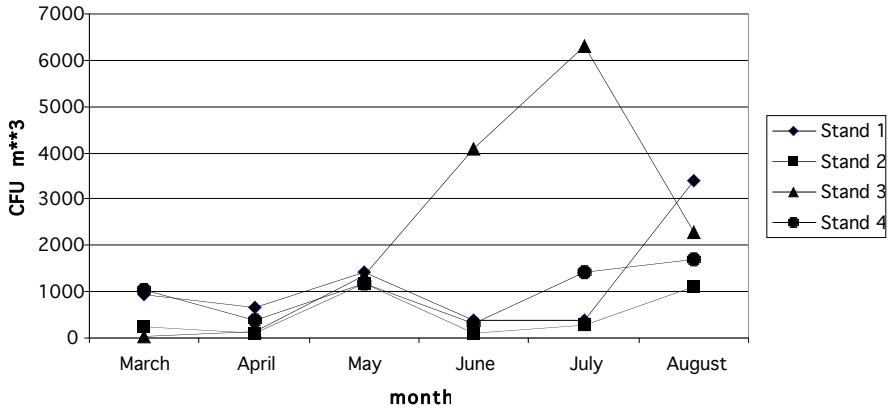


Fig. 1: The number of total bacteria in atmospheric air on the premises and in the vicinity of the sewage treatment plant

The number of the indicator bacteria *Pseudomonas fluorescens* ranged from 0 to 22 CFU m^3 (Tab. 1).Similarly, the most of them were detected at sand catchers and at the maturing

compost piles. They mostly brought about moderate air pollution. *P. fluorescens*belongs to the group of specific bacteria, which determine the range of the effect of municipal facilities on the air quality and pollution level more clearly. Among the bacteria of the genus *Pseudomonas* one can distinguish both saprophytic forms, actively participating in the processes of organic matter decomposition, and pathogenic and relatively pathogenic species. Infections with *Pseudomonas* often occur as complications together with other microorganisms. A frequency of infections caused by Gram-negative bacilli, including those of the genus *Pseudomonas* has increased considerably for the last decade [4,9].

Tab. 1: The concentration of indicator bacteria Pseudomonas fluorescens in atmospheric air on the premises and in the vicinity of the sewage treatment plant

Stand	Concentration (CFU m**3)					
	March	April	May	June	July	August
1	22	14	10	3	0	0
2	4	2	4	5	5	8
3	0	4	7	6	20	2
4	3	4	10	2	4	0

Bacteria of the genus Enterococcus occurred in the tested air in small quantities (Tab. 2). Their number did not exceed 24 CFU m^3. Fecal streptococci were most often isolated at stand 1, while at stands 2 and 3 they were detected only in the summer months (from June to August). At point 4 their presence was not observed throughout the research period. In the case of fecal streptococci there are not threshold values determining the permissible content of these bacteria in the air. Their mere presence in the air indicates the pollution which poses a health hazard for people and animals.

Tab. 2: The concentration of fecal streptococci in atmospheric air on the premises and in the vicinity of the sewage treatment plant

Stand	Concentration (CFU m**3)					
	March	April	May	June	July	August
1	12	24	8	0	10	0
2	0	0	0	2	10	3
3	0	0	0	22	4	1
4	0	0	0	0	0	0

Both Polish and foreign authors indicate that areas adjacent to aeration chambers are most exposed to the effect of biological aerosols [1,5,8]. According to Piekarska and Traczewska [10], the highest share in aerosol emission is attributed to the thin surface layer of sewage. The concentrations of pollutions in this layer and in aerosols emitted to the air exceed the mean concentration in the fluid collected from the middle part of the sewage tank by 10 to 100 times. On the basis of the results it was stated that it was not aeration chambers but sand catchers and the maturing compost storage site that were the most serious source of air pollution. Also in previous studies conducted by Breza-Boruta and Paluszak [2,3] at the municipal sewage treatment plant in Toru the highest emission of *P. fluorescens* and *Enterococcus* was found at the sand catchers.
Meteorological conditions play an important

role in spreading pollutions around the sewage treatment plant. The most essential of them are the temperature, air humidity, and the direction and speed of winds. The results obtained concerning the number of the tested bacteria may have been influenced by the season of the year when the measurements were made. The summer months were characterized by a high air temperature. Such weather conditions favours microbial growth even in such disadvantageous environment for them as the air, where they are deprived of nutrients and at the same time are exposed to a range of unfavourable factors such as solar radiation [12].

Conclusion

The highest bacterial emission at the Municipal Sewage Treatment Plant in Toru was observed in the atmospheric air at the sand catchers and at maturing compost storage site. Sewage aeration chambers did not cause the strong air pollution with the tested groups of bacteria.
In the air on the facility premises the indicator bacteria *P. fluorescens* and fecal streptococci were detected, whose presence indicates the potential health hazard both for people working in the sewage treatment plant and temporarily staying on the premises and in the vicinity.
The concentration level of bacteria in the air obtained at the point located 100 m from the sewage treatment plant indicates that the facility tested does not pose a hazard in respect of the emission of the tested bacteria.

Acknowledgement

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Biological hazard resulting from wastewater treatment related of bioaerosol concentration

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Summary

Exposures to bioaerosols in the occupational environment of wastewater treatment areassociated with a wide range of health effects, including infectious diseases, acute toxic effects, allergies and cancer. This air will often contain micro-organisms, such as viruses, bacteria, and fungi, but antibiotic resistant organisms too. The results revealed that high-grade airborne contamination existed at several of the studied sites. The highest counts of microorganisms were observed in pre-treatment and primary treatment stages, namely at coarse treatment on rakes and sieves and fine mechanical treatment by sand trap. In this section of pre-treatment, in the enclosed warm and humid environment where bioaerosol accumulates in a confined space at limited exposure to sunlight that could also devitalize the micro-organisms, there were evidently almost ideal conditions for their survival.

Introduction

Bioaerosols are defined as a collection of aerosolized biological particles which may affect living things through infectivity, allergenicity, toxicity, pharmacological or other processes. They can vary greatly in size (Cox and Wathes, 1995).

The composition, size, and concentration of the microbial populations comprising the bioaerosol vary with the source, dispersal mechanism in the air, and, most importantly the environmental conditions prevailing at a particular site.

Increasing temperature and decreasing humidity can increase bacterial bioaerosol concentrations (Ondraovi et al., 1996).

Relative humidity is often associated with bacterial bioaerosol survival as well. Increase in relative humidity is known to increase survival (Marthi et al.,1990), especially under the influence of direct sunlight (Handley and Webster, 1995). Under foggy conditions (100% humidity) bacterial bioaerosols are thought to propagate in the air, supporting the assumption that a higher humidity increases survival (Fuzzi et al., 1997).

Bioaerosols originating from potentially pathogenic sources may have the potential to cause disease in humans and animals if transported by air currents. Bioaerosols have been linked to highly publicized problems, such as sick building syndrome (Slotova, 2006), humidifier fever, farmers lung, tuberculosis, brucellosis, legionnaires disease, Organic Toxic Dust Syndrome and others.

Material and Methods

In the experiment, samples were collected by means of a sampler MAS-100 Eco. The MAS-100 Eco air monitoring system is a compact sampler intended for use with standard Petri dishes. Petri dishes with respective nutrient media (meat-pepton agar, endoagar, Sabouraud agar, MCC agar) are placed on top of the dish support of the sampler and after aspiration of a preset volume of air, they are incubated at appropriate temperatures (37 °C meat-pepton agar, Endo agar for one day and 24 °C Sabouraud agar for four days and room temperature). The plate counts were recalculated per 1 m^3 of air.

Susceptibility (MIC - minimal inhibitory concentration) was determined by colorimetric broth microdilution method according to CLSI guidelines using ampicillin, ampicillin and sulbactam, ceftiofur, ceftriaxon, ceftazidime, ceftazidime and clavulanic acid, apramycin, gentamicin, streptomycin, neomycin, spectinomycin, nalidixic acid, enrofloxacin, ciprofloxacin, chloramphenicol, florfenicol, tetracycline and cotrimoxazol.

Results

Samples of bioaerosol from different locations of a municipal wastewater treatment plant were analyzed in winter and spring seasons. Simultaneously we measured relative humidity and temperature of the air.

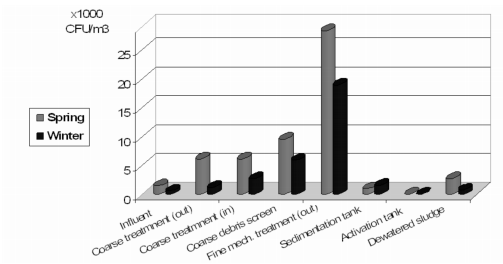


Figure 1 - mean total count of bacteria at different sampling sites

In the wastewater treatment plant the municipal wastewater is treated by mechanical, chemical and biological processes so it can be discharged into the recipient.

The results revealed that high-grade airborne contamination existed at several of the studied sites. The highest counts of microorganisms were observed in pre-treatment and primary treatment stages, namely in coarse treatment on rakes and sieves and fine mechanical treatment by sand trap (see figures). In this section of pre-treatment, in the enclosed warm and humid environment, where bioaerosol accumulates in a confined space at limited exposure to sunlight that could also devitalise the micro-organisms, there were evidently almost ideal conditions for their survival.

Samples collected in the influent location showed increased numbers of micro-organisms associated with the respective contamination of wastewater in this location.

The numbers of aerial micro-organisms were lower in the location of aeration tanks and the tank for storage of treated liquid sludge which is most likely related to the previous treatment of waste-water and sludge. Measurements in the outer environment can also be affected essentially by atmospherical conditions. In the building for dewatering of sewage sludge we recorded increased temperature and decreased relative humidity. The latter factors resulted in development of dry bioaerosol.

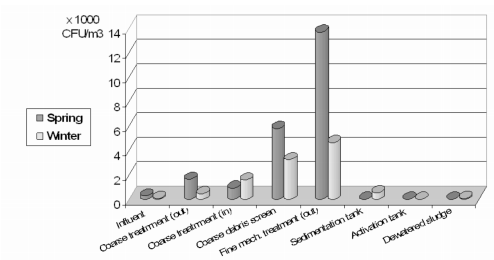


Figure 2 - mean counts of total coliforms at different sampling sites

We detected seasonal variations in the concentrations of bacteria. In spring the total count of microorganisms, total coliform and moulds (Fig. 1, 2, 3,) were higher. This may be related to better condition for survival of microorganisms, namely higher temperature and humidity.

In workplaces with the highest concentration of microorganisms (coarse treatment, fine mechanical treatment) we detected also antibiotic-resistant bacteria. Antibiotic resistant bacteria were found also in tertiary treatment section, in dewatering of sludge.

Samples from coarse treatment contained one isolate of *E.coli* resistant to nalidoxic acid, tetracycline, cortimoxazol.

In samples from fine mechanical treatment we detected one isolate of *E.coli* resistant to ampicilin, nalidoxic acid, tetracycline and one isolate resistant to tetracycline and cortimoxazol.

In air sample from dewatering of sludge we detected two isolates of *E.coli* resistant to nalidoxic acid, tetracycline cortimoxazol.

In spring samples we isolated E. coli with ESBL (Extended spectrum -lactamase), responsible for bacteria resistance to all beta-lactam antibiotics.

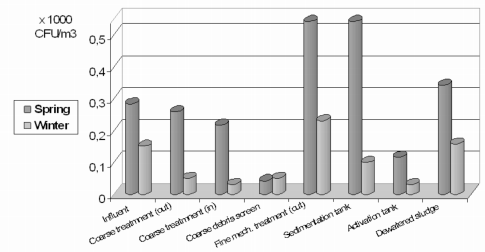


Figure 3 - mean counts of moulds at different sampling sites

Discussion

Sewage and wastewaters are known to contain human pathogens. Using mechanical, chemical and biological means, wastewater treatment plants prepare our wastewater for disposal into the environment. Treatment of wastewater involves agitation, aeration and mechanical movement which is associated with splashing and popping of bubbles and release of aerosols laden with microorganisms (termed bioaerosols) into the air. Thus workers at wastewater treatment plants are exposed to pathogenic organisms through the air they breathe (Stampi et al., 2000).

Some areas of wastewater treatment plants produce higher concentrations of bioaerosols than others. The proximity to an aeration source, and its aeration rate have been linked to the level of bioaerosols produced (Brandi et al., 2000). Temperature, relative humidity, season, time of day, sunlight, and particle size are all thought to affect bioaerosol survival (Seltzer, 1994).

Concentration of bacteria in seasonal influents varies. For example, in the study from Finnish waste treatment plant there was no seasonal variation in the concentration of bacteria, but the concentrations of fungi were highest in autumn at the waste incineration plant and the bacteria identified did not include any potent pathogens (Rahkonen, 1992).

Using personal samplers it has been shown that sewage treatment plant employees that had higher incidence of headaches, tiredness and nausea were exposed to more rod-shaped and more total culturable bacteria (Melbostad et al 1994). The prevalence of self reported gastroenteritis and headaches were higher in activated sludge wastewater treatment workers compared to rates for college maintenance and oil refinery workers. Workers, who spent more time in areas where the potential for higher exposure existed, had higher rates of illness than those who did not (Khuder et al., 1998).

The most frequently reported health complaints include eye, nose, and throat irritation, headache, nausea, diarrhea, hoarseness, sore throat, cough, chest tightness, nasal congestion, palpitations, shortness of breath, stress, drowsiness, and alterations in mood. Typically, these symptoms occur at the time of exposure and remit after a short period of time. However, for sensitive individuals such as asthmatic patients, exposure to odors may

induce health symptoms that persist for longer periods of time as well as aggravate existing medical conditions (Shiffman et al., 2000).

In the recent years there were reports about increased use of antibiotics by people and in livestock breeding on a worldwide scale. From the entire production antibiotics almost half was used in livestock breeding and vegetable production. The micro-interval adaptability of prokaryotic cells and selection pressure of antibiotics on these cells in the outer environment resulted in development of biological, genetic and molecular mechanisms that caused resistance (Langadl, 2000).

Resistant microorganisms that exist in bioaerosols in different environments pose hazard to animals and people particularly through exposure to infection resistant pathogens and related potential mortality and failure of therapy.

Acknowledgement

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Characterisation of the endotoxic and proinflammatory activity of different airborne dust fractions from animal houses

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Summary

Organic dust is considered as a carrier of endotoxins, microorganisms, gases and other substances. Exposure to organic dust is associated with a variety of acute and chronic respiratory symptoms, for example the toxic pneumonitis and chronic obstructive bronchitis. The aim of this study was to determine the endotoxic and proinflammatory activity in different organic dust fractions (inhalable dust, respirable dust, PM10, PM2.5 and PM1.0) from various animal houses and work places at animal husbandry. The endotoxic activity in the sampled dust fractions were determined by using a Limulus Assay and the proinflammatory activity by using a Human Whole Blood Assay. The Limulus Assay is limited to the detection of endotoxins whereas the Human Whole Blood Assay is able to detect abroad spectrum of proinflammatory active substances simultaneously. The highest endotoxic (EU/m) as well as the highest proinflammatory activity (EEU/m) was found in the inhalable dust. In other dust fractions these activities showed a relatively evenly distribution. Furthermore a significant positive correlation between the concentration of airborne endotoxins (EU/m) and proinflammatory activity (EEU/m) among the different investigated dust fractions was found.

Introduction

Organic dust (bioaerosol) is considered as a carrier of endotoxins, microorganisms, gases, and other substances. Inhalation of endotoxins and other proinflammatory substances, which are present in bioaerosols, causes transient as well as chronic lung dysfunction in humans. Acute symptoms after inhalation of bioaerosols are cough, decrease in lung function, fever and malaise. A prolonged exposure to different bioaerosols can cause different diseases of lungs for example the toxic pneumonitis and chronic obstructive bronchitis [1, 2, 3, and 4]. The cause of these diseases is proinflammatory substances, such as endotoxins and glucans, which activate the alveolar macrophages [5]. As a result of this process cellular proinflammatory cytokines such as IL-1ß, IL-6, tumour necrosis factor (TNF)- are released from the above cells (Fig. 1). These cytokines play a major role in the inflammatory process [2, 6]. The aim of this study was to determine the endotoxic and proinflammatory activity in different airborne organic dust fractions (inhalable dust, respirable dust, PM.10, PM. 2.5 and PM.1, 0) from various animal houses.

Material and Methods

In this study different dust fractions (inhalable dust, respirable dust, PM10, PM2.5 and PM1.0) were sampled by using standardized filtration techniques from various animal

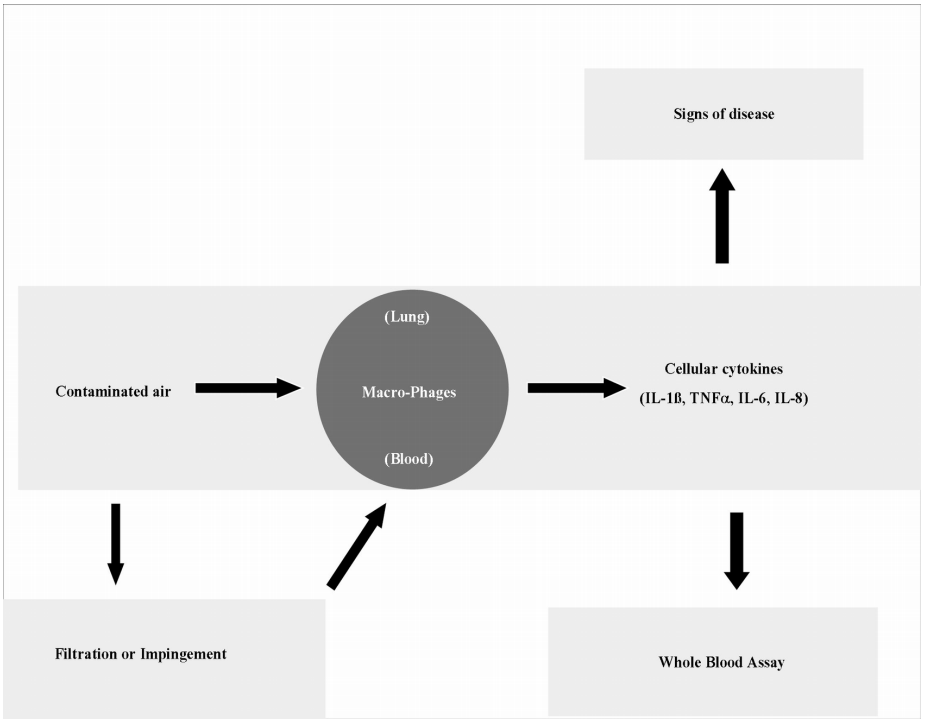


Fig.1: Effect of the contaminated air on respiratory health and its detection by Human Whole Blood Assay.

houses (4 pigs, 4 ducks, 3 sheep and 2 horses) as well as duck hatchery and slaughterhouse (n =16). The exposed filters were washed with pyrogen-free water for 2 hours, and then in the extracted fluids the concentration of endotoxins and proinflammatory active substances were determined. The LAL assay QLC-1000 (Cambrex/lonza Walkersville, USA) was used to determine the concentration of endotoxins in the bioaerosol samples. The concentration of proinflammatory active substances was determined by using a human whole blood assay as described by Zucker et al. 2006 (8(. The basic principle of the human whole blood test is shown in figure 1. Bioaerosol samples (washing fluid of exposed filters) 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. 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;, Cambrex/lonza, USA). The activity in the LAL assay was expressed in Endotoxin Units (EU), in the whole blood assay in Endotoxin Equivalent Units (EEU).

Results

The highest endotoxic activity (EU/m) as well as the highest proinflammatory activity (EEU/m) was found in the inhalable dust (Fig.2). In the other dust fractions these activities showed a relatively evenly distribution. Furthermore a significant positive correlation (Spearmans r=0,844, =0, 01, n=88) was found between the concentration of airborne endotoxins (EU/m) and the proinflammatory activity (EEU/m) among the different investigated dust fractions.

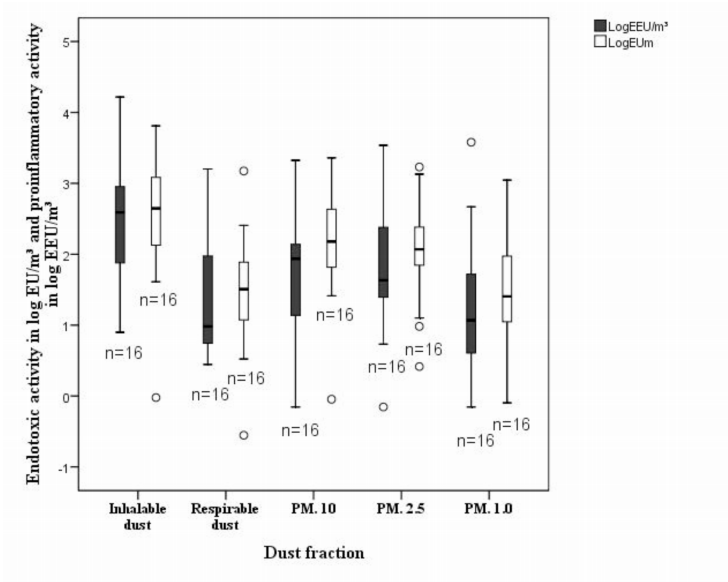


Fig. 2: endotoxic and proinflammatory activity of stable air in the different fractions of airborne dust.

Discussion

These results indicate that endotoxins and other proinflammatory active substances were bound mainly at particles with an aerodynamic diameter of >10 m and therefore they are unable to penetrate in deep areas of the respiratory tract.

Conclusion

From the previous results, we observed that the highest concentrations of the endotoxic activity (EU/m) and proinflammatory activity (EEU/m) were associated with the inhalible dust fraction, while these concentrations were nearly equally distributed in the other dust fractions

Acknowledgement

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Quantitative detection of Newcastle Disease Virus in air samples

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Summary

In this study the detection of Newcastle disease virus (NDV) strain LaSota in generated aerosols as well as in air of stables is investigated. The efficiencies of two systems, impinger and gelatine filter, for virus isolation from air samples were determined. Virus detection using gelatine filters and subsequent real-time PCR was shown to be the most efficient method, furthermore it is very precise and fast. Nevertheless, virus in the air of experimental stables after vaccination could only sporadically be found; for an investigation in commercial stables larger sample volumes might be necessary.

Introduction

The transmission of infectious diseases presents a very important topic in medicine. Especially airborne viral diseases such as Influenza [1], SARS [2], Newcastle disease [3] or foot-and-mouth disease [4] are of great danger and consistently attract public attention.

The development and improvement of different methods for quantitative virus detection in air samples is, therefore, very important. In contrast to bacteria and fungi, there are fewer studies about viruses because of the difficulties in collecting and detecting airborne viruses.

Animals, Material and Methods

In this study the detection of Newcastle disease virus (NDV) strain LaSota in generated aerosols as well as in air of stables is investigated. Two systems for detecting airborne viruses, sampling via impinger AGI-30 and gelatine filter, are compared and the virus detection rates were calculated. For this purpose the virus was nebulized in an aerosol chamber, and air samples of the same volume (200 l) were collected simultaneously with both instruments. To determine the virus titer the samples were titrated in embryonated chicken eggs. In parallel, virus was quantified by real-time PCR established in this study. Target gen was the fusion protein. The quantitative real-time PCR was performed by normalization of all samples.

For studying airborne viruses in the air of model henhouses, 50 animals were vaccinated with the LaSota strain of NDV via drinking water and by the intraocular route. Air samples (1000l - 2000l) with both instruments were collected daily for two weeks and analysed by quantitative real-time PCR. Also swabs of 20 animals were taken dailly where 10 swabs were pooled to one sample and analysed by real-time PCR.

Furthermore, air samples (1000 l) were collected using gelatine filters in various commercial henhouses 4 - 6 days after vaccination.

Results

Both sampling methods could detect infectious virus as well as virus genome in aerosols, however, with varying efficiencies (fig.1). Virus detection using gelatine filters and subsequent real-time PCR was shown to be the most efficient method with an efficiency of 22.3%/3.1 (geometrical mean value and geometrical standard deviation) for virus genome. The detection rate of infectious virus, however, was better using the impinger (4.0% /1.7).

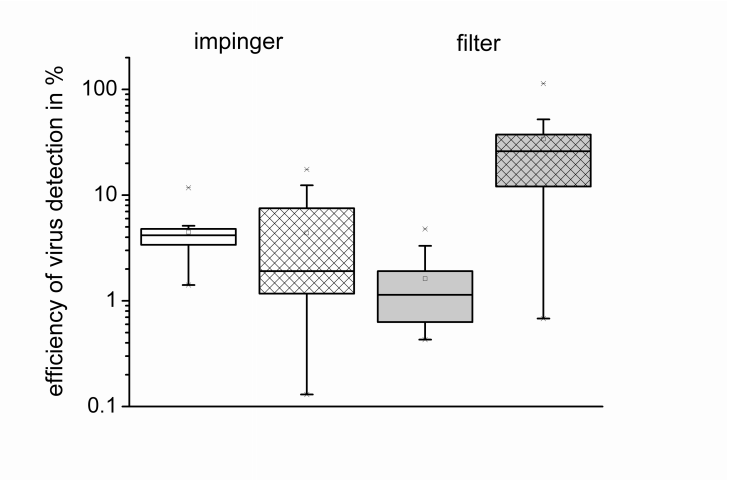


fig. 1: Efficiencies of virus detection in aerosol samples with both collection instruments. The white boxes present the results of the impinger, the grey ones present the results of the gelatine filter. Boxes without shading show the virus titration in embryonated chicken eggs, boxes with shading the virus titration by real-time PCR.

Under experimental conditions Newcastle disease virus in the air of henhouses after vaccination could sporadically be found. Only three air samples were positive in real-time PCR. The virus titer varied from $10^{1.5}$EID$_{50}$/m^{3}to $10^{4.3}$EID$_{50}$/m^3. In contrast virus detection from swab samples of vaccinated animals was generally successful (fig.2).
Under field condition, no virus could be detected in the air samples of commercial stables.

Discussion

Results showed a significant lower detection rate for infectious Newcastle disease virus than for virus genome. During the sampling process using both instruments the virus looses its infectivity. The fragile lipid envelope of NDV may be most responsible to that substantial loss of infectivity [5].
Virus quantification by real-time PCR was performed with normalization of all samples. This method is so far not commonly used for analysing air samples, but it appears to be very powerful. By means of normalization the different efficiencies of nucleic acid isolation and reverse transcription of various air samples are compensated. Furthermore the different inhibition of PCR by the various air samples taken by several collection instruments are corrected. As a result the air samples are better comparable and the quantification is more exactly [6]. Under experimental conditions only few air

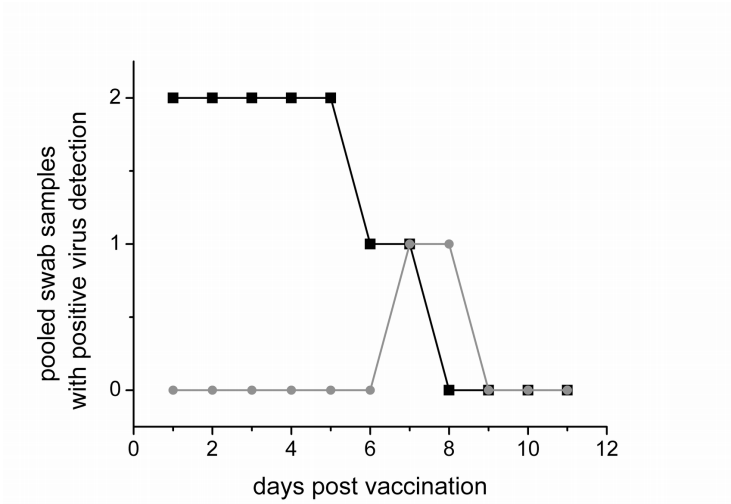


fig. 2: Results of pharyngeal and cloacal swabs analysed by real-time PCR and taken daily after vaccination (two pooled samples from ten swab samples each). Pharyngeal swabs are presented in black, cloacal swabs in grey.

samples from the stable were virus positive and no virus detection in commercial stables was successful. It may be conclude that the small amount of virus released by the vaccinated birds is diluted far below the detection limit, for instance, by the large air volume of the stables as well as by the ventilation.

Conclusion

Gelatine filters in combination with a real-time PCR analysis is a qualified method for virus quantification from air samples. This method is both precise and fast, nevertheless it is a quantification of virus genome and the interpretation regarding infectious virus particles has to be done carefully. The airborne viruses in commercial stables might be investigated by taking larger sample volumes.

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Part X

Disinfection

Chapter 29

Block 7B

Disinfection, an Integral Part of Animal Biosecurity on Farm

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Summary

Preventive precautions in animal husbandry are directed to prevent the communication of diseases from outside and to reduce the pathogens inside the farm. The aim of our work was the assessment the effect of preventive disinfection to minimise the potential disease causing micro organisms onto the farm and the evaluation of the economical effect of stable disinfection. We proved that the Biosecurity programme TEKROCID enables to decrease the infection pressure by special techniques, the special formulation of livestock disinfectants and unambiguous application methods.

Introduction

The infection pressure in stables is proportionally increased by the animal concentration, length of their stay in the animal house. This may cause growth depression and health problems in livestock. Therefore the sanitary control measures should be incorporated in all houses, so that a good hygiene standard can be easily maintained. Therapeutic arrangement is usually too expensive. The use of good breeding practices and correct Biosecurity rules usually prevents the herd from health disorders. All farmers solve essential profitability questions for their farms, however the profitability of every farm not only depends on animal genome (20 %) nutrition (55 %) but also on the environments influence (25%) [1]. Spending on feedstuffs forms around.65% of the farm budget and decisive influences over efficiency are farm staff and above all neglect of official duties and incorrect breeder practice (as far as 80%). The aim of project TEKROCID 2007 was to identify and create the list of Critical Control Points (CCP) with practical check in cross - country conditions in pigs farm and prove so, that the correct precautionary measures are lower cost than treatment.

Material and Methods

The field trial was carried out on commercial swine farm with 1200 sows of basic herd. Consequential weaning pigs were in the same reproduction-part. Fattening - farm was placed 20 km's faraway.
There were two periods under consideration: before (I-X/2006) and after (I-X/2007) implementing the hygiene programme Tekrocid in the farm. After internal risk analysis an internal Biosecurity Index was developed, listing all the steps to control the CCP. There were controlled indicators of swine efficiency - direct losses (deaths) and indirect losses (low feed conversion and animal growth, high medication cost and consumption of water and chemicals for hygiene procedures). The Biosecurity programme TEKROCID, developed in cooperation between companies Tekro (CZ), Evans Vanodine (UK) and Pfizer (USA) was

used as the control protocol.
There were used special cleaners and disinfectants for livestock produced by Evans Vanodine (UK). All the farm staff was trained to follow the procedure correctly according to CCP plan. The microbiological assessments of surface were done through taking microbiological swabs (10cm^2 moulds) and air control was being done by a Cassel gas sampler with air-supply 22 l/min.

Results and Discussion

Infectious disease outbreaks occur after reaching the threshold concentrations of pathogens, that in conjunction with non-pathogenic microorganisms and negative factors cause immunity suppression in pigs.
Our objective was to prevent any spread of disease / contamination to other parts of the farm and to a new herd and moreover maintain low numbers of airborne microorganisms in animal houses all along the presence of animals.
On tested farm were identified and solved CCP's as mentioned in the Poster submission "Farm Animal Biosecurity, Significant Preventive Precautions in the Engagement against Zoonosis in Swine Herds; Tittl(1) K; Novak(2) P."
Results of studied dynamics of microbial contamination during hygiene process between check-in and during the crop are showed in Table 1.

Table 1: Dynamics of microbial contamination during hygiene process

	Stages	CFU	Enterobacteriaceae	Entero/CFU
A	After checking out (floor)	9.0E+9 /cm**2	7.8E+9 /cm**2	86,67%
B	Under high-pressure washing >120 bar (200m far, in the air)	2.1E+7 /cm**3	1.3E+7 /cm**3	61,90%
	Under low-pressure washing <100 bar (200m far, in the air)	8.0E+3 /cm**3	9.1E+2 /cm**3	11,38%
C	After washing (dry surface, floor)	4.8E+7 /cm**2	9.5E+6 /cm**2	19,79%
D	After disinfection by GPC8 (dry surface, floor)	2.3E+2 /cm**3	1.4E+1 /cm**2	6,09%
E	1 minute in the air in the presence of animals (5th week)	3.1E+5 / m**3	6.5E+4 / m**3	20,97%
F	Feed bulk tank (silo, 5th week)	4.0E+8 /cm**3	-	-
	Drinking system (3rd month)	3.3E+5 /cm**2	-	-

Table 1 shows that swine houses are naturally dirty environments which could be potentially harmful for health in case of hazardous behaviour of farm personnel. Disinfectants do not operate in an unwashed hall. The reason is the mass of the organic mass with an incredible number of microorganisms (Stage A).
Traditional high pressure washing of these dirty surfaces unfortunately leads to strong airborne surface recontamination in wide surrounding areas 1-3 km's [2],[4] (Stage B) – this action causes rapid spread of diseases during only one crop. To reduce the risk of the spread and transmission of disease by air, a system of so-called "low pressure detergent foam washing" (<100 bar) was implemented in the farm. As the result of this recommendation there was a marked limitation of respiratory and virus diseases (APP, Mycoplasmosis, TGE, etc.) on the farm. An important side effect was the noticeable reduction of water consumption by 1/3 and reduction of washing time by even half the time [3].
Huge amounts of pathogenic microorganisms cause life threatening infections. The infectious pressure could be easily transmitted on following crop. These numbers are rapidly lowered by dry cleaning and washing up to 99% (Stage C).

One of most effective methods to minimise the risk of disease transfer from one crop to the next is to reduce harmful micro-organisms to a level which is not harmful to health (<1.0E+3 CFU/cm^2, Stage D). This rule must be accomplished on any surface including absorbing materials (wood, concrete, plaster, plastic materials and bedding) [4]. The most fundamental determinant of the use of disinfectants beside composition is the method of their application.

A suitable composition of disinfectant influences not the only solution consumption and thus the costs directly; it treats an absorbing surface; but it also extends the exposition time even 40 times! Disinfectants without a high content of non-ionic surfactant or with an unsuitable one (anionic or cationic) are not only deactivated in hard water, but also by organic matter.

The most of pathogenic microorganisms are destroyed usually after 15 minute action of the suitable efficient substance; the most resistant ones after 30 minutes. If a disinfectant is applied without incorporation of a non-ionic surfactant by spraying, It is not possible to achieve a longer action time on a vertical surface than 1 minute. If we apply, however, e.g. the disinfectant GPC8 (with 8% non-ionic wetting agent), we are able to extend the exposition to 30 - 42 minutes. The foam application of the TEKROCID system chemicals also enables to discover places dirty by organic matter so that absorption occurs in 2 seconds, and it is easy for the applicator to add more of foam to the relevant places.

An additional contamination factor can be found in dust. Dust levels in livestock housing have a direct influence on the spread of respiratory diseases by the size of dust particles. Already sterile small dust particles 2.5-10 micro-meter can cause pneumonia. Contaminated particles are more dangerous without compare. Amongst the most harmful risks belongs the transfer of airborne and windborne pathogens in the presence of animals (Stage E) and nutrient transmission (Stage F).

Table 2: Effect of programme TEKROCID on decrease of medication cost

ITEM	I-X '06 (before Tekrocid)	I-X '07 (after Tekrocid)	Comparison 2007 – 2006
Medication cost	134 463 EUR	104 594 EUR	-29 869 EUR
Disinfection cost	15 543 EUR	19 501 EUR	3 958 EUR
Death in sucking pigs	17,14%	13,33%	-17 939 EUR
Death in weaning pigs	12,52%	7,90%	-48 656 EUR
Death in fattening pigs	6,51%	4,22%	-30 254 EUR
SAVINGS:	-	-	-122 761 EUR

At the conclusion in Table 2 is a visible decrease of indirect cost after starting the programme TEKROCID. Slightly increasing expenses on hygiene programme were completely negated by savings in medication cost, expressed in EUR, and by the reduction in mortality in each category. The total farm profitability was increased about more than 122 thousand EUR during the compared periods (January-October) interannually.

The farm owner especially valued the decrease of seasonal fluctuation in numbers of check-out pigs as well as the reduction in mortality (see Fig. 1).

Conclusion

Intensive livestock production is bringing new disease challenges. There are required more attention to Biosecurity procedures for the entire farm. Farms following these procedures are the

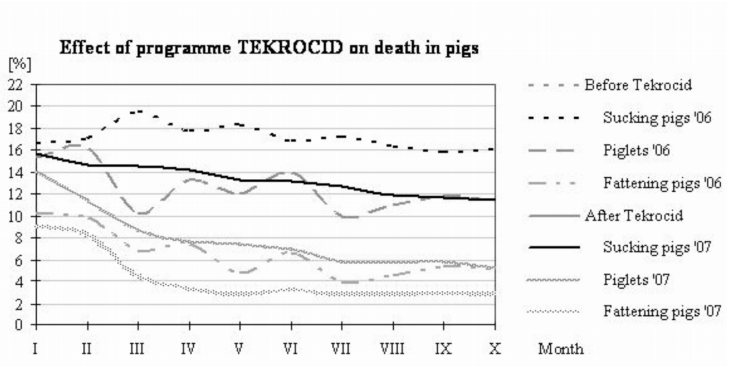


Figure 1: Influence on decrease of death and balance in swine

most successful. The elimination of such risks can only be delivered through high quality disinfection programme, such as Tekrocid, which allows establishing controlled farming techniques. This programme enables to decrease the infection pressure by special techniques, the special formulation of stable disinfectants and unambiguous application methods. Disease outbreaks are preventable if farm staff adopts health management practices to prevent the introduction and spread of diseases in swine herds. The future is purchased by the present! (Samuel Johnson)

Acknowledgement

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Effects off using disinfection preparation d-v to reduce the number of bacterial and somatic cells in the conditions of the farm

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Summary

Mastitis continues to remain a problem for Kosovo farmers and brings big economic losses. Our research includes milking cows of the farm of the Faculty of Agriculture and Veterinary in the University of Pristina in order to study the effect of disinfection matters, which affect environment pathogens and reduce the number of somatic cells as a result. During this period samples from 10 milking cows of different breeds and lactation phases were analysed. All animals were treated under the same environmental conditions. Samples were taken in the morning and evening in accordance with ICAR method in order to evaluate the hygiene and quality conditions of the milk produced by these cows. All physical and chemical parameters, total bacterial cells (CFU) and somatic cells (SCC) were analysed. Our study shows that the use of disinfection matter strongly affects the reduction of CFU and SCC in fresh milk. Nevertheless, influencing factors, such as genetic dispositions, animal feeding and breeding in hygienic conditions should be taken into account for the reduction of SCC.

Introduction

The consumption of milk products in Kosovo is estimated to be nearly 170 kg per capita per year. Kosovo farms supply 76% of the total demand for milk products and 24 % are provided by imports.

Due to the bad hygienic conditions, the number of milking cattle suffering clinical and subclinical mastitis causes big economic losses [5,3,1]. Up to now farmers have been provided with different techniques and methods to maintain hygiene in the cowshed and during milking. Several studies have been made in regard to economic losses, udder damages and reduction of productivity caused by mastitis

The purpose of the study was to identify the situation in the farm and analyse the effect of disinfection matter in the reduction of microbial load and SCC in fresh milk. Based on this purpose, the study considered the analysis of individual milk samples for 6 weeks.

Material and Methods

General conditions of the research: The experiment included milking cows of the farm of the Faculty of Agriculture and Veterinary. The experiment took 6 weeks divided respectively into 3 periods of time of 2 weeks each. Milk samples were taken based on ICAR system (ICAR, 2007).

Phase A (first period): the actual situation of the farm without any intervention was analysed.

Phase B (second period): during this period,

udders were disinfected before milking (1% D-V – in accordance with the instructions of the producer), milking equipment was disinfected and special importance was given to the hygiene of the workers who were responsible for milking and husbandry.

Phase C (third period): the same procedure of phase B was continued, but the disinfection was carried out even after milking with 24% D-V solution and the cows were not allowed to lay down up to half hour after milking.

The chemical preparation used for disinfection is D-V. 100ml of this solution contain 4 g of chlorhexidine gluconate. This preparation is used to disinfect udders before and after milking. At the same time, this preparation affects both gram-positive bacteria and gram-negative bacteria including bacteria which cause the incidence of mastitis.

Data registration, manipulation and sample analysis: Samples were taken in the evening and morning in a quantity of 40-50 ml of milk poured in sterile bottles with conserving material (AZIDOL). The bacterial content in milk was analysed using BactoScan Foss equipment and the laser method of infra red spectrophotometrics of counting the colonies of bacteria. Somatic cells were analysed using Fossmaticminor equipment.

Statistical analyses: Statistical analyses were carried out through PROC-GLM procedures using JMP-starter package, [4].

In general, the model with specific effect with one dependent variable was used to evaluate the effect of independent variables potentially affecting the bacterial load and the number of somatic cells in fresh milk. Differences between the means of various variables were tested using Duncan test.

Results

Analyses of milk samples in proportion to the bacterial load (CFU/ml) per sum of least squares mean (LSM), standard error (SE) and standard deviation (Std DEV) in different phases of the experiment (A, B and C) are shown in Tab. 1:

Results achieved from the laboratory analyses show big variances between the phases of the experiment ($(P < 0.0001)$, clearly showing that in phase C2 the bacterial load in fresh milk was much smaller (CFU=257.95). The highest number of bacteria was registered in Phase A2 (CFU=2576.25), followed by phases A1, B2, B1 and C1, respectively having CFU = 1194.35, CFU = 756.60, CFU = 518.98 and CFU = 423.70.

Tab. 1. Least squares means (LSM) and the analysis of variances (Pr>F) on the effect of phases on the bacterial load in fresh milk (CFU/ml)

Effects	Bacterial load per farm – in 000)		
	N	LSM±SE	STD DEV
Phase effect			
Phase A1	10	1194.35±219.31	402.75
Phase A2	10	2576.25±219.31	1604.87
Phase B1	10	518.98±219.31	100.81
Phase B2	10	756.60±219.31	323.69
Phase C1	10	423.70±219.31	81.92
Phase C2	10	257.95±219.31	61.01
Analysis of Variance	df	Pr>F	
Phase effect	5	0.0001	

If we compare the phases of the experiment in regard to the bacterial load, a significant reduction is noticed from Phase A to Phase B, at a rate of 77%. A bigger decrease has been registered in the last phase of the experiment

in proportion to the beginning of the experiment (Phase A) at the rate of 82%.

Based on the laboratory analyses and their results, it can be noticed that there are big differences between different phases in regard to the SCC present in the fresh milk analysed. Even though not statistically significant P (< 0.8028), differences were figuratively meaningful. Results obtained show an evident ongoing decrease in the number of SCC from phase A to phases B and C. SCC = 356.95 was registered in Phase A1 followed by phase A2 with SCC=321.40. In phases B1 and B2 there was a decrease in the number of SCC=329.55 and SCC=329.100 respectively.

A rapid decrease in the number of SCC per 1 ml of milk was registered in Phase C1 (SCC=293.45), respectively C2 (SCC=217.00).

Tab. 2. Least squares means (LSM) and the analysis of variances (Pr>F) on the effect of phases on somatic cells in fresh milk (SCC/ml).

Effects	Bacterial load per farm (SCC/mil – in 000)		
	N	LSM±SE	STD DEV
Effect of phase			
Phase A1	10	356.95±171.81	337.62
Phase A2	10	321.40±171.81	464.55
Phase B1	10	329.55±171.81	274.58
Phase B2	10	329.10±171.81	290.35
Phase C1	10	293.45±171.81	164.11
Phase C2	10	217.00±171.81	116.72
Analysis of Variance	df	Pr>F	
Effect of Phase	5	0.8028	

If we compare these phases to each other, it comes out that from Phase A (the beginning of the experiment) to phase B, SCC decreased by 3%. From Phase A to C (the completion of the experiment), SCC decreased at the rate of 25%.

Discussion

This results lead to the conclusion that bacterial content in fresh milk in our diary farms is very high, compared to existing standards. This is an issue to be worried about for all interested parties linked to milk industry chain, considering that 26.72% (CFU/ml < 200.000) in 2008 and 47.48% (CFU/ml < 100.000) in 2009 do not fulfil the standards for fresh quality milk. There are big differences between different farms, included in the study, we can see managerial divergences for many aspects [2] which in many cases are not linked only to expensive infrastructure (closed milking system etc.) but they derive directly from negligence and improper management. It is noticeable that there is a negative correlation between cattle breeds with high performance (i.e. HF and HK) and bacteria in milk. There is a concern that if the dairy commercial farms continue to produce low quality milk, some of these farms will not be able to sell their milk legally (to dairies, milk processors, etc) and in this way they will be obliged to sell their milk illegally (green market, smaller milk processors, etc).

Conclusion

Use of disinfection matters is a necessary measure in the milking practice in order to have hygienic milk. This practice is still lacking in almost all our farms in Kosovo. Our research presents several preventive measures which influence in the prevention of the development

of bacterial cells and somatic cells at the same time. In order to make the research more comprehensive, further studies should include longer time periods and analyse in more detail the causes of contamination of the fresh milk with such agents. The identification of the type of bacteria present in fresh milk as a possibility of treating mastitis properly and efficiently is of a special importance.

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Biosecurity and disease prevention measures in the European poultry sector - an Avian Influenza perspective

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Summary

To gain more insight into prevalent farm management structures and biosecurity practice applied at poultry farms, farm data were gathered by using questionnaires and logbooks in 343 farms in Germany, The Netherlands, and Austria. To evaluate the results, an international expert panel defined and weighted the most important risk factors for AI introduction within a three-stage Delphi study. Accordingly, all farms were assigned to three classes of risk. 19.8 % of all farms considered were assigned to the highest risk class. This class represents farms with a high potential for AIV introduction. Comparing the different prevalent structures of poultry production in the three countries, the results can also be used in regard the EU zoonosis directive.

Introduction

The ongoing outbreaks in Asia, Africa, and Europe demonstrate that the *Avian Influenza virus* (AIV) still represents a global threat to the international poultry industry. As experiences from many AIV outbreaks have already shown, a complex interaction of many risk factors at farm level was involved [1]. The overall aim of the farm level analysis within the EU project "Healthy Poultry" was to investigate prevalent farm management structures and biosecurity measures and to gain more insight into the contact structure in poultry farms.

Animals, Material and Methods

Participants. Within the survey a total of 343 poultry farms with different poultry species and different farm sizes were analysed. As can be seen from table 1, data were collected in 84 farms in Austria, in 37 Dutch farms as well as in 222 poultry farms in Germany. Main participants were farms with layers, broilers and turkeys. Additionally, 26 farms with other poultry species like ostriches, pullets, ducks and geese participated in Germany (Table 1).

Most of the farms are located in the main poultry production areas of the countries. The poultry stocks analysed represent 3 % of all Dutch farms (2.8 mill. birds), 11.2 % (1.5 mill. birds) of the Austrian farms and 12.2 % (13.1 mill. birds) of the poultry stocks in Germany. In these densely populated poultry areas, an outbreak of AIV would cause severe economic consequences for the whole production chain within the poultry industry.

Tab. 1: Overview of participating farms. (Q = Questionnaire, LB = Logbook)

Country	Germany			Austria			The Netherlands		
Poultry species	Q	LB	in %	Q	LB	in %	Q	LB	in %
Broilers	76	68	89.5	29	29	100.0	16	12	75.0
Layers	65	49	75.4	29	29	100.0	21	11	52.4
Turkeys	55	35	63.6	26	24	92.3	-	-	-
Pullets	12	12	100.0	-	-	-	-	-	-
Other poultry species*	14	4	28.6	-	-	-	-	-	-
Total	**222**	**168**	**75.7**	**84**	**82**	**97.6**	**37**	**23**	**62.2**

*Three layer breeder farms, one farm with geese, one farm with ostriches, nine farms with pedigree poultry

Survey instruments. Based on a literature research, the main risk factors for AIV introduction were identified. At farm level, risk factors can be differentiated in those, which are related to farm management and factors, which deal with business contacts at the farm. Therefore, a questionnaire was developed to obtain data on prevalent farm management structures. Furthermore, a logbook was designed, where the farmers recorded all type of business and private visitors for a period of four weeks. The data included the information whether the persons had access to the poultry house and if they had already visited other poultry farms at the same day. Subsequent to a pretest in 12 poultry farms the questionnaire consisted of 68 questions related to the categories "farm location", "management" and "disease prevention".

Risk assessment. Based on a literature research, the main determinants for AIV introduction were identified within a three-stage Delphi study, where an expert panel of 21 international experts with a special expertise on AIV was involved. The experts rated the relative importance of single risk factors, which was used to derive the weights for 32 risk factors. Within the evaluation process, the participants' answers were checked on the occurrence of risk factors. For each risk factor present, a specific score was attributed to the farm's account. Subsequently, for each farm the total score of each category was attributed to one of three risk classes by assuming that the number of risk factors is correlated to the risk of AIV introduction. To communicate the results in a comprehensible way, a traffic-light-scheme was applied to visualise the farms' risk status in regard to AIV introduction.

Results

A selection of factors which were examined within the questionnaire survey is summarized in table 2. Since a high quality of biosecurity constitute an important barrier to the incursion of AIV into poultry farms [2] a variety of basic measures undertaken in the participating farms were analysed. As can be seen from table 2, a set of security gaps such as no hand washing or no disinfection of footwear before entering the poultry house can be found in different shares of the considered countries. This shows that the efficacy of biosecurity plans greatly depends on the compliance of the farmer and his personnel [2].

The logbook results display clearly the high and different amount of visitors, who enter the premises during the poultry production cycle. Practising a direct sale at the farm or keeping different domestic species results in more visitors per day. The more visitors come to a farm, the higher the potential for the introduction of infectious diseases to the farm. The number of visitors registered during the investigation period averages between 33 (Austria), 46 (Netherlands) and 47 visitors in Germany. The majority of these contacts are deliveries

of feed, litter and other materials as well as private contacts which are considered low risk contacts. Austrian farms had fewer contacts, 3 +/- 49 contacts per four weeks of which on average 7.1 contacts entered the sheds.

Tab. 2: Selection of risk factors examined within the questionnaire survey

Risk factors	Unit	Austria	Germany	Netherlands
Direct sale of poultry and poultry products	Yes [%]	40.5	14.0	40.5
Farm is part of a vertically integrated production system	No [%]	54.8	18.5	89.2
Additional keeping of ducks / geese	Yes [%]	8.3	11.3	0.0
Usage of a multiple age system	Yes [%]	36.3	47.9	43.2
Type of housing	Indoor: Cage [%]	6.0	9.5	10.8
	Indoor: Barn [%]	75.0	65.3	81.1
	Outdoor: Freerange [%]	13.1	17.6	0.0
	In- and Outdoor [%]	6.0	7.2	8.1
Storage of litter	Open for wild birds, cats, and rodents [%]	57.2	60.3	20.0
Information of the staff about AI and the associated special caution and care required?	No [%]	13.1	8.6	37.8
Disinfection of housing equipment?	No [%]	70.2	60.4	37.8
Regularity of vermin control?	If required [%]	32.2	12.3	0.0
Carcass container on the farm?	No [%]	20.2	8.1	0.0
Rendering truck drives onto the premises?	Yes [%]	58.3	28.4	18.9
Machinery sharing?	Yes [%]	28.6	19.4	24.3
Cleaning and disinfection of the machinery before delivery to owner?	On own premise after delivery [%]	29.6	13.6	0.0
	Not regulated [%]	25.9	34.1	11.1
Disease prevention plan at the farm?	No [%]	82.1	37.4	n.d.

As described above, the data on risk factors and risk contacts per farm were evaluated. The final scores range between 11.1 and 49 points. The theoretical maximum to reach was 77 points. Only farms with both documents handed in for analysis were considered (table 1). The more risk factors and risk contacts apply to the farm, the more points were assigned to a farm. In the last step, the final scores were classified on the basis of equal intervals and all farms were assigned to the three risk classes.

Table 3 summarizes the results of the farm assessment by countries. As can be seen none of the Dutch participants, 4.9 % of the Austrian and 13.1 % of the farmers in Germany reached the lowest risk class 1 (less than 16 points in total). Risk class 2 is defined by a score between 16 and 32 points, where between 69.9 % (Germany) and 78.3 % (The Netherlands) of all farms can be found. Poultry farms with more than 32.1 points were attributed to the highest risk class 3. As shown in the table this was the case for 24.4 % of the Austrian, for 17.3 % of the German and for 21.7 % of the Dutch poultry farms.

Discussion

Despite the limitations of this study and the relatively small data set considered in relation to the total number of poultry farms in the countries, the system developed can provide an outline for the prevalent AI introduction risk status of livestock and poultry farms. For future research and use within disease prevention it is recommended to investigate a representative sample of farms in different European regions. In combination with the

Tab. 3: Final assessment of the participating farms by country

	Austria		Germany		Netherlands	
Risk classes (RC)	farms	in %	farms	in %	farms	in %
RC 1 (<= 16.0)	4	4.9	22	13.1	0	0
RC 2 (16.1 - 32.0)	58	70.7	117	69.6	18	78.3
RC 3 (>= 32.1)	20	24.4	29	17.3	5	21.7
Total	**82**	**100**	**168**	**100**	**23**	**100**

regional risk maps developed within the EU project "Healthy Poultry" [3] this information could be of use for European decision makers in terms of disease prevention.

Conclusion

In this study, poultry farms were analysed in regard to farm management and disease prevention practices. One result of the study was that the farms perform worst in the category "hygiene / disease prevention". As described above, the investigation revealed a set of biosecurity gaps which are related to basic hygiene measures. On the one hand, this means that more education of the farmers is necessary and on the other hand it demonstrates that most of these security breaches are easy to bridge.

Since the evaluated variables represent a poultry farm's first line of defence, the results are not only relevant for the introduction of the AIV, but also for the introduction of any pathogens. Especially with regard to the EU zoonosis directive 2003/99/EC and for the realisation of the commission regulation No. 1168/2006, most of the farms investigated need to adjust their hygiene management in order to achieve the defined prevalence level by each country. Concluding, a high level of biosecurity practice during the whole poultry production cycle can be one of the main keys to improve food safety and to be prepared for future challenges within food production.

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Using an innovative laboratory based system to evaluate the efficacy of cleaning procedures used on livestock farms

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Summary

A study evaluating the efficacy of cleaning procedures to be used in livestock buildings was conducted in South Australia. The cleaning methods assessed included degreasing, hosing, incineration, liming and scrubbing. It was found that degreasing and burning resulted in better elimination of bacterial loads when compared to stand alone scrubbing, hosing or liming. It was also revealed that the efficiency of liming and incineration could be improved by using them in conjunction with scrubbing. The study results also suggested that further investigation is required to optimise liming and incineration procedures for better adoption by pig producers.

Introduction

Previous investigations on pigs kept in buildings with improved hygiene levels revealed enhanced production efficiency coupled with reduced respiratory problems detected in the animals (3). Improved hygiene can also contribute to a reduced occupational health and safety risk on livestock farms (1). Despite the evidence presented by a number of authors (5; 6), there are few investigations in the literature that specifically evaluated different cleaning procedures and their efficacy in reducing bacterial load on the flooring used in livestock buildings. Studies were therefore designed to assess the efficiency of different cleaning methods to ensure a high level of animal welfare and productivity on farms.

Material and Methods

Four controlled experiments were performed as listed in Tab. 1.

Tab. 1. Comparison of cleaning methodologies evaluated in this study

Experiments	Description
1	Hosing vs. Hosing + Degreasing
2	Hosing vs. Scrubbing
3	Scrubbing vs. Scrubbing + Lime
4	Hosing vs. Scrubbing + Burning

The individual experiments were conducted on concrete paver blocks (CPB) using pig manure to mimic the animal activity (Fig. 1a.) following previously published methodology (2; 4). The cleaning effect was evaluated based on reduction in the microbiological load of the CPBs. Each experiment used 8 CPBs with the dimension of 80 mm x 45 mm. All the CPBs were cleaned with detergent, disinfected and dried prior the experiments. CPBs were soiled prior treatment using fresh pig manure. 500 g of pig manure was collected from the Roseworthy piggery, suspended in 750 mL of tap water and mixed well to create slurry. 15 mL of the slurry was applied on each of the paver block using a spatula (Fig.1b). The blocks were allowed to dry for 8 hours prior to cleaning process to mimic a natural baking effect occurring in pig shelters.

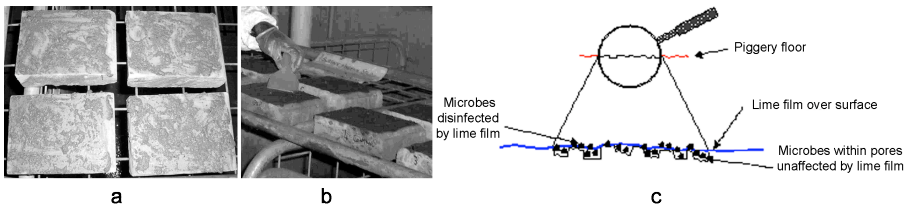


Fig. 1. Application of pig manure (b) on experimental paver blocks (a) and post liming bacterial growth on paver surface (c)

Several cleaning procedures (as listed on Tab. 2) were applied in the current study. At the end of the cleaning, each paver block was sampled at four specific corners using a Perspex sheet with 4 square windows (20 mm x 20 mm) to obtain a representative sample of the bacterial load. The samples were collected using a sterile swab (Rowe scientific Ltd. Adelaide) dipped in 1% sterile peptone water.

Tab. 2. Choice of cleaning procedures currently applied in piggeries

Cleaning method	description
Hosing	Individual CPBs were hosed using mains water for 10 seconds
Degreasing	CPBs were hosed briefly for about 10 seconds before the degreasing agent (Farm mate™) was applied diluted in water at 1:3 volume ratio.
Scrubbing	Heavy duty nylon brush was used to clean the CPB surface with hand pressure mimicking on farm cleaning
Liming	Thin slurry of builders lime (11%w/v) was evenly spread over the CPBs using a paint brush.
Incineration	Hand held LPG gas burner was used in flaming the CPB surface. Flame was moved to ensure an effective contact time of 5 seconds.

The samples collected on the swabs were diluted serially to 10^4 dilution and plated on HBA (Horse blood Agar) plates (Oxoid scientific Ltd. Adelaide). 100L of diluted sample was used for plating and evenly spread on the plate.

Results and Discussion

Prior to the application of different cleaning process; soiling over the CPBs appeared as a hard crust containing particulates and visibly dirty. Different cleaning processes resulted in

varying degrees of visual cleanliness. Degreasing process led to the best post cleaning status visually followed by less appealing appearance after liming. CPBs after dry scrubbing and burning resulted in the least appealing visual cleanliness. Fig. 2 shows post cleaning bacterial loads for various cleaning methods studied. The difference in residual bacterial loads between scrubbing and hosing was around 13% and was not significant. This result suggests that after hosing significant bacterial population remains within micro crevices of the CPB surface despite of visual cleanliness. The non-significant difference in residual bacterial loads between scrubbing and scrubbing plus liming was around 16%. However, the relative bacterial loads of the CPBs after incineration and degreasing were significantly reduced by 30% and 70%, respectively. The incineration and degreasing processes were compared against hosing as control. Burning seems to destroy residual bacteria but the elimination of cells may depend on the heat generated and its contact time with the contaminated surface.

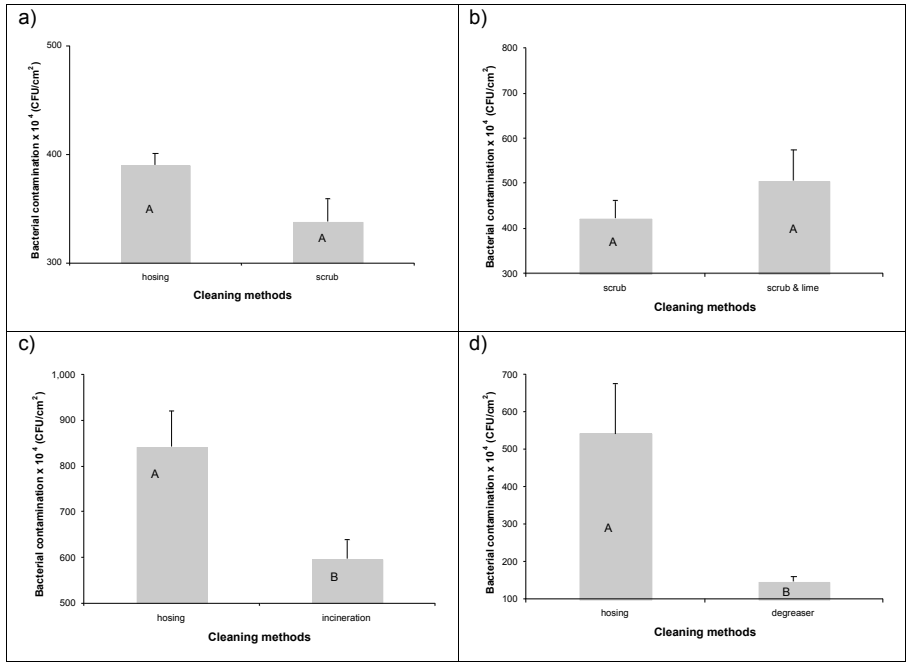


Fig. 2. Post cleaning bacterial loading on the pavers: a) hosing vs. scrubbing b) scrub vs. scrub & lime c) hosing vs. incineration and d) hosing vs. degreasing. (Bars with different letters indicate a significant difference in the residual bacterial load $P<0.05$)

Although liming is believed to have a killing effect on bacterial cells, it tends to form a protective film during its contact over the contaminated surface thus encouraging further microbial growth underneath it. Fig. 1c shows a schematic explanation for possible bacterial growth in micro crevices on the surface of CPBs.

Conclusion

Degreaser use on concrete floor surfaces resulted in very good level of cleanliness both visually and microbiologically. Hosing resulted in a visually more appealing cleanliness compared to

scrubbing but may not be suitable as a stand alone cleaning method if a microbiologically clean surface is required. Further investigations are required to optimise incineration and liming techniques in order to improve microbiologically acceptable post cleaning standard. This would include investigation of minimum contact time required for microbial killing and effects of pre-scrubbing to improve burning efficiency. Our study indicated that liming concentration needs to be optimised to provide maximum bacterial killing efficacy within the micro-crevices on the CPBs.

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Resistance induction against disinfecting agents at coagulase-positive *Staphylococcus* isolates.

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Summary

The results of this investigations show that the use of sublethal concentrations of disinfectants can increase the minimum inhibitory concentration value at different *Staphylococcus* spezies including MRSA strains, whereas the acquired disinfectant resistance than is mostly consistent.

Introduction

Disinfection is an important factor in the prevention and control of infectious diseases. Resistance to antimicrobial substances represents a prevalent problem worldwide, as well as a limiting factor in the control management.
The use of active agent concentrations below the minimum inhibition concentration (MIC) in objects of the daily necessities leads to the suspicion that active agents in sublethal concentrations reduce sensibility and raises adaptation to active substances, whereas the effectiveness is diminished [1, 2].

Material and Methods

10 isolates of each examined coagulase positive *Staphylococcus* ssp. such as Methicillin-resistant and –sensible *S. aureus* (MRSA and MSSA; n=10 each), *S. intermedius* and *S. hyicus* were included. These isolates were tested against following disinfecting agents: Triclosan (TLN), Chlorhexidin (CHX) and Benzalkoniumchlorid (BKC).
The MIC and the sublethal concentration (concentration of a substance below the MIC at which the individuals still show growth) was determined for each active agent. The Staphylococcus isolates were treated ten times with the sublethal active agent concentration. Thereafter the MIC values were determined again, and compared to the original MIC values.

Results

A substance- and species-dependant induction of disinfectant resistance could be detected at all *Staphylococcus* ssp. The sensitivity reduction could be shown for the MSSA and *S. intermedius* isolates at 73,3%, for the MRSA isolates at 43,3%, and for the *S. hyicus* isolates at 36,6%. Regarding to the active substances resistance induction was shown for Triclosan at 72,5%, for BKC at 62,5%, and for CHX at 35% of the *Staphylococcus* isolates (Tab. 1).

Tab. 1: Number of disinfectant resistances induced by sublethal concentrations.

disinfectant	MRSA (n=10)	MSSA (n=10)	S. intermedius (n=10)	S. hyicus (n=10)	
Triclosan	6	10	10	3	**29 (72.5 %)**
Chlorhexidine	1	7	3	3	**14 (62.5 %)**
Benzalkoniumchloride	6	5	9	4	**24 (35 %)**
	13 (43.3 %)	**22 (73.3 %)**	**22 (73.3 %)**	**10 (36.6 %)**	

Finally a high percentage of the tested *Staphylococcus* isolates showed a consistent acquired disinfectant resistance (Fig. 1).

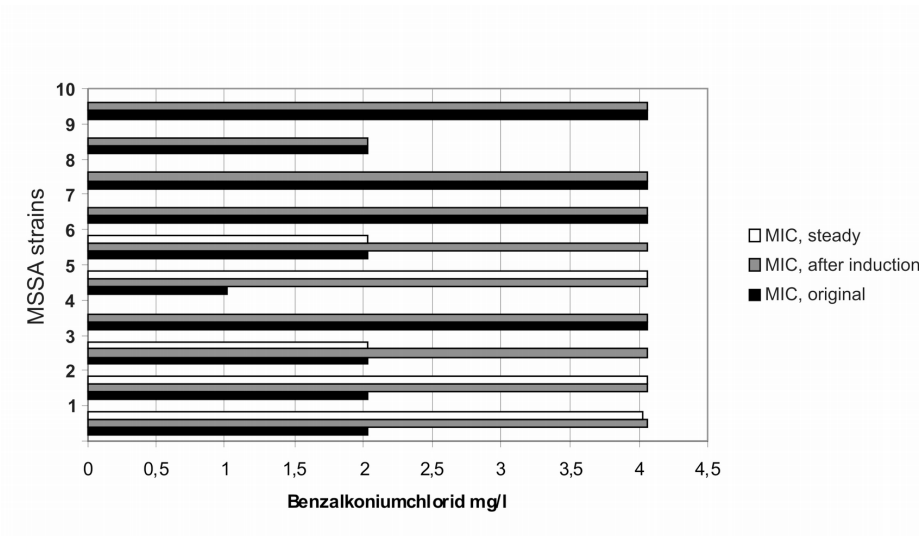


Fig. 1: Resistance induction against Benzalkoniumchloride at methiciline sensitive S. aureus (MSSA) strains.

Discussion

Our results show that the use of sublethal concentrations of disinfectants can increase the minimum inhibitory concentration value, whereas the acquired disinfectant resistance than is mostly consistent.

The boadly practiced use of disinfectants in sublethal doses (for instance Triclosane for conservation purposes in the majotity of toothpastes) must therfore assessed critically.

Firthermore, there are some hints that such an induction of disinfectant resistances also can lead to cross-resistances to antimicrobial agents resulting in an ineffective disinfection of pathogens which than possibly have an increased resistance to antibiotics [2].

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Chapter 30

Block 8B

African swine fever biocontainment adoption in Ibadan, Nigeria: problems, needs and opportunities

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Summary

To study adoption level of ASF biocontainment technologies and factors associated with pig farmers' adoption behaviour, a sample of 60 pig farmers from six local government areas were selected through "stratified two–stage random sampling" method. Data were gathered with the use of structured interview schedule. Assessment criteria in forms of frequency, percentage, mean and product moment correlation coefficient were calculated. Majority of pig farmers (86.67%, n=60) were at low level of adoption behaviour, followed by 10% (6/60) at medium level and 3.33% (2/60) at high level of adoption of ASF biocontainment technologies. This result indicates that majority of pig farmers did not adopt ASF biocontainment technologies. Farmer's income from piggery, knowledge of biocontainment strategies and extension contact had positive and high significant relationship with overall adoption by pig farmers. Specific needs and emerging opportunities for improved cooperative extension activities were identified. They included services for early detection of ASF virus (ASFV)– infected pigs by University based officers, need to produce and distribute ASFV–free pigs by the state Ministry of Agriculture and Natural Resources (MANR), and use of ASF specific extension materials by Oyo State Agriculture Development Programme (ADP) officers.

Introduction

Pig production problem in Ibadan. Pig production is a source of employment and livelihood in Nigerian agriculture (3). During the past decade (1997-2007) pig rearing that had been one of the livestock industries growing most quickly in southwest Nigeria became one at a high risk of losses from African swine fever (ASF), an infectious disease recently introduced into Nigeria (1). In East and South African countries ASF is one of the most serious obstacles to the development of pig production (5). West Africa has recently had the same experience as Southern Africa.

The first outbreak of ASF was reported in Ibadan in June 2001 (1). The disease continued to spread from farm to farm through contact with infected and carrier pigs during stock trade, breeding activities and farm visits. The cost of mortality losses in Ibadan and environs owing to ASF outbreaks in 2001 alone were estimated to be US$941,491.67 (1). This severe and devastating disease of domestic pigs requires a firm control and eradication programme to ensure profitable agriculture.

All available strategies to prevent the escape of a disease agent currently on a farm or in an operation are collectively termed biocontainment practices. Biocontainment technologies include providing a facility to isolate pigs on farms, testing for ASF infection, removing infected pigs, disposing of dead pigs, disinfecting premises, and other indirect containment techniques, such as ensuring the hygiene of feed delivery vehicles. Biocontainment practices are emphasized in infectious diseases that are enzootic in a population of animals, such as ASF is in Ibadan. This author therefore embarked

on a multidisciplinary research project to implement early detection, tracking and technology transfer for the biocontainment of ASF-infected pigs in Ibadan, southwest Nigeria, in 2007. The objective of the research and education project was to develop and disseminate to pig farmers effective and acceptable biocontainment technologies for ASF control in collaboration with Oyo State Agriculture Development Project (ADP) in Ibadan, southwest Nigeria. (MacArthur Multidisciplinary Grant Ref. No: 800/406/54/2006/MRG/3).

Material and Methods

The study area was the city of Ibadan and its environs in Oyo State (Latitude 7° 23^1 and Longitude 3° 56^1), where outbreaks of ASF had been reported by members of the Pig Farmers Association of Nigeria (601 registered members in Ibadan) and the Ministry of Agriculture and Natural Resources (MANR). Ten percent (60) were selected. In the first stage, six local government areas (LGAs) were selected out of 12 in Ibadan. The second stage was a random selection of ten farmers in each of the six LGAs selected. The sample size of 60 respondents was obtained, comprising both farmers having contact with the ADP in Ibadan and those without extension contact. Questionnaire was developed to obtain relevant information covering all aspects of the objective of the study. Primary and secondary data were employed. Secondary data were obtained from the literature, project reports, official documents, publications, and personal consultations. Primary data were collected through the use of a validated questionnaire consisting of both open and closed-ended questions to obtain information from the target respondents. Three trained enumerators who have the knowledge of the dialect of the farmers were used to assist in the collection of the information required.

The survey instrument was divided into four parts;

(A): Socio-personal characteristics (B): Income from piggery, ASF mortality losses and state compensation (C): Sources of information on biocontainment practices and extension activities (D): Biocontainment technologies adopted

The instrument for data collection was subjected to preliminary testing among farmers that were not included in the sample. Validity and reliability tests were carried out on the pre-test evaluation group.

Depending upon the extent of adoption of biocontainment practices, the respondents were categorized as follows:

1. Low adopters (up to 33%)
2. Partial adopters (34 – 66%)
3. High adopters (67 – 100%)

Results

Pig Farmers' Association of Nigeria (PFAN) (93.3%) and friends/neighbors (83.3%) were the most important sources of biocontainment information to pig farmers in Ibadan (Table 1). These sources provided weekly information (friends/neighbors, 73.3%) and monthly information (Pig Farmers, Association, 86.7%) to the respondents. Members of the PFAN met once a month during which they discussed relevant issues for improving their practices. Invited resource persons also informed and trained farmers on new topics. ADP officers did not visit many of the respondents (36.7%) in 2007. This appeared to be in conformity with current policy of the agency to visit the sub-urban (outskirt) farmers more often than urban (city) farmers.

As a result of the disposition of pig farmers, the overall adoption behavior was low. Majority of the respondents 86.7% (52/60) were at low level of adoption behavior. Only 10%

Tab. 1: Sources of information on ASF biocontainment among respondents

Source of information	Not usually informative		Weekly information		Monthly information		Yearly information	
	Freq	%	Freq	%	Freq	%	Freq	%
Contact farmers	14	23.3	33	55	7	11.7	6	10
Extension agents	31	51.7	0	0	6	10	23	38.3
Friends/neighbors	10	16.7	44	73.3	6	10	0	0
Pig Farmers' Association (PFAN) meetings	4	6.7	0	0	52	86.7	4	6.6
Newspapers/magazines	51	85	0	0	1	1.7	8	13.3
Radio	17	28.3	0	0	8	13.3	35	58.3
Television/video	45	75	0	0	0	0	15	25
Research institutes/ Universities	21	35	0	0	8	13.3	31	51.7

Source: Field Survey, 2007

(6/60) of the respondents showed partial adoption behavior, and a minority of 3.3% (2/60) showed high level of adoption behavior (Table 2). All the biocontainment practices had cost implications to the pig producers (1).

Tab. 2: Overall adoption of biocontainment technologies by respondents

S. No.	Level of adoption	Score index	Frequency (N=60)	Percentage (%)
1	Low adopter	Up to 33%	52	86.7
2	Partial adopter	34-66%	6	10
3	High adopter	67-100%	2	3.3

The most adopted practice was disposal of dead pigs and disinfection of premises. The range was from 3.3% at high, and 75% at partial levels (disposal of dead pigs and disinfection of premises); 1.7% at high, and 23.3% at partial levels (indirect spread containment facilities); 5% at high, and 16.7% at partial levels (removal of infected pigs); and, 5% at high, and 10% at partial levels (isolation facility); to 0% at high, and 5% at partial levels (testing for ASF, that was the least adopted practice).

Discussion

The job experience and age were not significantly related to adoption behavior of a pig farmer (r = 0.299 and -0.095 respectively). However, a farmer's income from piggery, the frequency of extension contact and the knowledge of biocontainment technologies were positively and significantly related to adoption level. It is imperative to build strategies for promoting veterinary extension on this basis. Formal veterinary research though generated a vast amount of knowledge and insights into pattern of spread of ASF and ways to enhance its control (4), did not improve adoption by smallholder farmers, especially in Ibadan, which has remained below expectations. It may be concluded that the traditional

method of extending biocontainment technology to farmers is not sufficiently adequate at the moment in the circumstance of pig farmers in Ibadan. A new approach is needed in which pig farmers are actively involved in the acquisition of biocontainment skills, geared to the specific physical, economic and social circumstances of these farmers.

Conclusion

Farmer Field School (FFS) is a learner-centered approach, whereby farmers through understanding, observation, experimentation and evaluation are equipped to address challenges and make appropriate changes in their farm management practices. (2). It was used to promote malaria control among crop farmers. The approach should be adopted in ASF control in Ibadan. FFS is a form of education that uses experiential learning methods to build farmers' expertise, and has proven farm level disease control effectiveness.

Acknowledgement

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Experience with the biosecurity status in chinese pig farms

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Summary

Pig production is steadily increasing during the last decade in China. It is estimated that about 50 % of the world pig production happens in China. With increasing productivity biosecurity measures gain more and more importance. This paper rerports on experiences with biosecurity on chinese pig farms.

Introduction

In the year 2008 the pig meat production in the world was 95,8 Mio [2] tones total and in China 48,0 Mio tons (50,1%).Since the year 2008 the "Peoples Republic" (PR) of China is a member oft the WTO [1],for production of meat and food with WTO standard .Since some years the PR China is a member oft the following organizations as FDA,FAO and OIE [6].In the year 2006 by the government of the PR China is installed a disease control system for the main epidemic diseases.The breeders and the veterinarians protect the herd health by biosecurity programs.

Material and Methods

In the PR China we have mostly private pig farmers with a few sows and with more then 1000 sows for breeding,piglet production and for fattening.Some international breeding organizations as PIC or TOPIGS have build up breeding farms with there one genetic in the SPF status.In this high health farms they produce gilts and boars for the private pig farm organizations in China.In some big closed farms with sows and fatteners we find different races as landrace,large white sows and boars and duroc boars for fattening.The sow farms - with more then 1000 sows with fatteners - produce normaly at one place with closed systems.Till now in only 1% of the farms we find a two or a three side production.The most pigs in China were produced in the provinces along the Yangze river,as here is much production of harvest etc..In the north part of China 22,5% and at the cost line provinces 16,8% of the pig production is installed by private agriculture organization with feed miles and sometimes slaughter houses.In the year 2008 in two provinces during our visits in pig farms (>2000sows/farm) with fatteners we controlled the hygiene - and the health status,the results of the serology blood tests and the vaccination programs. During the farm visits we looked for the problems with the PRRSV infection and other pig diseases.

Results

Feeding. Until now there are many farm workers and that' why there is no automatic feeding system for the sows,the piglets and the fatteners.All the different pig groups get pellets produced in feed mills.The feed is produced by grain as ,barley, wheat,maize and soya meal with amino acids,vitamins and minerals.As in the grain are often mycotoxins the mills mix a mycotoxin absorber as Biomin* Mycofix* in the pellets for sow and piglets feed.

Cleaning, disinfection. The farm workers take the excrements during the day in the cesspit by shovel and broom.With the all in and all out system the stables were cleaned with a high presser machine and then disinfected by different chemical substances.In many farms the stable walls were painted by quicklime.

Biosecurity. In all big farms the farmers and the visitors must change there clothes,wash there hands and shower there body before entering the stables.Transport cars for feed,for pigs etc.must be disinfected before entering the farm ground.The gilts and the boars were tested for farrowing quantity,uniformity,survival,lean meat and back fate rate. The health status of gilts and boars is tested by blood samples for different diseases.

Health status program. Since the year 2006 the chinese pig farms had problems with a high infected PRRS virus in different provinces with a intensive pig production.Some times there are outbreaks with other endemic pig diseases,as listed in the OIE,in the farms.In the year 2008 by the government there was installed a "Disease Control Program" and a vaccination program for some diseases as CSF;MFD,PR,PRRSV [5] .We found other vaccinatons,if there were problems with special diseases as EP,RA,GD or APP after the results of sections or blood tests.Against PRRS outbreaks with the disease there are produced live vaccines in China with special PRRS virus phylums for the pigs.By these live vaccines the farmers try to reduce the problems with PRRSV in the breeding herds.As the success to catch the virus is not very high more and more pigs were vaccinated with the licensed Boehringer Ingelheim (BI) Ingelvac* MLV Vaccine.By the BI PRRSV live vaccine the sows were vaccinated two or three times a year and the piglets were vaccinated in the third week after birth.In the "Bolai Agrar Group" there is a strict vaccine program [4] during the different production steps from gilts,sows,boars,fatteners for diseases (Table1,2,3).The pigs were vaccinated by the vets.At the slaughter house the pigs were controlled for antibiotics,sulfonamides, clenbuterol,diazepam or chlorpromazine and other residues.

Production control. In big farms with a computer program were collected the number of live and death borne piglets,the losses,during breeding,the return to oestrus dates,the days for fattening,the pig prices and the feed or medicine and vet costs.The slaughter weight was 120 kg body weight in more then 200 days fattening. During the visits the results for the piglets fluctuated between 18 and 24 in the farms.The return to oestrus was from 10 to 30% By the government was
installed the program "Food Safety" for food residues.The feed mills were controlled for Hg,Pb,As,Cd,Cr,Lindan,DDT etc..

Tab. 1: vaccine program for gilts, Ho Heichii,5/28008

group	day/age	vaccine	KV/LV	company
	130	PRRSV	LV	Boehringer
	140	RA	KV	Intervet
	150	FMD	KV	home
	160	M. Auj	LV	Boehringer
	170	Jap. Enceph.	LV	home
	180	PPV	KV	Intervet
gilts	190	PRRSV	LV	Boehringer
	200	Jap. Enceph.	LV	home
	210	PPV	KV	Intervet
	220	PRRSV	LV	Boehringer
	230	CSF	LV	home
	240	FMD	KV	home

Tab. 2: vaccine program for boars,sows, Ho Heichii,5/20008

group	day/age	vaccine	KV/LV	company
	3.9.12. month	FMD	KV	home
	3 x a year	CSF	LV	home
	3.9. month	Jap. Enceph.	LV	home
	3 x a year	PRRSV	LV	Boehringer
sow/boar	October	T - P	KV	home
	30 days a.p.	RA	KV	Intervet
	40-15 days a.p.	K 22 E.coli	KV	home
	10-20 days p.i.	PPV	KV	Intervet

Tab. 3: vaccine program for fattener, Ho Heichii,5/2008

group	day/age	vaccine	KV/LV	company
	15 - 20	PRRSV	LV	Boehringer
	25 - 30	CSF	LV	home
	25 - 60	M. hyo	KV	Boehringer
	60 - 70	FMD	KV	home
fatteners	70 - 85	CSF	LV	home
	85 - 100	M. Auj.	LV	Boehringer
	100 - 110	FMD	KV	home
	110 - 130	CSF	LV	home

Discussion

During the visits in the pig farms we found a hygiene program for the pigs and the farm workers [3].The pigs were produced with segregates early (3 weeks) weaning,all in and all out,artificial insemination,farrow by farrow feeding and with quarantine and sanitation for gilts and boars in the most farms.

The reasons for different results in pig farming,health status and management we found in:

(1) the races as landrace,large white,duroc,hybrids as PIC or TOPIGS etc,

(2) differences in hygiene and cleaning,
(3) all pigs from sows to piglets to fatteners at one place with crowding disease,
(4) instrumental insemination with differences in the sperma quality,
(5) heat control mistakes,

(6) reduced laboratory tests for health status and diseases,
(7) uncontrolled antibiotika treatment without resistant tests,
(8) uncontrolled vaccination programs without blood tests,
(9) feeding and mycotoxin problems,
(10) minimal production dates for sows, piglets, fatterners.
(11) mycotoxin problems.

Till now in China the pig farm organizations with there farmers work hard for getting the international health and hygiene status in the pig stables. In more then 100 schools of engineering the farmer study "Animal Health".

Conclusion

More than 50 % of the world pig production takes place in China with a growing tendency in the next years, as more chinese people eat porc and poultry meat. Since some years the PR China is a member of international world organizations for producing "Safe Porc" with biosecurity rules and high health standards for the farms. However, there are also considerable gaps in knowledge and in the execution of the practical application. Therefore more than 100 schools of engineering "Animal Health" were fouded to raise the knowledge and skills among farmers and farm workers

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Preliminary research of sows' udder hygiene on teats' bacterial reduction

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Summary

Research was conducted in order to determine efficiency of sows' udder sanitation concerning reduction of bacteria, which enter through contaminated teats in piglets' organism. The research was carried out in farrowing pen with 90 sows, which were separated into three equal groups. The first group, control, was not treated. In the second group sows' udders were treated with water and in the third group with healthy and ecologically acceptable commercial antiseptic Oxyl$^{(R)}$with powerful oxidising bactericidal activity. During the period of 24 days microbiologic purity of the teats was determined by the method of swab before and after teat treatment, and cultivation on nutrient media. Samples were analysed for presence of mesophilic aerobic bacteria which were expressed as their total number per cm^2 (CFU/cm^2) of teat. Study results showed in both treated groups, compared to the control, significantly reduced number of bacteria. Besides, significantly lower bacteria number was determined in the third group treated with antiseptic, compared to the second group, treated only with water. Accordingly, everyday use of udder sanitation, which is commonly component of ruminants' milking hygiene, could also significantly reduce sows' teat bacterial contamination and consequently entering excessive bacteria in the piglets' alimentary system. The following researches should provide the evidence if there exists connection between udder sanitation, reduced number of bacteria as potential causes of piglets' alimentary diseases, and diseases' appearance.

Introduction

In modern pig production most significant losses occur in suckling and weaned piglets. Furthermore, 60-80% of total mortalities in the perinatal period occur during the first three days of piglets' life [1]. Alimentary diseases represent one of the significant causes of such losses. Piglet organism is very sensitive to bacteria from environment and it can be expected, depending on number and species of bacteria and piglet individual resistance, that excessive entering of bacteria through contaminated teats makes predisposition for outbreaks of alimentary diseases. This raises question in what way effectively reduce entering of bacteria in piglets' organism through contaminated teats and their harmfull effect. Sows' udder hygiene during piglets' suckling period could answer to what extent udder cleanless reduces teats' bacterial contamination and consequently entering bacteria in the piglets' alimentary system.

Material and Methods

Research was done in farrowing pen with appropriate housing conditions and included 90 sows, which were separated into three equal groups. The first group was not treated and represented control. In the second group sows' udders were treated with water and in the third group with healthy and ecologically acceptable commercial antiseptic Oxyl$^{(R)}$ with

powerful oxidising bactericidal activity. The antiseptic was used diluted 1:20 according to producers' instructions. Sows were housed in a farrowing pen for 24 days and every morning the swabs were taken from surface of one cranial teat of each sow by pattern of 1 1 cm. In the control group swabs were taken once, and in the second and third group before and after udder treatment with a napkin for one-time use immersed in water or antiseptic. The total number of aerobic mesophilic bacteria was determined in the standard way by culturing them on nutritious agar and incubating at 37 °C within 24 hours. The results were expressed as the total number of aerobic mesophilic bacteria per cm^2 (CFU/cm^2) of teat. Statistical analysis was performed using statistical software Statistica 8.0 (Statsoft Inc., 2008).

Results

Results are presented in Tab. 1. and Fig. 1.

Tab. 1: Total number of aerobic mesophilic bacteria per cm^2 (CFU/cm^2) of teat before and after treatment with water or antiseptic

Day	Control group (n=30)	Group treated with water (n=30)		Group treated with antiseptic (n=30)	
		Before treatment	After treatment	Before treatment	After treatment
1.	202,15 +- 19,81	237,58^{a} +- 20,62	112,76 +- 7,77	248,10^{a} +- 20,59	18,187 +- 3,22
2.	225,59 +- 19,20	248,68^{a} +- 20,68	117,87 +- 6,28	253,74^{a} +- 18,33	19,51 +- 4,04
3.	254,15^{a} +- 20,86	260,21^{a} +- 17,49	122,65 +- 8,07	262,51^{a} +- 15,41	20,61 +- 3,55
4.	273,40^{ab} +- 23,71	278,30^{a} +- 19,94	128,14 +- 10,26	263,09^{b} +- 14,33	21,77 +- 4,06
5.	293,56 +- 23,47	273,45^{a} +- 14,42	127,86 +- 9,73	264,91^{a} +- 1150	20,80 +- 3,16
6.	317,08 +- 22,16	269,08^{a} +- 15,35	122,15 +- 8,07	265,12^{a} +- 9,38	21,95 +- 4,10
7.	343,08 +- 22,99	272,09^{a} +- 10,94	125,36 +- 7,91	263,63^{a} +- 9,07	22,26 +- 3,85
8.	369,01 +- 27,55	269,06^{a} +- 9,58	126,21 +- 7,75	263,01^{a} +- 12,27	21,68 +- 3,22
9.	391,10 +- 26,39	272,05^{c} +- 9,71	125,60 +- 7,25	260,77^{c} +- 8,26	22,68 +- 3,68
10.	418,40 +- 26,03	272,65 +- 8,58	125,44 +- 9,42	261,06 +- 9,29	21,62 +- 3,32
11.	446,31 +- 28,77	271,80^{a} +- 10,12	125,25 +- 8,29	263,66^{a} +- 10,46	21,42 +- 3,61
12.	470,86 +- 29,25	263,80^{a} +- 6,13	123,17 +- 7,84	263,73^{a} +- 15,06	21,06 +- 4,01
13.	495,73 +- 27,30	268,77^{a} +- 9,74	125,75 +- 7,98	265,31^{a} +- 10,96	21,82 +- 4,37
14.	517,79 +- 23,85	270,90^{a} +- 10,42	127,71 +- 9,06	262,80^{a} +- 12,29	21,27 +- 4,08
15.	542,12 +- 22,99	273,29 +- 10,23	125,58 +- 7,04	262,47 +- 8,98	21,49 +- 2,81
16.	562,16 +- 25,28	278,37 +- 9,53	128,61 +- 7,16	265,21 +- 9,52	22,64 +- 4,17
17.	583,23 +- 26,41	276,41 +- 9,77	125,24 +- 7,06	259,46 +- 85,35	21,71 +- 3,97
18.	606,38 +- 25,41	269,37^{a} +- 10,48	126,30 +- 8,45	262,41^{a} +- 8,51	22,54 +- 3,49
19.	628,61 +- 24,18	268,42^{a} +- 10,09	125,69 +- 7,58	263,78^{a} +- 9,20	22,41 +- 3,45
20.	651,49 +- 23,58	271,91^{a} +- 9,57	126,11 +- 7,65	263,79^{a} +- 10,59	21,49 +- 3,16
21.	673,97 +- 27,36	274,09^{a} +- 9,31	126,96 +- 8,11	265,54^{a} +- 9,94	21,65 +- 3,00
22.	698,09 +- 29,44	270,62^{a} +- 9,30	127,03 +- 8,52	268,56^{a} +- 12,69	21,54 +- 3,80
23.	722,86 +- 26,30	275,25^{a} +- 9,00	126,39 +- 5,82	270,78^{a} +- 14,04	22,74 +- 4,00
24.	745,98 +- 29,65	274,87^{a} +- 10,12	127,20 +- 7,92	269,84^{a} +- 13,27	22,56 +- 3,97

Values are expressed as mean (x) ± SD; for average microorganism number within the same day means in a raw with no common superscript differ significantly ($P<0.01$); ^{ab}means between groups (within the same raw) statistically differ ($P<0.01$) except values marked with the same letter; ^{c}means marked with the same letter (within the same raw) statistically differ on the level of $P<0.05$

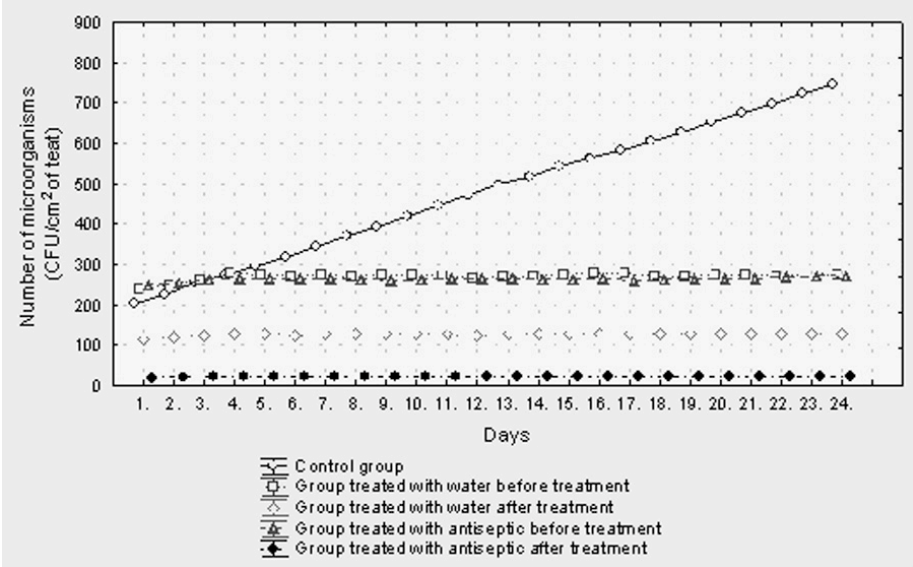


Fig. 1: Total number of aerobic mesophilic bacteria per cm^2 (CFU/cm^2) of teat before and after treatment with water or antiseptic

Discussion

Hygiene is a scientific discipline investigating how to preserve health and prevent disease [6]. In ruminants the right udder hygiene is the basic measure in preventing mastitis and reducing postsecretion contamination of fresh milk. It is performed by disinfection measures as the most significant form of medical sanitation [2,4]. In a wide sense disinfection includes procedures of removing and destroying of microorganisms. In the limited sense it includes procedure which reduces number of microorganisms below infection dose and most often is performed by chemical sanitisers [3,5]. In this research the influence of sows' udder hygiene on teats' bacterial reduction during suckling period of piglets was investigated (Fig. 1). According to the results, a significantly lower total number of aerobic mesophilic bacteria was recorded after treatment of teats with water or antiseptic ($P<0.01$). Besides, significantly lower bacteria number was determined in the group which was treated with antiseptic, compared to the group treated only with water ($P<0.01$) (Tab. 1).

Conclusion

1. Everyday use of udder sanitation, which is commonly component of ruminants' milking hygiene, could also significantly reduce sows' teat bacterial contamination and consequently entering excessive number of bacteria in the piglets' alimentary system
2. Following researches should provide the evidence if there exists connection between sows' udder sanitation, reduced number of bacteria as potential causes of piglets' alimentary diseases, and diseases' appearance.

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Disinfection use in two finishing pig systems: effects on air quality, health and productivity

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Summary

This study compared the use of disinfection in All-In/All-Out and Continuous Flow systems of pig production during the finishing phase against a negative control of continuous flow without disinfection. Disinfection significantly reduced bacteria on pen surfaces ($P<0.001$) and had an influence on aerial concentrations of ammonia, but had no effects on any other air quality parameters, pig health or productivity. Ventilation rate, pen hygiene and the pig numbers per room influenced concentrations of total dust. The pen hygiene also influenced concentrations of ammonia and airborne bacteria, the latter of which was additionally influenced by humidity and feed inputs. The AIAO room had lower concentrations of ammonia ($P<0.01$) and airborne bacteria ($P<0.05$) compared to the CF rooms. Pig health and productivity did not differ between the systems.

Introduction

Cleaning and disinfecting is regarded as one of the most important and effective methods of disease control. Performed between movements of pigs, disinfection removes, or at the very least reduces, the exposure of younger batches of pigs to the pathogens of their older predecessors [1]. Combined with all-in/all-out (AIAO) management, disinfection is able to break disease cycles and improve growth rates of batches of pigs [2]. Acute and chronic exposure of pigs to aerial pollutants is considered a contributor to the aetiology of multifactoral respiratory disease in pigs [3]. The cleaning of AIAO buildings prior to a batch entering enables the reduction of the levels of aerial contaminants [2] relative to a continuous flow (CF) system of production, which harbours a build-up of contaminants. With many pig producers working with CF buildings, the aim of this study was to investigate the effect of disinfection in a CF system on air quality, pig health and performance, relative to a full AIAO system.

Animals, Material and Methods

The research was conducted in a finisher building comprising three adjacent rooms, identical in size and layout, each containing four pens (3.07 x 3.26m) with part slatted flooring. Each room was equipped with separate ventilation systems controlled by a thermostat set at 19'C. Pigs were fed ad-libitum pelleted diet with water available from nipple drinkers over the slats.

The rooms were each designated to a system - AIAO with disinfection (AIAO), CF with disinfection (CFD) and CF with no use of disinfection (C). Over three replicates, 36 pens of 12 Large White x Landrace finishing pigs balanced for gender and weight (53.1kg), were allocated between the rooms. AIAO rooms were filled from single batches and were cleaned and disinfected between each batch of pigs. CFD,

and C rooms were formed from sequential pens of successively younger pigs. CFD room pens were cleaned individually as they cleared for slaughter at 95kg, and were refilled upon drying with another experimental group. C pens remained uncleaned. Older seeder pigs created a disease pressure in CFD and C rooms prior to first experimental groups.

Pig health and productivity measures. At two week intervals all pigs were weighed and the feed input per pen recorded. All pens were checked daily for signs of ill health and individuals displaying clinical signs were treated and recorded. Once each week all pens were scored for the prevalence of coughing, sneezing and scour. In addition, pens were assigned a pen health score based on the total number of pigs showing clinical signs that week. Pen hygiene (the percentage of the pen lying area covered in faeces) and pig cleanliness were also scored. At slaughter, the lungs of individual pigs were scored for pneumonic lesions, and paired blood and saliva samples were collected for further analysis.

Air quality measures. Total and respirable dust, ammonia and the total viable bacteria per m^3 of air were measured in each of the three rooms once each week. Temperature, humidity and ventilation rate were also recorded. Total and respirable dust samples were collected simultaneously with a flow rate of 2L/min over a 24 hour period using glass fibre filters and multidust foams loaded together in a cassette, held in an IOM sample head. Flow rate was taken at the start and end of each sampling period. Spot concentrations of aerial ammonia were measured using a portable gas monitor. Measurements of dust and ammonia were taken at a height of 1.5m within the air flow of the ventilation system. Serial dilutions were performed from total dust for measurement of bioaerosols.

Swab samples were taken from each pen before and after disinfection. Serial dilutions of the swabs up to 10^8were performed. Replicate Tryptone Soy agar plates were inoculated with the bioaerosol and the surface sample dilutions. Plates were incubated at 37'C for 24 hours, after which the average bacterial counts were calculated.

Data Analysis. A general linear model analysis was used for pig productivity and health measures, with system (room) and replicate as factors in the analysis. The mean air quality parameters over the course of the trial weeks were analysed by system with the washing activity as a covariate. All residuals were checked for normality and where necessary data were transformed. Correlation analysis was used to examine individual relationships between all the variables and multiple regression analysis was then used to determine the principal environmental factors influencing the air quality.

Results

Disinfection significantly reduced the median levels of bacteria within pens: pre vs post treatment cfu = 11.5 x $10^3/cm^2$ vs $7.8/cm^2$ ($H = 24.8$, d.f. = 1, $P<0.001$). Concentrations of ammonia and airborne bacteria were significantly higher in the CFD and C, compared to the AIAO room. All other parameters measured did not differ (Table 1).

Disinfection influenced concentrations of ammonia, but no other aerial environmental parameters. Investigation of the determinants of air quality showed a negative correlation between the ventilation rate and log of total dust ($r = -0.423$; d.f. = 33, $P < 0.05$) and log of respirable dust ($r = -0.349$; d.f.= 33, $P < 0.05$). The mean pen hygiene score showed

a positive correlation with ammonia concentration (r_s = 0.479; d.f = 63, P < 0.01) and a negative relation to airborne bacteria (r_s = -0.316; d.f.= 42, P < 0.05), with the latter also being related to humidity level (r_s = 0.607; d.f. = 42, P <0.01). Results of a stepwise multiple regression analysis are shown in Table 2.

Tab. 1: Pig performance, health and air quality parameters (mean and pooled s.e.)

Measurement	AIAO	CFD	C	Pooled SE	*P*
Average gain per day (kg)	0.71	0.72	0.72	0.02	0.874
Feed consumed/pig/day (kg)	1.97	2.05	2.12	0.04	0.063
FCR	2.81	2.90	2.94	0.09	0.628
Pneumonia (pigs/pen)	0.17	0.50	0.42	0.18	0.567
Lung score (per pen)	3.29	4.63	5.51	0.99	0.297
Scour (pigs/pen)	3.08	3.00	2.42	0.53	0.631
Total dust (mg/m²)	2.37	2.88	2.41	0.16	0.289
Respirable Dust (mg/m^3)	0.14	0.21	0.17	0.02	0.539
Ammonia (ppm)	1.84	2.93	3.11	0.23	0.006
Airborne bacteria (CFU/m^3)	1.7×10^5	3.8×10^5	4.1×10^5	0.81	0.014

Tab. 2: Factors affecting air quality parameters (regression coefficient and level of significance)

Predictor	Total dust (mg/m^3)	Respirable dust (log mg/m^3)	Airborne bacteria (log CFU/m^3)	Ammonia (ppm)
R-sq (adj)	58.0%	38.6%	50.8%	31.0%
Washing activity	NS	NS	NS	0.969*
Ventilation (m^3/hour)	-0.00075***	-0.00039***	NS	NS
No. pigs/room	0.098***	0.031**	NS	-0.034*
Pen hygiene	-0.036***	NS	-0.040***	0.056***
Feed input in 24 h (kg)	NS	NS	-0.00449*	NS
Temperature (°C)	NS	0.213*	NS	NS
Humidity (%)	NS	0.035**	0.146***	NS

Pig health measures showed there was a difference in sneezing (counts/5min) across the rooms (AIAO: 0.11, CFD: 0.35, C: 0.27; H = 6.47, d.f = 2, P< 0.05) but there were no other room differences of pig health or productivity (Table 1). Of the ailments recorded, scour was the most prevalent with 23.6% of pigs showing symptoms of scour at some time, compared to 3.01% for pneumonia. Lung scores were significantly higher across all rooms in replicate one than in replicates two and three (Replicates, 1: 7.89, 2: 3.07, 3: 2.48, s.e.m.1.00; P = 0.001). At the pen level (N=36), cough scores were positively correlated to the prevalence of clinical pneumonia symptoms in live pigs (r_s = 0.549; P = 0.001), and log of lung scores at slaughter (r = 0.355; P < 0.05). A high pen health score was negatively correlated to gain per day (r_s = -0.430; P < 0.01).

Discussion

Previous studies have reported improvements in the growth of pigs housed in AIAO disinfected buildings [2]. In this controlled study, whilst disinfection did reduce the bacterial count in pens, it did not significantly improve the health and productivity of the pigs. Surprisingly, the unwashed pens did not have a depressive effect on productivity, as has been found elsewhere [2]. This suggests that the challenge presented by an unwashed pen was

not great enough to affect production in this particular scenario.

The systems tested are believed to help prevent (AIAO) or contribute (CF) to respiratory disease spread. The figures on the percentage of pigs displaying clinical signs of pneumonia and lung scores indicate there was little effective

pneumonia challenge to pigs at this production stage in any of the rooms and the reduction in lung scores over the course of the three replicates suggests the pneumonia infection pressure reduced over time. This could be a problem of trying to create a controlled system on a small scale. However, it also coincides with a seasonal change from winter to summer, giving an increase in ambient temperature and ventilation rate, which is known to reduce pneumonia.

The high prevalence of scour that occurred may well have eliminated any potential growth differences between the rooms. The outbreaks were related to an existing *Brachyspira* pathogen within the herd, which was exacerbated by the experimental allocation and relocation procedures.

The correlations seen between the cough scores and subsequent lung lesions indicate that these can be a useful on-farm monitoring system.

Whilst it is recognised that, at high concentrations, environmental contaminants can be limiting factors for productivity and negatively affect pig health [3], this was not evident in this study. This study found contaminant levels that were comparable with other reports [3], but not in the concentrations known to cause health problems. Ventilation rate, humidity, pig numbers and level of pen hygiene primarily influenced concentrations of aerial contaminants, and this emphasises the importance of building management to alleviate poor air quality.

Conclusion

Disinfection did not significantly improve air quality, pig health or productivity in this study. Results suggest that maintaining a good quality ventilation system and correct stocking density would manage aerial contaminants better than cleaning in a CF building. Simple cough scores can be used by producers to monitor pneumonia severity.

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A targeted hygiene concept to reduce Streptococcus suis in pig herds

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Introduction

The disease complex caused by the bacterium *Streptococcus suis* can cause very high economical losses in pig production. *Streptococcus suis* belongs to the bacterium which is most founded in pigs.
They are located in the first part of the respiratory tract (nose, tonsils), in the intestinal tract on the mucose of the vagina and on the skin.
Piglets are infected during or immediately after birth. After that the bacterium colonizes their bodies.
The second important way for infections are wounds. They are often a result of injections, castration, cutting the tail, grinding the teeth, fights between piglets and so on.
If there is a situation or environment which induces stress for the pigs (for example wrong climate, changing pens, rearing, changing food) the bacterium can ingrease enormously. The result could be very often a dramatically disease with very high losses.
The first question is how to combat *Streptococcus suis*?
Mostly treatment is done by using antibiotics both as prophylaxis and treatment (during the symptoms). The disadvantages of this approach are: high costs, resistance problems and often disappointed results.
It is nearly impossible to eliminate the agent completely in the herd. Therefore it is really important to reduce the level of infections and stress to a minimum level immediately after birth and during the whole suckling and rearing period. Thats why a hygienic concept to reduce this problem has been developed and implemented in several herds, which have problems with *Streptococcus suis*.

Material and Methods

The concept has 3 pillars:

1.) Cleaning and disinfection of the farrowing pens:
For all the compartments but especially the farrowing apartments use the "all in / all out system".
Before bringing in the sows a cleaning and disinfection concept based on reducing the *Streptococcus suis* problem is developed.
This means:
+ Soak the compartment
+ 30 minutes before cleaning use the gel detergent BIOGEL. Only on this way it is possible to destroy the biofilm.
+ Disinfection with Iodine.

2.) Cleaning and disinfection of the sows closely before farrowing.
In and on the skin of the sows there are a lot of pathogenic germs, for example *Streptococcus suis* and *Clostridia*. The main target is to destroy these germs. Thats why it is necessary to wash and disinfect the sow before entering the farrowing compartment.
For a quick and efficient cleaning the sow has to be washed with an animal shampoo (Kenopro) and afterwards washed up. In the following the sows has to be sprayed with a concentrated spray based on Iodine. This product boosters

and stabilises the health of the skin.
Once per day in the week of farrowing the udder and the wounds could be sprayed with Kenodin from three days before until two days after farrowing. Thereby the wounds on the udder disappears and the quality of the udder increases. Higher milk production, better quality of the reared pigs and less problem are the results.

3.) A management system that increases the health and immunity of the piglets such as:
+ Maximum of colostrums intake
+ Prevention and reduction of wounds on the piglets
+ Prevention of any stress for the piglets

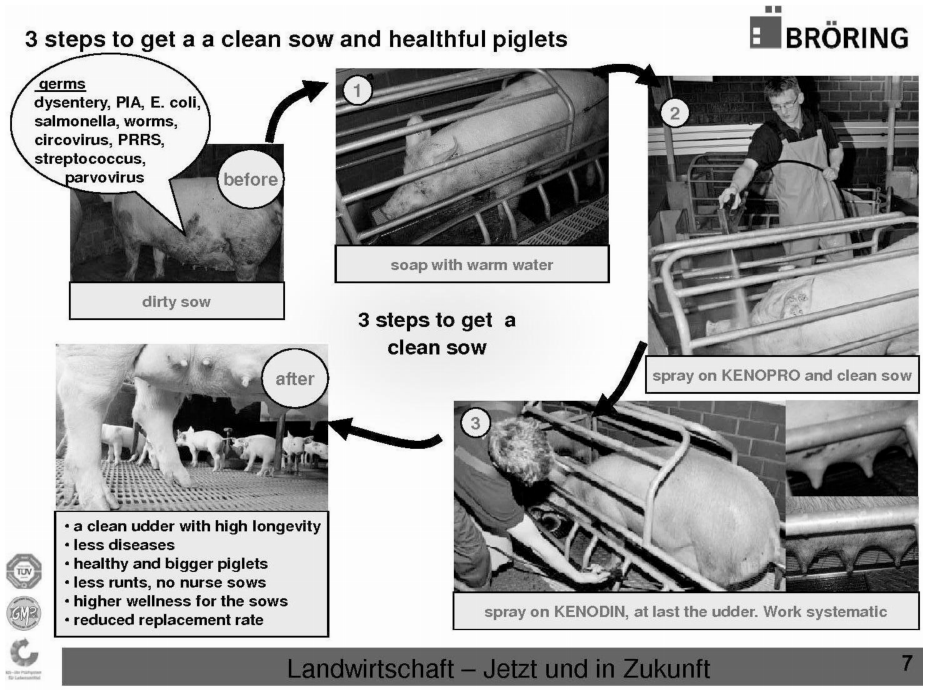


Fig. 1: Farmer 1 is cleaning a sow in the farrowing pen

Results

Farmer 1 (fig. 1). At first there was a higher weight of the piglets at weaning day (+0.6kg per piglet). Furthermore there was a better quality of the udder of the sow (less wounds), which induced a higher milk production. So it was not necessary to use nurse sows, caused by a higher milk production of the sows. This reduced the losses of piglets during suckling period (minus 3%). In general there were less problems with *Streptococcus suis*.

Farmer 2 (fig. 2). The treatment reduced the losses of piglets during suckling period (minus 3%). The spezial udder treatment realized in the rearing period further advantages: the losses of piglets were reduced (minus 2%) and the costs for antibiotic treatments were lowered (minus 50%), caused by less problems with *Streptococcus suis*.

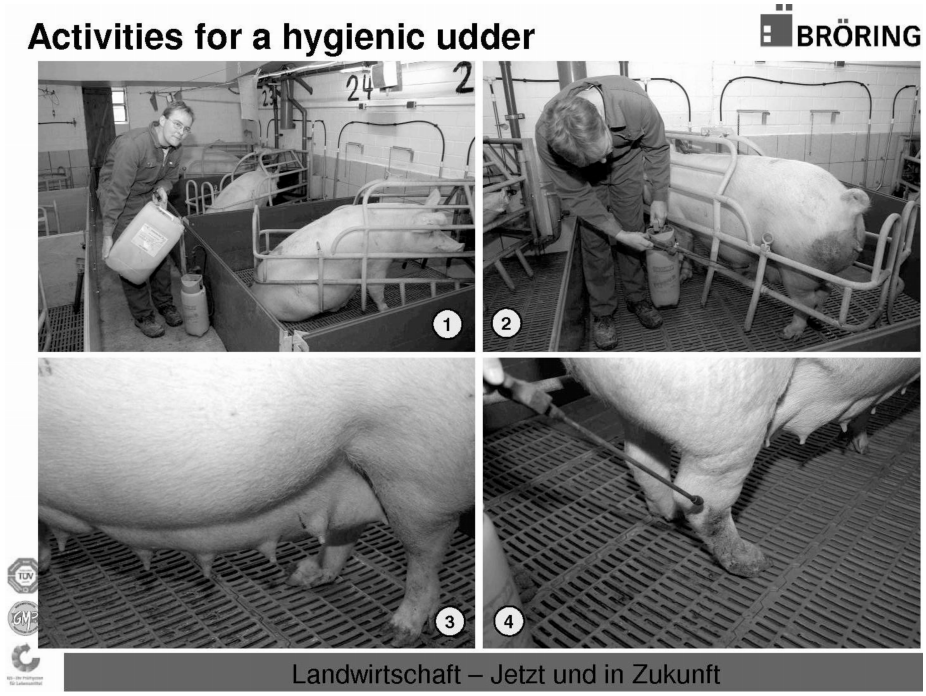


Fig. 2: Farmer 2 cleans his sows in a special shower and disinfects the sows during the first week in the farrowing pen

Conclusion

With this system we reduced enormously the problem of *Streptococcus suis*. Additionally we have seen that problems with wounds in areas like legs and udder were remarkable decrease. This means that the longevity of the sows is better and thats why she can produce more piglets. But this great advantage is only to achieve when the procedure is done consequently. Furthermore it must be accepted that it is an investment in labour but with a high profit (Much better than the profit of investments done by the bank).

References

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Chapter 31

Poster

Comparative Evaluation of the Efficacy of Disinfection by Two Microbiological Methods

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Summary

This paper is referring to the results of the comparative evaluation of the efficacy of disinfection in animal shelters using two tests approved for common use in Romania: the coliform bacteria test (CBT) and the microbiological field test (MFT). The results obtained by the two microbiological methods are pretty similar, the difference being statistically insignificant. It was noticed that after disinfection, 20.3% of the samples taken from the shelter surfaces and examined by CBT, and 22.9% of the samples examined by MFT, respectively, were positive as a result of not following the disinfection procedure correctly. MFT demonstrated a less costly and much simpler practical application, the transportation of samples to the laboratory not being necessary.

Introduction

Disinfection is one of the most important measures of prevention and control of transmissible diseases. Usually, disinfections are costly and require interruptions of the shelter use for certain periods of time. The assessment of the efficacy of decontamination is necessary in order to guarantee the reduction of the infectious pressure after each production cycle, and especially to ensure the disappearance of the pathogen agents from the animal environment, after the evolution of some highly contagious diseases, just before repopulating the shelters with disease-free animals.

To assess the efficacy of disinfection microbiologic methods are used based on evidencing the presence or absence of some test-germs on the surfaces subjected to decontamination [2]. Amongst the test bacteria having a sanitary indicator value, the coliform bacteria [2, 7, 8, 10] especially, and the enterococci [4] were recommended and used for the control of the efficacy of prophylactic disinfection; whereas for the disinfections needed as a measure of controlling transmissible diseases, staphylococci [2, 8] and atypical mycobacteria [6] were recommended. In Romania, the following procedures–also named *sanitation tests* are officially recommended for controlling the efficacy of disinfection on the surfaces from animal shelters: coliform bacteria test, staphylococci test and microbiological field test [2,8].

To control the efficacy of disinfection on surfaces in contact with food products, other microbiological methods are used, which are based either on conventional bacterial cultivation, or on image-analysis microscopy after staining with acridine-orange or with LIVE / DEAD stains. More recently, ATP measurements are being used to detect the presence of germs on surfaces [1].

This paper is referring to the results of a comparative evaluation of the efficacy of disinfection in animal shelters using the CBT and the MFT.

Material and Methods

The efficacy of disinfection processes was followed over a three year period in different cattle and swine shelters. A total of 443 samples were examined, of which 29 were taken prior to the disinfection and 414 after disinfection with various biocides, using the concentrations recommended by their manufacturers and approved by the National Veterinary Authority.

In the case of the CBT, the sampling was done using special swabs soaked in sterile physiological solution by wiping a surface of approximately 100 cm^2; later on, the tampons were placed in test tubes containing sterile saline solution, and then taken to the laboratory. There, the test tubes were shaken and 1 cm^3 of physiological solution was drawn and inoculated in another test tube containing *Kessler-Swenarton* culture medium. After 24 hours of incubation at 37'C, the test tubes with visible turbidity, color alteration and gas discharge were considered positive.

For the MFT, the sampling was performed in a similar manner. The sampling cotton swab was placed in a plastic bottle containing a specially designed, selective and concentrated culture medium, previously patented by Decun and Oprin [2]. For medium rehydration, sterile water was added, approximately 50 cm^3. The inoculated bottles were incubated at room temperature (22 – 25'C) until the next day. The samples with visible turbidity and blackened content -regardless of intensity - were considered positive, indicating the presence of hydrogen sulfide producing bacteria pertaining to the *Enterobacteriaceae* family (Fig. 1). The MFT principle is similar to that of the medium recommended by Manja et al. [9] for the detection of fecal pollution in drinking water. The obtained results were statistically analysed by the Mann Whitney test.

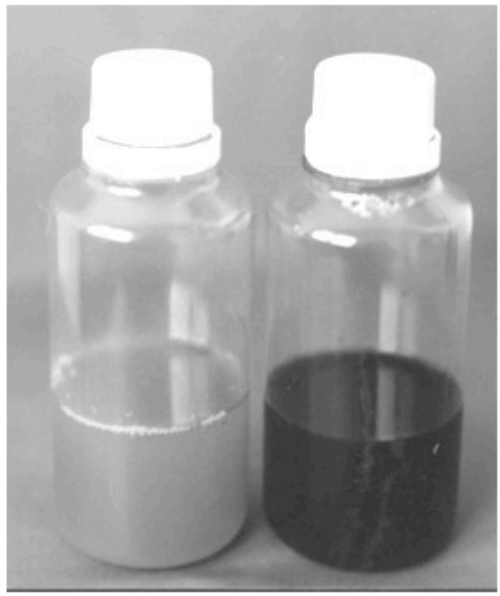


Figure 1: Microbiological field test: left - negative sample, right - positive sample

Results

After disinfection, from the 414 examined samples, 84 (20.3%) were positive when using CBT vs. 95 (22.9%) by MFT.

Discussion

The research results draw attention on the fact that mechanical cleaning and washing with water under pressure are not sufficient to eliminate the risk of pathogen agents' persistence, the surfaces requiring therefore a complementary treatment with disinfectant substances, applied by effective methods.

The relatively high ratio of positive samples

Table 1: Results of the microbiologic control of the efficiancy of disinfection of surfaces of animal shelters

Sampling place	Number of examined samples	Positive samples				Statistical significance
		CBT		MFT		
		Nr.	%	Nr.	%	
Floors and walls **before disinfection**	29	29	100	29	100	-
Floors and walls **after disinfection**	414	84	20.3	95	22.9	Insignificant (U=1,299)
Total	443	113	25.5	124	27.9	Insignificant (U=1,503)

shows the failure to correctly follow the methodology to prepare the surfaces and apply the disinfectant substances, and, in some cases, it is the consequence of the inappropriate selection of disinfectants and their concentrations in relation to the nature (smooth or rough) of the surface and to the shelter's temperature. The failure of disinfection was due especially to the poor mechanical cleaning and insufficient washing, to the low temperature in shelters during winter, and to the general, indiscriminate use of the recommended concentration of the disinfectant on surfaces regardless of their type (rough or smooth). In some cases, the explanation for the inefficiency of disinfection was found in not ensuring the needed minimal contact time (10 -15 minutes), especially for vertical surfaces, during summer, when low air humidity determined the rapid evaporation of the sprayed solutions.

Numerous situations of disinfection efficacy's failure are provided in literature, which calls for a necessary ongoing perfection of disinfection techniques, as well as for a better assessment of disinfection efficacy in animal shelters, especially in the cases where disinfection is a means to control the major transmissible diseases. Drouin et al. [5] established that the main causes of failure in disinfections towards *Salmonella enteritidis* come, at first, from a lack of motivation, a poor method of work, insufficient equipment and procedures; and secondly, from the bad design of equipment and buildings which make dismantling and accessibility difficult.

Comparing the two control methods of the efficacy of disinfection applied in our research, it resulted that both tests generated similar results. From a total of 443 samples in the CBT 25.5% were considered positive vs. 27.9% positive when using MFT. Statistically, the difference between the results obtained by the two methods is not significant (p=0.1).

Conclusion

1. Considering the great number of factors influencing the efficacy of disinfections, the high failure rate and the high costs of disinfection (by interrupting the production cycle, substance consumption and work), it is necessary to systematically assess the efficacy of decontamination, especially after disinfections performed to fight the major transmissible diseases.
2. The microbiological field test demonstrated a less costly and a much simpler practical application, the transportation of samples to the laboratory not being necessary.

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Ultraviolet disinfection with 222 nm wavelength - New options to inactivate UV-resistant pathogens

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Summary

Photo-inactivation of different micro-organisms and three enzymes were investigated under laboratory conditions. Bacteria, molds, yeast, phages and enzymes were irradiated with 222 nm (protein absorption maximum) UV-radiation emitted by a KrCl-excimer lamp or with 254 nm (DNA absorption maximum) UV-radiation produced by a low-pressure mercury lamp. The inactivation rates of the micro-organisms and relative enzyme activities were determined. The results show that a wavelength of 222 nm is more effective for inactivation of UV-resistant micro-organisms and enzymes compared to 254 nm. This indicates that a wavelength of 254 nm mainly affects radiation sensitive micro-organisms by DNA damage whereas a wavelength of 222 nm affects the radiation resistant micro-organisms at higher UV-fluences by protein damage.

Introduction

The irradiation with UV-light is an approved method for disinfection, it is still little used in life stock production. The effectiveness of killing micro-organisms varies markedly, depending on the wavelength used [7]. This is caused by varying degrees of UV-light energy absorption of different bio-molecules such as DNA, membranes or proteins due to the kind of chemical bonds between the elements of the molecules [9]. In most cases, the energy absorption results in damage of the molecules which were accumulate and thus in case of sufficiently high UV-fluence even kills the organism. Due to the absorption maximum of DNA a wavelength of 254 nm is traditionally used for disinfection and the effectiveness is reported in many publications [6 p. e.].

However, a lot of pathogens are highly resistant against UV-radiation due to a multiplicity of special DNA protecting proteins and/or different DNA repair mechanisms, in which a multitude of proteins are involved [10]. Additionally there are other highly UV-resistant pathogens such as prions, protein agents that are held responsible for the *bovine spongiforme enzephalitis* and *Scrapie* [1].

In the UV-range proteins have an absorption minimum at 254 nm wavelength but a strong maximum at 220 nm wavelength, due to the absorption of the peptide bonds and of the amino acids tryptophan and tyrosine. UV-radiation in this range can be easily generated with a KrCl-excimer lamp with a maximum emission at 222 nm. Therefore, 222 nm KrCl-excimer lamps could be used as an effective alternative to the 254 nm mercury lamps, especially for the killing of the highly UV-resistant pathogens. In the following, the effectiveness of photo-inactivation of 222 nm compared to 254 nm was investigated for different types of micro-organisms and three selected enzymes.

Material and Methods

A collimated beam device (WEDECO AG Water Technology) with interchangeable lamp units was used for the irradiations as described previously [2]. One lamp unit is equipped with four low-pressure mercury lamps with an irradiance of 20 W/m, the other one is equipped with two KrCl-excimer lamps with an irradiance of 3.6 W/m^2.
For the photo-inactivation experiments 25 mL test suspension with different micro-organisms (see figure 1) were irradiated for different periods of time in Petri dishes without intermixing and shade effects. After irradiation micro-organisms were further processed by pour-plate method on adequate culture media. The evaluation of the results was done according to the regulations of the DVGW and the ÖNORM as described previously [3]. The dose reduction factor was determined and plotted logarithmically as a function of the irradiance.
For irradiation of the proteins 100μl each of Amylase (preparation from *Aspergillus oryzae*, 31.8 units/mg protein, Sigma Aldrich GmbH), Katalase (preparation from *Aspergillus niger* (in 3.2 M $(NH_4)_2SO_4$ pH 6; 35.8 mg protein/ml; 4550 units/mg protein, Sigma-Aldrich Chemie GmbH) and Urease (preparation from *Canavalia ensiformis*, 1 unit/mg, Carl Roth GmbH & Co) were irradiated for different periods of time and enzyme assays were performed as previously described [4]

Results

Here there is a large variety in the response of the micro-organisms to irradiation with 222 nm or 254 nm wavelength. Figure 1 shows, that a wavelength of 222 nm is more effective compared to 254 nm for inactivation of especially germs with higher UV resistance as e. g. spores of *Bacillus cereus*, *Thermoactinomyces vulgaris* or mold spores of *Aspergillus niger*, as well as the very radio-resistant bacterium *Deinococcus radiodurans* or the phage MS2. In contrast, UV-sensitive bacteria can be inactivated better with 254 nm.

Figure 2 show, that enzymes can be also inactivated more efficiently by a wavelength of 222 nm. After irradiation with a wavelength of 222 nm and an UV-fluence of 1000 J/m, the relative activities of amylase and urease dropped to under 15 % and the relative activity of catalase to about 55 %. In contrast, after irradiation with 254 the relative activities for the three enzymes are still above 85 %.

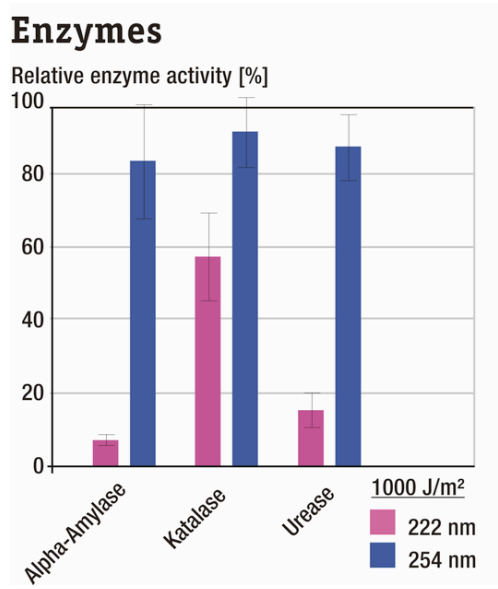


Fig 2: Photo-inactivation of amylase (22μg enzyme in 100 l aqua dest), catalase (3.5 g enzymes in 100μl aqua dest.) and urease (100 mg enzyme in 100μl aqua dest) irradiated with a UV-fluence of 1000 J/m with a 222 nm and a 254 nm.

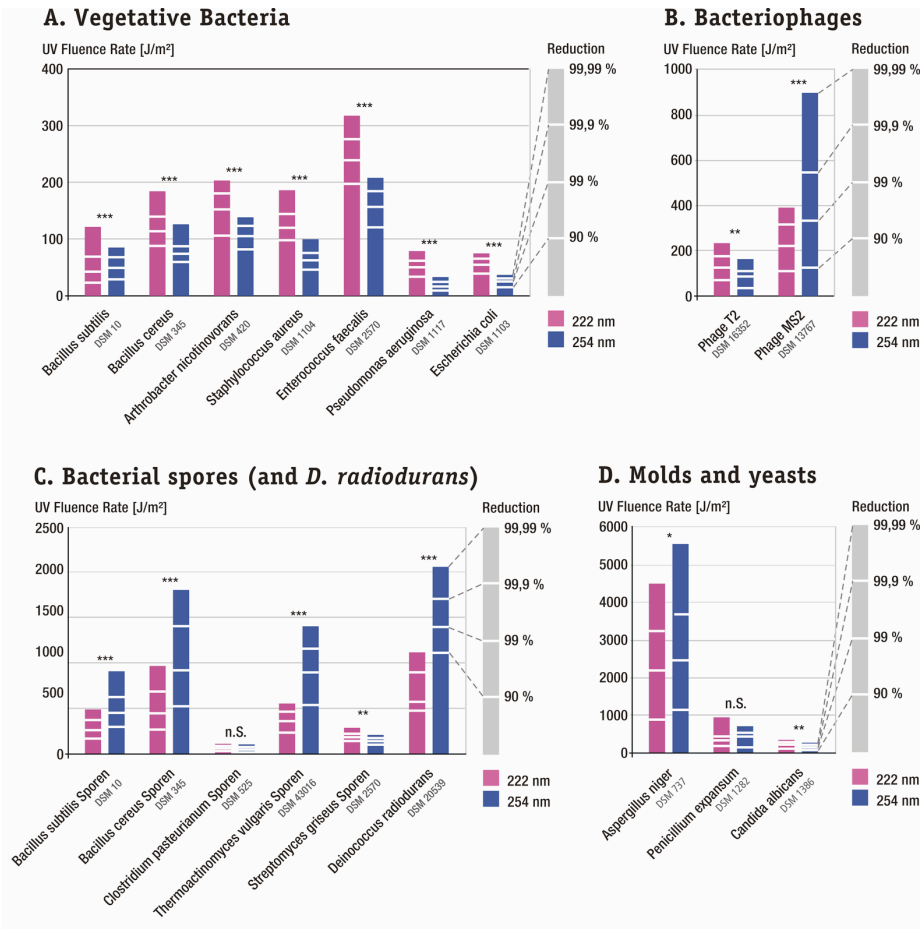


Fig 1: Photo-inactivation of different micro-organisms irradiated with 222 nm and 254 nm; n.s. $p > 0.1$; * $p < 0.1$; * * $p < 0.01$; * * * $p < 0.001$ (Students T-Test).

Discussion

The results indicate that a wavelength of 254 nm mainly affects radiation sensitive micro-organisms by DNA damage whereas a wavelength of 222 nm affects the radiation resistant micro-organisms at higher UV-fluences by protein damage. If the UV-fluence is high enough, proteins are damaged to such an extent that there is no possibility for the cell to maintain the metabolism. This finally leads to cell death, no matter how effective the repair mechanisms are. Another indication for this assumption of protein and repair mechanism damage is a lower rate of photo-reactivation performed by the enzyme DNA-photolyase after irradiation with 222 nm [2]. These results confirm earlier results that enzymes were effectively degraded by 222 nm radiation which was shown by SDS-Page [4].

Conclusion

The higher effectiveness of photo-inactivation of resistant micro-organisms with a wavelength of 222 nm due to the damage of proteins provides a new option for the inactivation of pathogens

in laboratory and the animal environment.

Acknowledgement

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Biosecurity and hygiene routines in pig farms: a descriptive study in 121 French farrow-to-finish herds

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Summary

The purpose of the study was to describe some biosecurity measures as well as common hygiene routines in a sample of 121 French farrow-to-finish pig farms. Information related to farm characteristics, biosecurity measures and hygiene routines were collected by means of a questionnaire. Two sets of variables were formed in respect to biosecurity measures as well as the cleaning and disinfection. For each set, descriptive statistics were performed. For the biosecurity data set, a multiple-correspondence analysis method was used to describe the relationships between the different variables. A cluster analysis was performed to establish groups of farm according to their practice pattern. As for the biosecurity dataset, three groups were constituted. A group of 44 farms was mainly formed by small size farms ($\leqslant$150 sows), characterised by a low frequency of pig transport trucks coming in, no rendering plant vehicles entering inside the farm perimeter, no control of flies proliferation, the absence of a room for visitors to change their clothes and a proper maintenance of the farm surroundings. A second group consists in herds of medium size with no visitor room, no foot baths, low level of fly control. The place where dead animals are stored before rendering was located at less than 50 m from the piggeries. The third group was characterized by larger herds (>250 sows), with a visitor room, foot baths, fly control, frequent pig trucks coming in but no truck from the rendering plants. The most frequent steps of the hygiene procedures applied in farrowing, post-weaning and finishing compartments comprised: soaking (90.1%, 85.1%, 90.9% respectively), washing (100%, 97.5%, 99.2%) and disinfection (96.7%, 95.9%, 95.9%). Current cleaning and disinfection routines did not significantly vary in the different sections of a farm.

Introduction

On farm biosecurity and hygiene routines are of paramount importance in disease transmission and prevention [1]. Low levels of biosecurity and hygiene routines applied in pig units are often identified as factors associated with epizootic or enzootic diseases like classical swine fever or respiratory diseases [4, 5, 7]. The term of biosecurity encompasses a broad spectrum of external and internal farms' measures aiming at protecting a herd from infectious agents (viral, bacterial, fungal or parasitic) [2]. The role of biosecurity is not only to prevent the introduction of new infectious diseases but also in combination with hygienic rules to prevent the spread of current infections of the herd. Although the importance of effective biosecurity is underlined in numerous studies to reduce disease spreading, few data on the implementation of such measures on pig farms are available [2]. Knowledge of currently biosecurity levels applied in farms might be helpful to determine to what extend appropriate measures are employed. This can in turn provide a baseline of information for veterinarians and producers to build herd health programmes in order to improve the degree of protection. Therefore, the purpose of the study was to describe some biosecurity measures as well as common hygiene routines in a sample of 121 French farrow-to-finish pig herds.

Material and Methods

The studied herds belong to a sample of 144 farms which were included in a survey designed to identify risk factors for respiratory diseases from October 2006 to February 2008. Farrow-to-finish herds located in the western part of France were selected leading to a sample size of 121 farms. Information related to the farm characteristics, the biosecurity measures and the hygiene practices were collected during a face to face interview with the farmer by means of a questionnaire or were assessed by the investigators through the one-day farm visit. All the questions, apart 5, related to the herd size, the number of pigs in the vicinity and the frequency of trucks coming in, were closed or semi-closed. The answers were coded into categorical data. Two sets of variables were formed in respect to biosecurity measures and cleaning and disinfection. For each dataset, in a first step an univariate analysis were performed (SAS 9.1, SAS Institute Inc.). Variables with sufficient variability (category frequency>20 %) were included in a multivariate analysis. This criteria was fulfilled for the biosecurity data set and a multiple-correspondence analysis method followed by a hierarchical clustering was used to describe the relationships between the different variables and to reveal clusters of pig herds with similar biosecurity practices (SPAD 5.6, DECISIA Inc.).

Results

A high proportion of herds were specialised in pig production (73.6%). When other animal productions were present on the farm, the association with dairy cattle was the most frequent one (17.4%). The mean herd size was 242 sows and for 57 % of the farms, part of the fattening pigs was reared on another site. As for the biosecurity dataset, three groups were constituted according to the hierarchical clustering from 19 out of 33 available variables. Details of the characteristics of the different clusters are presented in Tab. 1, 2 and 3. A group of 44 farms was mainly formed by small size farms ($\leqslant$150 sows), characterised by a low frequency of pig transport trucks coming in, no rendering plant vehicles entering inside the farm perimeter, no control of flies proliferation, the absence of a room for visitors to change their clothes and a proper maintenance of the farm surroundings. A second group consists of herds of medium size with no visitor room, no foot baths, low level of fly control. The place where dead animals are stored before rendering was located at less than 50 m from the piggeries. The third group was characterized by larger herds (>250 sows), with a visitor room, foot baths, fly control, frequent pig trucks coming in but no truck from the rendering plants, the latter keeping away from the facilities.

Current cleaning and disinfection routines did not significantly vary in the different sections of a farm. The most frequent steps of the hygiene procedures applied in these compartments comprised: soaking (90.1%, 85.1%, 90.9% respectively), high pressure washing (100%, 97.5%, 99.2%) and disinfection (96.7%, 95.9%, 95.9%). For 48 % of the farms a detergent is used either before or after washing and the farrowing rooms are mainly concerned. The slurry is more often removed during the cleaning programmes in farrowing and post-weaning sections than in fattening rooms (62 % *vs* 33%). The mean length of the down period is 4.7 (SD=3.8), 4.1 (SD=2.8) and 3.7 (SD=2.6) days in the farrowing, post-weaning and finishing stages, respectively.

Tab. 1 Characteristic biosecurity practices of the herds belonging to cluster 1

Characteristic variables	Category frequency (%)	
	In the cluster (44 farms)	In the sample (121 farms)
. Feed delivery frequency <= 3/month	77.3	**43.0**
. The rendering company trucks do not circulate along the buildings	97.7	**74.4**
. Herd size <= 150 sows	61.4	**33.9**
. Distance between the place where the dead animals can be picked up and the piggeries > 50 m	52.3	**30.6**
. Interval in-between successive batches = 3 weeks	81.8	**61.2**
. Lack of changing room for visitors	90.9	**72.7**
. No control of flies in gestating rooms	70.5	**49.6**
. The rendering company truck do not enter the farm perimeter	93.2	**76.0**
. No control of flies in farrowing buildings	65.9	**46.3**
. No control of flies in post-weaning stages	56.8	**38.0**
. Proper maintenance of farm surroundings	93.2	**78.5**

Tab. 2 Characteristic biosecurity practices of the herds belonging to cluster 2

Characteristic variables	Category frequency (%)	
	In the cluster (37 farms)	In the sample (121 farms)
. The farm surroundings are not kept clean	59.5	**21.5**
. Entry of the rendering company truck inside the farm perimeter	59.5	**24.0**
. The rendering company trucks run along the buildings	59.5	**25.6**
. Distance between the place where the dead animals can be picked up and the piggeries < 50 m	91.9	**69.4**
. No control of flies in fattening units	56.8	**34.7**
. Footbaths in the different sections of the farm	94.6	**77.7**
. Herd size :]150;250] sows	56.8	**36.4**
. Number of pig farms in a radius of 2 km <2	37.8	**21.5**
. No footbath at the farm entry	91.9	**76.9**

Tab. 3 Characteristic biosecurity practices of the herds belonging to cluster 3

Characteristic variables	Category frequency (%)	
	In the cluster (40 farms)	In the sample (121 farms)
. Interval in-between successive batches <= 2 weeks	50.0	**19.0**
. Herd size > 250 sows	65.0	**29.8**
. Control of flies in post-weaning section all over the year	42.5	**17.4**
. Control of flies in fattening units all over the year	47.5	**23.1**
. Changing room for visitors	52.5	**27.3**
. Frequency of vehicles delivering and collecting live pigs >4/months	82.5	**57.0**
. Proper maintenance of farm surroundings	97.5	**78.5**
. Control of flies in gestating units	72.5	**50.4**
. Footbath at the farm entry	42.5	**23.1**
. Clothes specific to each farm section	35.0	**19.0**
. Control of flies in farrowing rooms	72.5	**53.7**
. No entry of the rendering truck in the farm perimeter	90.0	**76.0**

Discussion

As the pigs constitute the main source of pathogens introduction into a herd, when dealing with biosecurity, the policies of replacement stock and quarantine management need to be considered. These factors were collected but were not taken into account in this first approach. They will be added in further analyses. However, the results of the study indicate that the importance given to biosecurity and hygiene varies among farms, the larger herds paying the highest attention. Improvements are specially needed in medium size herds to reduce the risk of disease introduction and spreading. In a study conducted in 116 Danish fattening herds, biosecurity was associated with the herd size and the health

status of the farms [3]. Ribbens *et al.*, [6] who assessed the level of biosecurity practices in Belgian pig herds, found that procedures for persons entering the herds are better applied in larger herds compared to smaller ones. In our study, the lack of control of flies is a main factor characterizing the groups. Since insects may be vectors of swine infectious agents like *Streptococcus suis* or PRRS virus special attention must be placed to insect control in the different rearing sections [1]. Dealing with decontamination procedures *per se*, current cleaning and disinfection routines did not significantly vary in the different sections of a farm. The major steps are theoretically applied in almost all farms. Boklund *et al.*, [3] found in Denmark that the majority of the interviewed farmers always washed and disinfected the rooms between successive batches of fattening pigs. Whether they are properly implemented and effective remain questionable and needs further investigations.

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Farm Animal Biosecurity, Significant Preventive Precautions in the Engagement against Zoonosis in Swine Herds

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Summary

A Biosecurity programme should describe management strategies to prevent the disease introduction to a farm, to control the spread of disease amongst animals within a farm [1]. One of the most important critical points for maintaining of good health of livestock is the hygienic programme. The aim of our work was the assessment of the main Biosecurity practices for swine herds, which can reduce the risk of Zoonosis in swine farms. The presented system of farm protection "TEKROCID" has been created in cooperation with companies Pfizer and Evans Vanodine. It is divided into 3 levels. The 1st level T1 helps farmers to choose the only recognized cleaners and disinfectants. The 2nd level T2 includes the individual hygiene programmes for separate species by identifying Critical Control Points. The 3rd level T3 is the complex Biosecurity programme with regard to each external and internal CCP in the farm [2].

Introduction

Livestock owners and the industry personnel who support their farms should be genuinely concerned with the health, well being and productivity of animals. They should know that disease outbreaks are preventable and adopt health management practices to prevent the introduction and spread of diseases in swine herds. Some breeders spend thousands of euros each year fighting disease outbreaks in addition to the costs of health care, valuable livestock and production. Due to those facts the farms turn to be unprofitable.

A suitable Biosecurity programme describes management strategies to prevent the disease introduction to a farm, control the spread of disease amongst animals within a farm. One of the most important critical points for maintaining of good health of livestock is animal-hygiene program. The prevention of the disease introduction and reduction of the huge amount of microorganisms in stable to an acceptable level have to be its objective.

Material and Methods

The aim of our work was to determine the main Biosecurity practices for swine herds, which can reduce the risk of Zoonosis in swine farms. Presented system of farm protection TEKROCID has been created in cooperation with companies Pfizer and Evans Vanodine. TEKROCID is divided into 3 levels: T1 - T2 - T3.

TEKROCID T1 - a Farm Cleaners & Disinfectants are aimed to reduce pathogens to a level which is not harmful to health ($<$1000cfu/cm^2) and exert antimicrobial activity on the internal surface for up to 8 weeks.

TEKROCID T2- Individual Hygiene Programmes are directed to the long-term reduction of infection pressure and solution of current medical condition.
TEKROCID T3 - a Farm Biosecurity Programme includes the black and white zone formation with the animal profitability guarantee, all risk HACCP analysis and personal training.
For the purpose of swine farm hygiene process are used following cleaners and disinfectants:
SHIFT - Heavy duty alkaline detergent cleaner; helps to remove organic soiling.
GPC8 - Farm surface foaming disinfectant with glutaraldehyde and QAC; patented formulation with efficacy up to 8 weeks.
VANODOX - Farm disinfectant with peracetic acid, hydrogen peroxide and QAC used for fogging, air disinfection and feed bulk tank disinfection.
BIOCID-30 - Iodophor foaming disinfectant with controlled release of iodine, which is effective up to 8 weeks; efficacy measured by colour change
ARID - drying powder for bedding is suitable as a part of prevention and protection against diarrhoea; helps in treatment of skin and foot wounds.

Results and Discussion

The 1st level TEKROCID T1 helps farmers to choose the only cleaners and disinfectants which are designed for farm use only. The farm staff must be trained how to use the T1 chemicals properly, how to use them through any high- or low-pressure washers, atomizers and how to recognize the successful application.
The farm cleaners and disinfectants chosen for programme TEKROCID T1 are:
1. Verifiably for full spectrum (bactericidal, fungicidal, sporicidal and virucidal)
2. Effective in farm conditions (see "Farm factors" in paragraph bellow)
3. Biostatic activity on surface for 8 weeks
4. Approved by DEFRA (in UK) for the purposes of the Animal Health
5. Verifiable in each point of use (visible or by test strips)
6. Non-toxic and non-hazardous in use
7. Non-abrasive and non-corrosive (protection of facilities and materials)
8. Fully biodegradable, stable and safe during storage
9. Easy to handle and controllable through the all cleaning process
10. Cost effective in use (per square meter)

Factors affecting the choice of disinfectant in farms. To be able to choose a suitable product it is necessary to know how the stable environment differs from others. All the chemicals should be approved for disinfection of livestock housing, fogging, water disinfection, equipment decontamination, foot and vehicle wheel dips/showers. The environment in animal houses is different from household in 5 points:
1) Water quality reduces the efficiency of disinfectant very noticeably due to the microbial contamination and water hardness.
2) Type of pathogenic microorganisms. A proper disinfection must kill the entire spectrum. Some chemicals are not efficient e.g. against uncovered viruses or against bacterial or fungal spores.
3) Surface quality is one of the most important parameters affecting the disinfection efficiency. The surface in animal house is markedly different from "the universal environment". For example, wood, concrete, plaster or bedding is porous (full of capillary cracks), where the efficient substance of the disinfectant without non-ionic surfactant does not penetrate at all due to its charge.
4) Environment temperature affects the efficiency and stability of some disinfectants. The suitable product should be verified for the farm environment and approved for efficacy at low temperatures (5–10'C). The efficient substances must be also stable for storage at higher temperatures (30 – 50'C).

5) Heavy organic soiling is a characteristic of all stables. A perfect disposal of dried excrements or bedding from all corners of the pens and drains is almost impossible. In case of continual breeds, it can be seen that the principle of prevention the airborne pathogen transmission, is extremely difficult. To achieve the hygienic standard, it is then necessary to choose such a disinfectant, which is efficient even in a thicker layer of organic soil (through foaming application).

The 2nd level TEKROCID T2, based on Biosystem 3000, includes the individual hygiene procedures for separate species / categories of swine by identifying internal Critical Control Points. Each farm is leaded by Biosecurity specialist individually for a year, till all the staff is thoroughly prepared to fulfil each step correctly. This level shows that disinfection is not only about chemicals (20%) but it is an integrated approach (80%). It divides hygiene programme into ten stages on any swine farm so that the staff is able to execute each step unambiguously, correctly and moreover easily revisable. After the disinfection risk analysis is created a regular CCP system and the staff is trained. It leads to reduction of the infectious pressure for long period [3].

Important risks solved through CCP hygiene protocol T2.

- High pressure washing >120 bar
- Disinfection on wet surface and insufficient coverage
- Cleaning tools / equipment
- Application of inefficient universal disinfection
- Non-observance of dilution rate
- Biofilm in water supply devices, fungal contamination of feed containers
- Incorrect dosage per square meter
- Microbiological evaluation
- Hazardous behaviour of farm personnel

Stages of TEKROCID T2 protocol for swine. I. Removal of Gross Soiling - emptying feeders, drinkers; brushing and scraping litter and equipment; removal of removable equipment; dry cleaning; ways of removing; quality control.

II. Pre-soaking of Stable Surface and Non-removable Equipment - shift application – all surfaces (floor, walls, ceiling, equipment) must be covered by white foam for 30 minutes; quality control.

III. Cleaning and Disinfection of Removable Equipment in a separate area; cleaning by Shift; drying; disinfection by GPC8; storage, quality control.

IV. Low-Pressure Washing of Stable – rinsing all the surfaces inside and outside the stable by low-pressure washer 50-100 bars; quality control.

V. Drying - all the surfaces must be allowed to dry for 12-48H; all the preparations shall be done; quality control using microbiological swabs.

VI. Cleaning & Disinfection of Water and Feed System by Biocid-30 and feeding system by Vanodox. Quality control.

VII. Preparation & Application of Disinfectant Solution GPC8 - all surfaces must be covered by visible foam for 15-42 min. Quality control.

VIII. Allow to Dry all surfaces (12-24H) & Install Desiccative Agent & Fogging - problematic parts (sucking piglet beddings) must be covered by Arid; reproduction area should be fogged by Vanodox. Quality control.

IX. Final Microbiological Assessment & Pest Control.

X. Check-in & Entry Disinfection Procedures using a solution of Biocid-30 and fill boot and wheel baths. Quality control.

The 3rd level TEKROCID T3 is the complex Biosecurity programme involves the control of micro-organism transmission into and across the farm from external and internal sources. An all risks analysis (Index Biosecurity 260_points) [4] should be developed with a specific plan to control risks at each point. The plan is implemented, and all staff is trained. All the actions are recorded and audited for 3years in 3phases). It involves the control of micro organism transmission into and across

the farm and their all sources. The aim of programme TEKROCID is to lower losses, gain more profit for the farm and produce the safety animal origin food for the consumers.

Conclusion

As to active substances there is no perfect disinfectant. Each disinfectant active has a different spectrum of kill. Some are better than others at killing but on the other hand they have harmful disadvantages (toxicity; carcinogenic). A suitable disinfectant must be efficient in stable conditions. Strict implementation of the 10-step cleaning and disinfection approach of Swine TEKROCID protocol as well as a thorough quality control will definitely compliment the farm Biosecurity and maximize production efficiency. Our part is to avoid the spread of disease and transmission of pathogens to a new herd. All the steps must be taken to protect livestock from diseases. The only standard practice keeps on good results for long time!

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The significance of the electro-oxygenated water application to the disinfection effectiveness

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Summary

The modern economic trends demand a reliable livestock production. Above all, establishment of a well-defined prevention, which also includes the reliable disinfection of the livestock objects, is of vital importance, thus it is necessary to select reliable biocides. Besides their effectiveness, one expects that they will also be ecologically acceptable and their usage non-toxic to people and animals. The idea of using the electro-oxygenated water (EOW) in livestock farming is gaining on its attractiveness owing to its ecological acceptability, its low toxicity and its mechanism of action. However, the meta-stable of EOW and the inadequate application of EOW to surfaces can cause its ineffectiveness. The market is alive with quite a few EOW producers. Steriplant N (SPN) is much more stable and consequently more effective. The company Swiss Steriplant AG has developed a special Zeljet CS* 1 mobile system in order to apply SPN to surfaces – the effectiveness of the new system of disinfection is presented in the following text.

For the experiment, SPN, as EOW, was applied to the surfaces. The effectiveness of EOW was monitored with the help of the redox potential measurements. Prior to its application, the redox potential value of the SPN was 930-950 mV. SPN was applied to surfaces by means of the Zeljet CS* 1 mobile system (Swiss Steriplant AG). To apply it to the surfaces, we used compressed air with the gas flow of 3.300 m^3/min. We applied the SPN spray to the target surface at various distances with the help of the Zeljet CS*1 mobile system in order to test its functionality. We also made a comparison between the effectiveness of the application and other ordinary modes of applying of the biocides.

Introduction

The modern economic trends demand a reliable livestock production. Above all, establishment of a well-defined prevention, which also includes the reliable disinfection of the livestock objects, is of vital importance, thus it is necessary to select reliable biocides. Besides their effectiveness, one expects that they will also be ecologically acceptable and their usage non-toxic to people and animals. The idea of using the EOW in livestock farming is gaining on its attractiveness owing to its ecological acceptability, its low toxicity and its mechanism of action [1].

The first methods of EOV winning were "two-chamber" systems, which resulted in the products acid fraction (pH about 1.0; redox 1.200 mV) and alkaline phase (pH about 12-13.0; redox -800 mV). The biocide effect has acid phase, whose weak properties are corrosiveness and instability of the end product. And the product of "four-chamber" system of EOV winning is based on winning of stable EOV (fit for use for 6 months) and with pH in neutral range (pH 7.3). EOV is meta-stable and some physical factors affect its stability. In stockbreeding production the common way of applying biocide solution to the breeding areas is by means of high-pressure device. The use of EOV in high-pressure systems seems to be unsuitable as it affects biocide properties essentially [2-9].

For the purpose of disinfection with EOV a special type of spraying nozzle for disinfection emerged in the market which we tested in practical conditions.

Material and Methods

The test was carried out in a poultry slaughterhouse. The area and equipment were cleaned and prepared for disinfection. In the test we used EOV Steriplant N of manufacturer Swiss Steriplant. To carry out cold fogging compressed air was used which created sufficient flow over spraying nozzle. Air flow over the nozzle was 3,300 m^3/min. The nozzle model 0/5 of the manufacturer Schlick Düsen was used for cold fogging in both cases.
To find out the presence of microorganisms on the surfaces smears were taken from the area of 20 cm^2. 50 smears were taken. Sampling points were placed on the parts of the equipment where slaughter carcases come in indirect contact with the equipment. Results are shown in CFU/cm^2.
Glass surfaces with test microorganisms *Staphilococcus aureus ATCC 6538* were placed parallel. The purpose of glass surfaces with the culture Staph. aureus was to find out the effect of EOV without the presence of dirtiness on the surfaces and to assess the importance of cold aerosolisation. The glass surfaces were placed in direct vicinity of sampling points from the equipment. The number of microorganisms on the glass surfaces was on average 5-7x10^6 CFU/cm*2.

Room 1. Building 1 (evisceration) has the volume of 1,100 m^3. The pressure of spraying nozzle was 3.0-3.3 bar. 12 l of undiluted biocide solution with redox value 870 mV was used for the fogging, which was sprayed in 6 minutes. We spent 10 ml/m^2 of undiluted biocide.

Room 2. Estimated volume of the room where poultry is slaughtered is 800 m^3. In cold fogging the nozzle model 0/5 was used. The pressure on the spraying nozzle was 3.0-3.3 bar. For the fogging we used 12 l of 80% biocide solution with redox value 870 mV which was sprayed in 6 minutes. We spent 15 ml/m^2 biocide with 50% concentration.

Results

Number of microorganism on the surface of slaughter equipment prior to the disinfection. Prior to the disinfection the number of microorganism on the surfaces in the building 1 was between 25-160 CFU/cm^2. And in the building 2 the found values were between 25-3.000 CFU/cm^2.

Number of microorganism on the surface of the slaughter equipment after the disinfection. After the disinfection the number of microorganisms on the surfaces in the building 1 was < 3 CFU/cm^2. The found values after disinfection in the building 2 were also mostly < 3 CFU/cm^2. Deviation is found value on the evisceration machine, where the found value after the disinfection was 1.950 CFU/cm^2.
The results of the decrease of microorganism number on the individual sampling point were also compared to test surfaces with starter culture *Staphylococcus aureus* ATCC 6538.
Test results are shown in the following graph:

Discussion

In evaluation of results on the surfaces of slaughter equipment we state relative low number of microorganism present. That indicates a good cleaning routine of the equipment in the slaughterhouse plant. However, this was

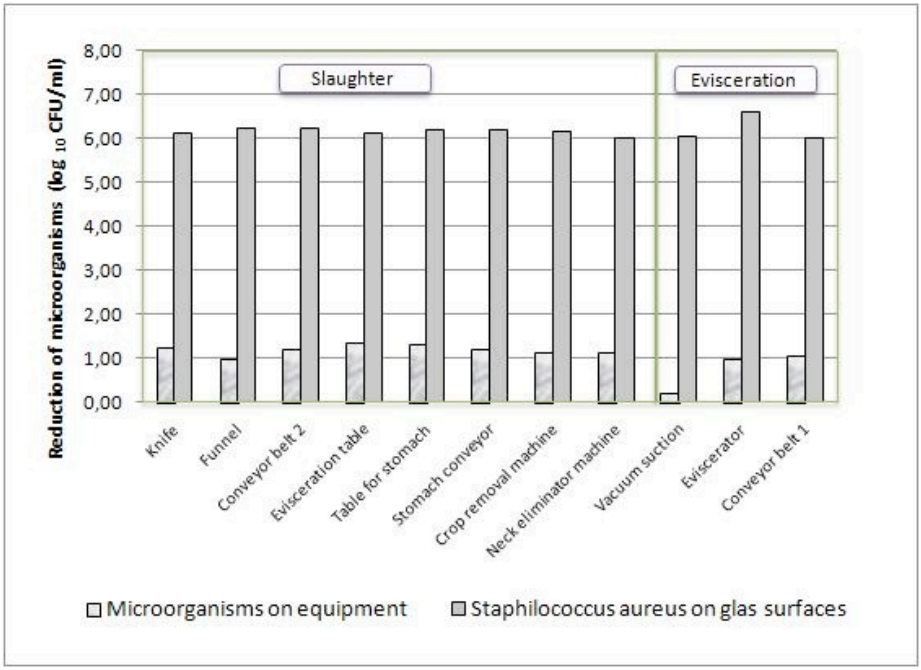


Fig. 1: Reduction of microorganisms on equipment and glass surfaces

the factor that made the evaluation of application (STN) with cold aerosolisation difficult. If we check the guidelines of the standards for biocide testing, where there is requirement for minimal reduction of microorganism on the surface 3 log10 CFU/cm^2, that was in fact impossible to achieve in our conditions, since the highest reduction we could achieve was by 90,0% or by 1log10 CFU/cm^2. However, the placed glass surfaces with test microorganisms Staph. aureus show the reduction 6 log10 CFU/cm^2. In the building 2 vacuum suction machine specially deviated, as in this case we achieved the reduction only by 35% or by 0.19 log10 CFU/cm^2. Possible cause of the lower reduction is in the structure of the vacuum suction machine. The smear was namely taken from the inner side of the tube-knife, where STN probably didn't penetrate in sufficient quantity. Because we found the highest CFU/cm^2 value on this spot too, we believe this part was also cleaned worse. Most likely the present uncleanness provided protection for microorganism and thus prevented the effect of STN.

Conclusion

In evaluation of effect of STN with cold fogging we can conclude as follows:
1. The use of STN with tested cold fogging shows the applicability in practical conditions.
2. Results of practical testing on glass surfaces show sufficient reduction of CFU, which demonstrates the reliability of the use of STN with the method of application of cold fogging.
3. For the disinfection of surfaces small quantities of STN are required.
4. It is necessary to evaluate the effect of STN in the presence of dirtiness more in detail, which is subject of further investigation.
5. It is necessary to study the effectiveness of cold aerosolisation of STN on the slaughter equipment more closely and to study the reliability and the possibility of other ways of applying biocide solution.

Acknowledgement

We would like to thank the Obisan Institution and the firm Schlick Düsen for technical support in realization of the test.

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Synergistic and Antagonistic Activity of Essential Oils – *Malaleuca alternifolia*, *Cymbopogon citrarus*, *Mentha arvensis*

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Summary

In vitro studies have demonstrated antibacterial activity of essential oils *Malaleuca alternifolia* and *Cymbopogon citrarus* (MaCc), *Cymbopogon citrarus* and *Mentha arvensis* (CcMea), against 2 Gram + *S. aureus* DSM-No.799, *E. faecium* DSM-No. 2918), 3 Gram – (*Ps. aeruginosa* DSM-No. 939, *E. coli, P. mirabilis* DSM-No.788) species and the yeast *C. albicans* DSM-No.1386 using the broth dilution method.

C. albicans, P. mirabilis, S. aureus and *E.coli* are sensitive for MaCc 0.05% high antibacterial activity was revealed for CcMea with bactericidal concentrations of 0.5%. Concentrations of 5% of MaCc have bactericidal activity and reduced total bacterial counts of*Ps. aeruginosa* MaCc 8% inactivated*E. faecium*. CcMea obtain 8% concentrations for *Ps. aeruginosa* and 10% for *E. faecium*.

Introduction

Essential oils are a potentially useful source of molecules with many biological activities. Essential oils or their components have been shown to exhibit antibacterial properties, antiviral, antimycotic, antitoxigenic, antiparasitic and insecticidal properties [1,2,3,5,8,10]. Numerous scientific reports have highlighted an important antimicrobial activity of essential oils [4,9]. Studies have also shown that certain components have lethal effects on microorganisms [2,6,7,11].

Various industries are now searching for sources of alternative, more natural and environmentally friendly antimicrobials and antibiotics as protection agents [3].

Material and Methods

Malaleuca alternifolia and *Cymbopogon citrarus* (MaCc), *Cymbopogon citrarus* and *Mentha arvensis* (CcMea)(Germany) were tested on 2 Gram + *S. aureus* DSM-No.799, *E. faecium* DSM-No. 2918, 3 Gram – *Ps. aeruginosa* DSM-No. 939, *E. coli, P. mirabilis* DSM-No.788 species and the yeast *C. albicans* DSM-No.1386 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) - using the broth dilution method. Standard bacterial broth cultures were mixed with different concentrations of essential oils in steps from 0.005 to 50% in order to evaluate the synergistic effect combined two-oils against Gram + and Gram – micro-organisms.

All tests were performed in nutrient broth Peptonwasser (Oxoid, LTD, Basingstoke, Hampshire, England). Micro-organism suspension 50 μl and 50 μl combined two-essential oils different concentration range 0.005-50%, was added to individual wells and incubated at 37 °C for 24h. Then100 μl micro-organism - Peptonwasser- essential oil suspension dissolved in tubes with 0,02% Tween 80 (Sigma).

100 μl aliquots suspension were plated onto agar plates: *St. aureus* DSM-No.799, *E. faecium*DSM-No.2918, *E. coli*, *P. mirabilis* DSM-No.788 were cultivated on Blutagar-Basis N.2 (Oxoid, England) 37 °C for 24h, *Ps. aeruginosa* DSM-No.939 were cultivated on Blutagar-Basis N.2 30 °C for 24h, *C. albicans* DSM-No.1386 were cultivated on Malzextrakt-Agar (Oxoid, England) at 25 ‘C for 48h. *C. albicans* were cultivated on Malzextrakt-Agar at 25‘C for 48h. Bacteria were cultivated on Blutagar-Basis Nr.2 37 °C for 24h.

Results

The concentration 0.005% of *Malaleuca alternifolia* combined with*Cymbopogon citrarus* (MaCc) were remarkably decreased *St. aureus* (84.3 ± 15.2)(p<0.001)*P. mirabilis* (88.2 ± 3.5) (p<0.001) *C. albicans* (74.02 ± 21.81)(p<0.002)*E. coli* (89.4 ± 8.5)(p<0.001) and MBC for this four bacteria was 0.05%.
The inhibitory effects of *Malaleuca alternifolia* combined with*Cymbopogon citrarus* were different on*P. aeruginosa* and *E. faecium*. MBC for *P. aeruginosa* used MaCc at a 5.0%, *E. faecium* 8.0%.The concentration MaCc 0.5% inhibit *P. aeruginosa* (30.6 ± 10.6)(p<0.003)and *E. faecium* (0.72 ± 0.33)(p<0.008).
The*Cymbopogon citrarus* and *Mentha arvensis* (CcMea) for >*St. aureus, P. mirabilis, C. albicans* and *E.coli* presented the same minimal bactericidal concentration (MBC = 0.5%). The concentration 0.005% CcMea inhibit *St. aureus* (90.2 ± 8.6) (p<0.0001),*P. mirabilis* (92.4 ± 4.09) (p<0.0001),*C. albicans* (76.5 ± 19.4) (p<0.001), *St. aureus* (90.16 ± 8.5) (p<0.001),*E. c*oli (97.06 ± 2.1) (p<0,001).The low sensitive species are *Ps. aeruginosa* and *E. faecium*. For*Ps. aeruginosa* MBC value was 8.0%. *E. faecium* has the highest MBC value 10%. CcMea 0.5%presented inhibition for*Ps. aeruginosa* (34.6 ± 8.8)(p<0,001) and*E. faecium* (0.82 ± 0.74)(p>0.07).

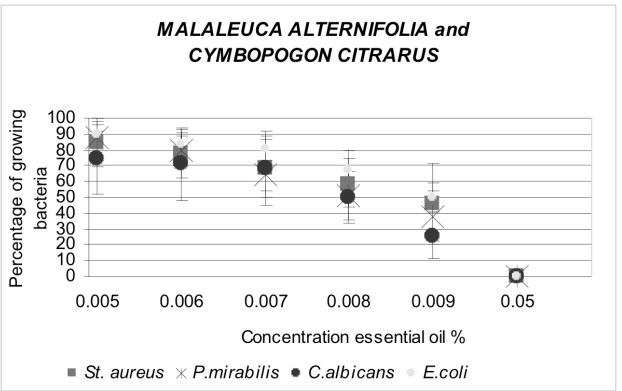


Fig. 1: Effect of *Malaleuca alternifolia* and *Cymbopogon citrarus* on *St.aureus, P. mirabilis, C. albicans* and *E. coli*

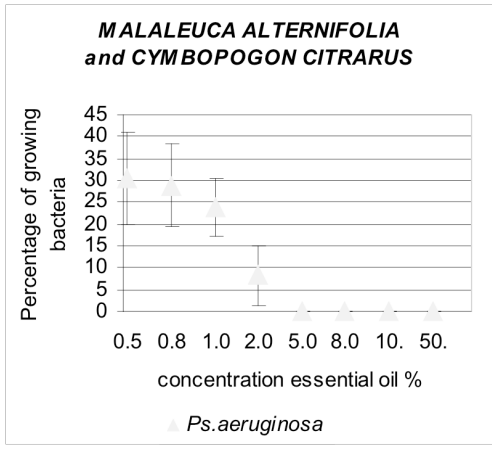


Fig. 2: Effect of *Malaleuca alternifolia* and *Cymbopogon citrarus* on *Ps. aeruginosa*

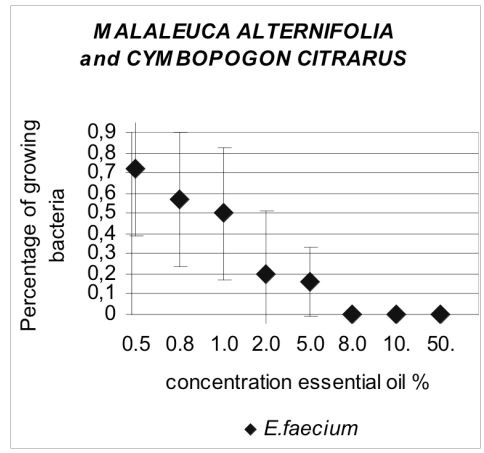


Fig. 3: Effect of *Malaleuca alternifolia* and *Cymbopogon citrarus* on *E. faecium*

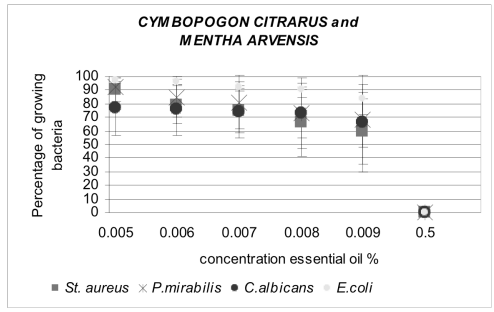


Fig. 4: Effect of *Cymbopogon citrarus* and *Mentha arvensis* on *St.aureus, P. mirabilis, C. albicans* and *E. coli*

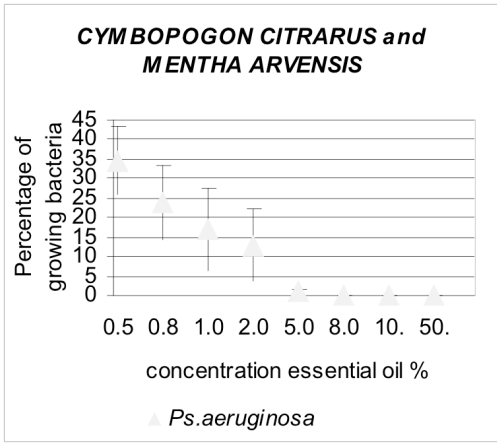


Fig. 5: Effect of *Cymbopogon citrarus* and *Mentha arvensis* on *Ps. aeruginosa*

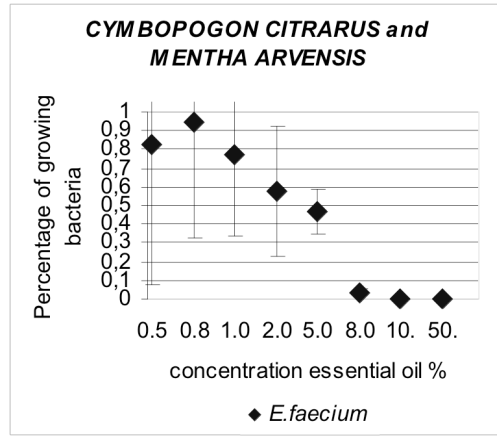


Fig. 6: Effect of *Cymbopogon citrarus*and *Mentha arvensis* on *E.faecium*

Conclusion

This study demonstrates that the essential oils cobinations - *Malaleuca alternifolia* and *Cymbopogon citrarus*, *Cymbopogon citrarus* and *Mentha arvensis*- definitely show antimicrobial effects on airborne bacteria and yeast. That combinations essential oils is greater than when individually applied. It seems that combinations essential oils may be a promising alternative to air disinfection.

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Sensitivity of *Staphylococcus aureus* isolates against biocides substances in udder-dip agents

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Summary

Quarter milk samples were collected from two groups (dipped and control group) of dairy cows with subclinical mastitis. Seventy strains of *Staphylococcus aureus* were isolated and tested in vitro to determine the efficacy of two types of teat disinfectants (Ujosan$^{(R)}$-dip; Nonoxinol-(9)-Iodine-Complex) and Eimue Chlorhexidine-dip$^{(R)}$ on *Staphylococcus aureus* isolates. The minimum inhibitory concentrations for all *staphylococcus aureus* strains were determined using broth macrodilution method which is indicative of the right line for examination of chemical disinfectants in Deutschen Veterinaermedizinschen Gesellschaft orientiert (DVG). All strains of *Staphylococcus aureus* were inoculated in a liquid medium serially diluted with Ujosan-dip and Eimue Chlorhexidine-dip. This study clarified that there was no significant differences between dipped and control group. Lack of positive results in this study could be explained by the fact that many of *Staphylococcus aureus* strains have already been established in the udder before using teat dips. It is not expected that teat dipping would have any effect on already established infections.

Introduction

In Europe, the problem of mastitis is a highly relevant issue not only for the economic losses to producers, but also for the hygienic production of milk and the safety of dairy products for human consumption [5]. Although several bacteria can cause this disease, *Staphylococcus aureus* is considered one of the most common etiologic agents [4]. Disinfection is one of the most important aspects of Mastitis prevention. Many disinfectants have been developed especially for the dairy industry to prevent the spread of infectious diseases [1]. Special preparations are used for teats disinfection and marketed as dip agent. In the last 25 years, teat dipping with a germicidal solution immediately after every milking has been proven to be an effective milking management practice to reduce the rate of new intramammary infections. Postmilking teat antisepsis is regarded as the single most effective mastitis control practice in lactating dairy cows and the reason for this is that teat dipping is a simple and economical way to dramatically decrease the bacterial skin populations on the teat [8]. The aim of this work includes determination the sensitivity of *Staphylococcus aureus* strains against biocides substances in udder dip agents.

Material and Methods

A total of 70 strains of *Staphylococcus aureus* were used in this study. All strains had been isolated after a half year of the beginning of teat dipping. 30 isolates were regularly dipped

with the preparation Ujosan dip (Nonoxinol-Iod-Komplex), (Fa. Kesla, Germany), Another 30 isolates were also regularly dipped with the preparation Eimue Chlorhexidin-dip, (Fa. Eimermacher, Germany) and 10 isolates without dipping in disinfectants (control group). The minimum inhibitory concentrations for all staphylococcus aureus strains were determined using broth macrodilution method which is indicative of the right line for examination of chemical disinfectants in Deutschen Veterinaermedizinschen Gesellschaft orientiert (DVG). Serial dilutions of disinfectant are made in a liquid medium which is inoculated with a standardized number of *Staphylococcus aureus* and incubated for a prescribed time. The turbidity of the actively growing broth culture is adjusted (by using Nephelometer) with sterile saline to obtain turbidity optically comparable to that of the 0.5 McFarland standards (ca 1 10^8 KbE/ml). The lowest concentration (highest dilution) of disinfectant preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). This method was repeated two times in the same manner for Ujosan-dip, Eimue Chlorhexidine-dip and control group and the results for each test were reported in tables.

Results

The minimum inhibitory concentration values of Ujosan-dip against *Staphylococcus aureus* strains isolated from subclinical cases of bovine mastitis in the dipped group were ranged from 41% to 50% and these values for the control group were ranged from 40% to 45%. The mean MIC value of Ujosan-dip for dipped and control group were 45, 41% and 42, 35% (fig. 1), respectively. While the minimum inhibitory concentration values of Eimue Chlorhexidine-dip against *Staphylococcus aureus* strains isolated from subclinical cases of bovine mastitis in the dipped group were ranged from 95% to 99% and these values for the control group were ranged from 96% to 98%. The mean MIC value of Eimue Chlorhexidine-dip for dipped and control group were 97, 08% and 96, 60% (fig. 2), respectively.

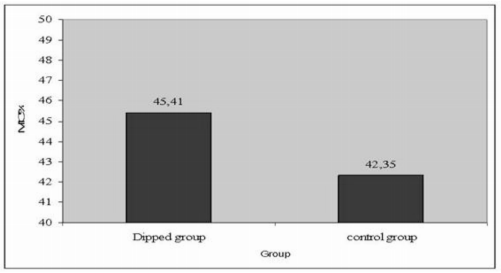


Fig. 1: The mean MIC values of Ujosan$^{(R)}$-dip against *Staphylococcus aureus* strains for both dipped and control group.

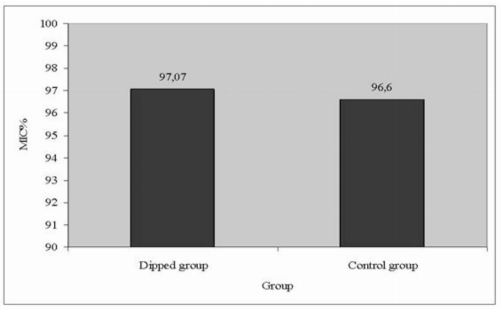


Fig. 2: The mean MIC values of Eimue$^{(R)}$Chlorhexidine-dip(TM) against *Staphylococcus aureus* strains for both dipped and control group.

Discussion

The current trial used two types of teat disinfectants Ujosan$^{(R)}$-dip (Nonoxinol-(9)-Iodine-Complexes) and Eimue Chlorhexidine-dip$^{(R)}$ and two groups of dairy cows (dipped and control groups) suffered from subclinical mastitis during a survey carried out for half year.

In the present study, there was no significant differences between dipped and control groups. Lack of positive results in this study could be explained by the fact that many of these bacteria have already been established in the udder according to [2]. It is not expected that teat dipping would have any effect on already established infections which is shown by [9], who identified higher somatic cell count in older *Staphylococcus aureus* positive cows dipped with iodine postmilking teat dip. The results of the present study are not in agreement with [5], [6] & [7].

Conclusion

In the present study, we found that there was no significant difference between the dipped and control groups for both Ujosan$^{(R)}$-dip and Eimue Chlorhexidine-dip$^{(R)}$. Lack of positive results could be explained by the fact that many of these bacteria have already been established in the udder before using teat dips.

Acknowledgement

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Importance of disinfection and validation of its effectiveness in fish processing facilities

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Summary

The study focused on hygiene and sanitation of premises for processing of fish. The premises were disinfected with preparation Topax 66, which was tested under laboratory conditions and directly in operation. In laboratory Topax 66 devitalised the test bacterial strains *E. coli* and *S. aureau* already at 0.1 % concentration and 5 min exposure time.
Under practical conditions we also observed good disinfectant effects of preparation Topax 66. Higher plate counts of *E. coli* were detected in swabs taken from surfaces of auxiliary equipment in the section of fish salads before operation.

Introduction

Food processing facilities have to comply with high requirements on cleanness and hygiene of all the equipment, premises, storage rooms and packaging materials. Failure to comply with these requirements and insufficient cleaning and disinfection results in negative influence on the final products. In order to evaluate objectively the hygiene level and sanitation in food processing facilities one must take into account the specificities of individual branches of food processing and relevant technological lines.

Material and Methods

The tested preparation Topax 66 – is aliquid alkaline disinfectant containing active chlorine and foam-producing substances intended for use in food industry in 2 % concentration. It is produced by Ecolab, s.r.o., Bratislava.
We used Standard Collection bacterial strains *E. coli* (CCH 5172); *S. aureus* (CCM 2012) and *B. cereus* (CCM 1999) as test strains.
Suspension test was used to test Topax 66 under laboratory conditions (AOAC, 1984). The disinfectant Topax 66 was diluted to prepare 100 ml aliquots of different concentrations (2.0 %, 1.0 %, 0.5 %, 0.1 %, 0.01 %). Into each of 5 test tubes we pipeted 0.1 ml of bacterial culture and added 9.9 ml of respective disinfectant dilutions. After 5, 20 and60 min we transferred a loopfull of the prepared suspension into a series of tubes containing 5 ml of nutrient broth. The tubes with broth were incubated in a thermostat at 37 C for 24 h.
Tests performed under practical conditions consisted of swabbing 100 cm^2 area with sterile swabs into sterile saline. Subsequently we carried out inoculation onto meat-peptone agar (TPC - total plate counts), Endo agar (*E. coli*) andSabouraud agar (moulds). After 24 h incubation in a thermostat at 37 C we counted colonies for TPC and *E. coli*. Moulds were

counted after 5-day incubation at 22 C. We also collected air samples using the sedimentation method and exposing plates with nutrient madia for 3 min. The number of colonies counted after respective incubation was recalculated considering the principle of sedimentation method that within 5 min exposure microorganisms from 10 L of air sediment to an area of 100 cm^2 [8].

Results

Cleaning of premises and technological lines in food production plays adecisive role in hygiene and affects directly the result of disinfection. Contamination of premises is affected decisively by residues of the processed materials that stick to floor, walls or technological equipment. In this form even the hygienically unobjectionable materials turn to waste and produce difficulties (slippery floor) or serve as nutrients for undesirable microflora in the environment. Layers of residues on surface of equipment of premises are referred to as biofilm [2, 3, 4, 7].

The investigated object was disinfected with Topax 66 warmed to the temperature of 40 C. Results of laboratory suspension testing showed that Topax 66 at 0.01 % concentration had no bactericidal effect on the test bacterial strains. However at 0.1 % concentration was effective against *B. cereus* at 20 min exposure. *E. coli* and*S. aureus* were devitalised by this concentration already at 5 min exposure (Tab.1).

Tab.1 Testing of bactericidal effectiveness of Topax 66 on selected bacterial strains by suspension test

Standard strains	Exposure (min)	Concentration (%)				
		0.01	0.1	0.5	1.0	2.0
E.coli	5	+	-	-	-	-
	20	+	-	-	-	-
	60	+	-	-	-	-
S.aureus	5	+	-	-	-	-
	20	+	-	-	-	-
	60	+	-	-	-	-
B.cereus	5	+	+	-	-	-
	20	+	-	-	-	-
	60	+	-	-	-	-

Tab.2

Swabbed place	No. of swabs	Before disinfection				After disinfection			
		CFU Ø	*E.coli* Ø	yeasts Ø	moulds Ø	CFU Ø	*E.coli* Ø	yeasts Ø	moulds Ø
Floor	8	NP	22	NP	0	0	0	2	0
Wall	8	NP	0	NP	0	4	0	2	0
Table	8	40	0	NP	0	0	0	0	0
Column	8	NP	0	NP	41	0	0	0	0

Table 2 shows results of microbiological swabs taken from surfaces in the acidic section of fish processing.

Swabs were taken from floor, wall, table and a support column. Before disinfection uncountable numbers of bacteria were present on floor, wall and column and the numbers of yeasts on all tested surfaces were also uncountable. Higher counts of moulds were detected only on the column. After disinfection the hygiene level of surfaces complied with the requirements.

Tab. 3 Mean levels of microorganisms (swabs from $10cm^2$ area) present on surfaces of auxiliary equipment in the section of fish salads after disinfection with Topax 66 and before work shift

Swabbed place	No. of swabs	After disinfection			Before work shift		
		CFU Ø	*E.coli* Ø	Moulds Ø	CFU Ø	*E.coli* Ø	Moulds Ø
Carriage 1	8	300	0	16	55	6	1
Carriage 2	8	250	1	7	0	11	0
Carriage 3	8	91	0	10	75	0	3

Tab. 4 Mean levels of aerial microorganisms in 1 m^3 before and after disinfection with Topax 66 ($CFU.m^{-3}$)

Place of sampling	No. of samples	Before disinfection			After disinfection		
		CFU Ø	*E.coli* Ø	Moulds Ø	CFU Ø	*E.coli* Ø	Moulds Ø
Acidic production	8	708	11	410	12	0	32
Fish salads	8	51	6	210	20	0	11
Brine section	8	25	11	10	10	0	8
Frozen products	8	608	10	161	4	0	6

Results of determination of aerial microflora in the section of acidic production, production of fish salads, brine and frozen products indicate good disinfection(Tab.4). Effectiveness of sanitation measures was reflected in CFU in air. After disinfection the total plate counts, coliforms, yeasts and moulds decreased significantly.

Conclusion

Cleaning preparations and disinfectants used in food production and processing should exhibit high effectiveness against microorganisms already at low concentrations, should leave no residua and put minimal load on the environment.

Testing of these disinfectants under laboratory and practical conditions is justified (some disinfectants or their incorrect concentrations do not ensure the desirable effect).

The disinfectant Topax 66 intended for use in food production devitalised the test microbial strains *E. coli* and*S. aureus* under laboratory conditions already at 0.1 % concentration and 5 min exposure time. Good disinfectant effect was noted also in practical conditions of fish processing, however, after its use we observed high counts of *E. coli* on surfaces of auxiliary equipment. Disinfection with this preparation was reflected positively on elimination of aerial contamination of processing facilities with regard to *E. coli* (negative).

The role of sanitation in protection of health of consumers and ensuring safety of food products as well as the extent and complexity of activities related to sanitation stress the importance of all measures that ensure high efficiency of all its individual stages.

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Fecal Streptococci as Indicators of Sanitation Efficiency of Meat Waste Processing

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Summary

According to the EU Directive 1774, animal by-products not intended for human consumption of category 3 may undergo biodegradation in processes of composting and anaerobic fermentation. In this study composting efficiency and fermentation in meso- and thermophilic conditions were assessed on the basis of elimination rate of D-group streptococci introduced into the waste biomass. Meat and bone carriers were used in the experiment, and a suspension of streptococci was introduced into them. The experiment made it possible to estimate the time necessary to the complete inactivation of streptococci in particular waste degradation methods. The number of bacteria decreased considerably during composting. However, they were still isolated in the last, 84th hour of the process. Daily decrease in the bacteria population ranged from 0.07 to 0.09 log. Also during mesophilic fermentation (37 °C) streptococci did not undergo inactivation and were detected on 21st day of the process. They were eliminated faster in the meat carriers. At 55 °C the total inactivation of streptococci occurred after 36 hours. Each of the processes tested resulted in a reduction of bacteria number of 7-10log, so there are not microbiological contraindications for using them as soil improvers.

Introduction

Recycling of organic waste of animal origin involves the risk of spreading pathogens. Biotechgnological methods applied for its treatment, as composting or anaerobic fermentation in thermophylic and mesophylic conditions, should guarantee that the product obtained is safe in respect of sanitation and that it can be applied for fertilization. It should not contain bacteria of the genus *Salmonella*, while the permissible number of bacilli of *Escherichia coli* or bacteria of the family *Enterococcaceae* must not exceed 5000 cells in 1g [Regulation of the Commission (WE) No208/2006]. For the monitoring of sanitizing effectiveness of different technologies, microorganisms are used, particularly thermo-resistant species such as fecal streptococci of D group (enterococci). It is assumed that if they are destroyed, also other microorganisms are most likely to be eliminated, including pathogens. The aim of the study was to compare the effectiveness of three sanitization technologies in respect of the elimination effectiveness of enterococci. Bacteria were introduced into special carriers imitating animal waste and placed in the biomass subjected to composting in the bioreactor and fermentation in thermophylic (55 °C) and mesophylic (37 °C) conditions, and then their number at different time intervals was determined.

Material and Methods

In the study, three technologies of animal waste were tested: composting in a closed bioreactor, fermentation in thermophylic and mesophylic conditions in experimental minireactors. Meat and bone carriers containing a suspension of streptococci were placed in the biomass of the reactors. The carriers were removed at regular intervals and the number of bacteria in them was determined. A drum bioreactor (composter) was used in the study, into which meat wastes of category 3 and sawdust were introduced. Bone carriers were fragments of the bone shaft and bone head of femur. After removing of bone marrow, polycarbon bags with

the bacteria suspension were placed into them. Pores of 0.01 μm in diameter in foil (Infiltec, PC-KN1CP81030) allowed microorganisms to come into contact with the composted biomass. Meat carriers were pieces of pork closed in cubic metal nets with sides of 3 and 5 cm, which were supposed to protect them from deformation during composting. The bacteria suspension was injected to them. Eighteenh culture of D-group streptococci in nutrient broth (Merck, nr 7882) with a density of 10^{8-10}cfu/ml was used in each sample. Small carriers (meat, 3 cm and bone shaft) were placed in the front, middle and back of the device. Additionally, large and small carriers were placed inside the biomass, in a metal perforated capsule. In the anaerobic bioreactors meat and bone carriers were also used, as well as ground meat [6]. Meat cubes was ground and 1 g samples were analysed. For bone samples, 1 ml of the bacteria suspension were taken to the analysis. The MPN method was used to quantitative determination of streptococci. One-gram or 1 ml samples was diluted in 0.9(NaCl. One ml suspension was added to 9 ml of the broth bouillon with glucose and azide (Merck, nr 1590). After 48 hours of incubation at 37 °C, a loopfull of culture was streaked to esculine and azide medium (Merck, nr 5222). The serological test was also made (Phadebac Strep D Test, Boule Diagnostics AB, Huddinge, Sweden). Statistical analysis was carried out using Statistica Microsoft Software.

Results

During the composting process the temperature inside the bio-reactor reached a maximum value of 62.9 °C. The temperature values at three locations were close. Streptococci number did not change for the first 36 hours in meat carrier and 48 hours in bone carrier. They were isolated from each sample after 84 hours (Tab. 1).

The theoretical time needed for the total sanitization was longer for the bone carriers than for the meat carriers (Tab. 2).

During the mesophylic fermentation streptococci were isolated in all the times analysed. However, a graduate decrease in their number was observed. The highest decrease occurred in ground meat and the lowest – in the bone carrier (Tab. 3).

The longest theoretical survival time of bacteria was recorded in the bone carrier - 46,65 days. The shortest survival time, and the fastest daily decrease in streptococci number was observed in ground meat (Tab. 4).

During thermophilic fermentation the number of streptococci decreased slowest in the small meat carrier and in the bone, and fastest in the large meat carrier. The total inactivation occurred in all types of the carriers after 36 hours of the process. The statistical analysis indicates that the theoretical survival time of streptococci and a decrease in their number was similar in the three carriers (Tab. 5,6).

Tab. 1: The number of group D-streptococci during composting process.

Type of	Sampling location	Time [h]				
		0	24	36	48	84
Meat 3cm	front	$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{8}$	$2.5x10^{3}$
	centre		$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{8}$	$2.5x10^{3}$
	back		$1.4x10^{10}$	$4.5x10^{9}$	$1.4x10^{6}$	$2.5x10^{3}$
Bone shaft	front		$1.4x10^{10}$	$4.5x10^{9}$	$1.4x10^{10}$	$2.5x10^{3}$
	centre		$1.4x10^{10}$	$1.4x10^{10}$	$1.5x10^{9}$	$2.5x10^{3}$
	back		$1.4x10^{10}$	$1.4x10^{10}$	$1.4x10^{10}$	$2.5x10^{3}$
Meat	capsule		n.e.	n.e.	n.e.	$2.5x10^{3}$
Bone	capsule		n.e.	n.e.	n.e.	$2.5x10^{3}$

n.e. – not examined

Tab. 2: Regression line equations describing the inactivation rate of streptococci during composting.

The part of bioreactor	Type of carrier	
	Meat 3cm	Bone shaft
front	y = - 0.08x + 11.65; x = 145.6	y = - 0.07x + 11.75 ; x = 167.8
centre	y = - 0.08x + 11.65; x = 145.6	y = - 0.08x + 11.76; x = 147.0
back	y = - 0.09x + 11.33; x = 125.8	y = - 0.08x + 11.86; x = 148.3

Tab. 3: The number of group D-streptococci in different type of carrier during mesophilic fermentation [MPN/g].

Type of carrier	Time [days]			
	0	7	14	21
Ground meat	1.4×10^8	9.5×10^4	2.5×10^3	0.9×10^2
Meat 3cm		2.5×10^8	2.0×10^7	4.5×10^3
Meat 5cm		4.5×10^8	9.5×10^4	1.5×10^3
Bone shaft		1.1×10^7	2.5×10^4	1.5×10^5

Tab. 4: Regression line equations describing the inactivation rate of streptococci during mesophilic fermentation.

Type of carrier	Regression equation	Total time of bacteria survival [days]	Decrease in bacteria number [log/day]
Ground meat	y=-0.29 x + 7.64	26.34	0.29
Meat 3cm	y=-0.20 x + 9.06	45.30	0.20
Meat 5cm	y=-0.27 x + 9.03	33.44	0.27
Bone shaft	y=-0.17 x + 7.93	46.65	0.17

Tab. 5: The number of group D-streptococci in different type of carrier during thermophilic fermentation [MPN/g].

Type of carrier	Time [h]				
	0	6	12	24	36
Ground meat	1.4×10^8	4.5×10^4	2.5×10^4	n.d.	n.d.
Meat 3cm		2.0×10^7	0.9×10^2	0.4×10^2	n.d.
Meat 5cm		4.5×10^3	n.d.	n.d.	n.d.
Bone shaft		1.5×10^5	n.d.	0.4×10^2	n.d.

n.d. – not detected

Tab. 6: Regression line equations describing the inactivation rate of streptococci during thermophilic fermentation.

Type of carrier	Regression equation	Total time of bacteria survival (h)	Decrease in bacteria number (log/h)
Ground meat	y=-0.22 x + 6.94	31.5	0.22
Meat 3cm	y=-0.23 x + 7.36	32.0	0.23
Meat 5cm	n.d.after 12 h	-	-
Bone shaft	y=-0.20 x + 6.04	32.0	0.20

Discussion

It has been stated that the temperature is the decisive factor of pathogen elimination during composting and thermophilic fermentation, while in the mesophilic fermentation, mainly ammonia, hydrogen sulphide or volatile acids can have bacteriocidal action. In the case of composting, the process should proceed for at least 3-4 weeks, and for 1 week the temperature in the compost biomass should reach at least 65 °C, although some researchers claim that the temperature of 55-65 °C is sufficient [5]. In the present study, the temperature of the composted biomass oscillated between 55 and 63 °C for about 10 days and it was a period sufficient to obtain a decrease of streptococci number by 7 log in all the carriers and places of the composter. The time needed for the total sanitization was longer for the bone carriers than meat carriers by several hours. This is in accordance with reports by Haug [2], who stated that the time needed for warming the inside of the bones to a temperature of 56 °C, while the surrounding temperature is 60 °C, is reported to be 40 hours. The temperature during thermophilic fermentation should remain within the range 50-55 °C, in order to effectively eliminate pathogenic microorganisms [4]. In the present study, waste sanitization occurred at 55 °C and proceeded very effectively. No bacteria were isolated from any carriers as early as at the 36th hour of the process. The

elimination rate of streptococci was not related to the size of the carrier, since they died faster in the large meat cube than in grounded meat or the small carrier. The survival time of streptococci and a decrease in their number in the three carriers was similar and amounted to about 32 hours and about 0.2 log/hour respectively. Full waste sanitization is not always obtained in mesophylic conditions [1,3]. In the present study, streptococci were still isolated in the 21st day of the process of fermentation, but at the 3-6 log lower level as compared with the control. Each of the three analysed sanitization processes led to the reduction in the number of fecal streptococci D-group to the safe level which allowed the product to be applied for fertilization.

Conclusion

During the composting process a decrease in the number of streptococci occurred in all the carriers, by 7 log; the mean survival of the bacteria in the bone carrier was longer by approximately 15 hours than in the meat carrier.
Mesophilic fermentation induced a slight decrease in number of streptococci at the order of 3-6 log/day. They survived longest in the bone carrier.
The process of thermophilic fermentation proved to be the most effective in respect of sanitization. No tested bacteria were isolated from any carriers at the 36th hour of the process.
There is no microbiological contraindication against using products of each bioprocesses as soil conditioners.

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Part XI

Wastemanagement

Chapter 32

Poster

Nutritive value of molasses distillers condensed soluble and its effect on growth performance of Lori Lamb

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Summary

This study was conducted to determine the digestibility and chemical composition of MDCS and its effect on growth performance of lori lambs. Twenty four male lori lamb (321.88 kg average body weight and 8 month of age) were used in fattening period. A completely randomized design was performed with 4 treatments (6 lambs in each group). The treatments were 0, %5, %10 and %15 of MDCS per Kg/DM of total ration. In-vivo and in-vitro digestibility of DM, OM, CP, EE, NDF and ADF of experimental diets were significantly affected by use of %15 MDCS compared to control group ($p<0.05$). Ingredients composition of experimental diets were alfalfa and barley. The average daily gain (ADG), feed conversion rate (FCR), dry matter intake (DMI) and final weight of animals were not significantly different between treatments.

Introduction

The manufacture of alcohol, yeast, citric acid, monosodium glutamate or other products by a fermentation process forms molasses distillers condensed solubles (MDCS) as a by-product [3,5]. The ethanol is produced by grain crop (corn, wheat, and barley), potato and molasses in Iran, however its production is depending on geographical location, cost and availability of these matters [2,6]. The water content, PH or the concentration of some of the mineral salts are unacceptable for administration of MDCS to animals [4,7]. Removing the water, however, is not sufficient to render MDCS very useful as an animal feed supplement because when the water content is reduced, the mineral salts tend to precipitate out of the mixture [3,8]. The precipitation has a crystalline character and forms a hard packed sediment cake that is difficult to remove and cannot be easily re-dispersed through the mixture. However removing the precipitation and related processing steps contribute to the cost of the final product [1,5]. As the recovered by product MDCS has a low pH (PH=4). It is advantageous to raise or even neutralize the pH. This pH adjustment can be achieved by adding sodium hydroxide or ammonia or any other feed grade alkali. Neutralization with ammonia is desirable, because the nitrogen can serve as a protein source in the animal feed [5,7].

Animals, Material and Methods

This experiment was conducted to determine the chemical composition of molasses distillers condensed solubles (MDCS) as dietary energy and protein supplement and its effect on digestibility and growth performance of lori lambs. Different stages of this research containing chemical analysis, digestibility and fattening period were performed

during June-November 2007. It was used 1000 liters of molasses distillers condensed solubles as a by product from alcohol fuels production. Because of high viscosity, MDCS was mixed with a determinate volume of water and added to alfalfa hay during several stages. It was used NAOH to raise and neutralize the PH of MDCS. First experiment was involved to determine the chemical composition of MDCS and digestibility of nutrients. Dry matters, crude protein, crude fiber, ether extract, and ash were analyzed by standard methods (AOAC.2000). The minerals Ca, P, Na, K, Mg, S and Cu were measured by atomic absorption spectrophotometer and gross energy was calculated by calorimetric bomb (par 2000 model). The digestibility coefficients of DM, CP, EE, NDF and ADF were determined by in-vivo and in-vitro experiments. With regard to the colour and weight of liver and kiddney of lambs and interaction effects of minerals, the effect of MDCS on animal health was investigated. In second experiment 24 male lori lamb (321.88 kg average body weight and 8 month of age) were used in fattening period at animal station of the research center of lorestan province. Lambs were randomly housed in experimentally controlled barn in individual pens with slatted floors. A completely randomized design was performed with 4 treatment (6 lambs in each group). The treatments were 0 (control), 5 (A), 10 (B) and 15 (C) percent of MDCS per Kg/DM of total ration. Ingredients composition of experimental diets were alfalfa hay and barley and the diets were balanced by use of NRC (2001) standard tables, with regard to animal requirements maintenance and daily gain(200 g/day). Experimental diets contained 3.2 Mcal Metabolizabel energy and 165 gr CP per Kg dry matter. Also the level of 5, 10 and 15 percent of MDCS replaced the barley. In this trials average daily gain (ADG), feed conversion rate (FCR), dry matter intake (DMI) and final weight of animals were determined. Data were analyzed using fit model procedure of proc ANOVA (SPSS, version 11) at the level of $P<0.05$ in completely randomized design and multiple Duncans mean test was used for comparison of treatments.

Results

The chemical composition of MSCD containing DM, CP, EE, CF, Ash, GE and ME were 63.5, 21.8, 0.5, 0, 28.5 percent and 3653 and 2576 Kcal/kg respectively. The minerals Ca, P, Na, K, Mg, S and Cu were 11.5, 5, 33.4, 65.5, 8.5, 22 and 3 g/kg (Tab. 1). The result indicated that in-vivo and in-vitro digestibility of DM, OM, CP, EE, NDF and ADF of experimental diets were significantly affected by use of %15 MDCS compared to control group ($p<0.05$). In-vivo digestibility of DM, CP, NDF and ADF of experimental diet in C treatment vs control group were 66.13 vs 57.59, 73.51 vs 66.51, 55.5 vs 49.12 and 57.8 vs 48.08 percent respectively but MDCS had no significantly effect on in-vivo and in-vitro digestibility of EE ($P<0.05$) In-vitro digestibility of CP and EE of MDCS were 96.6 and 59.5 percent. It was observed very high digestibility of DM, OM and CP of MDCS. In fattening period the dry matter intake in control group, A, B and C treatment were 1537.59, 1534.3, 1539.9 and 1538.83 g/day respectively. Also the average feed conversion rate were 9.22, 9.38, 9.31, and 9.61 in control, A, B, and C treatments respectively ($p>0.05$).

Average daily gain were 166.93, 163.58, 165.51, and 159.49 g/day in that same treatments ($p>0.05$) and final weights of animals were 47.09, 47.44, 47.01, and 46.5 kg in that treatments respectively (Tab. 2). The result indicated that final weights, DMI, ADG, and FCR were not significantly affected by use of MDCS ($P>0.05$). The liver and kidney weights of lambs were not significantly different ($p<0.05$) between treatment groups and the color of liver, kidney and meat of animals was normal.

Tab. 1: Chemical Composition of MDCS

Variable	Mean	S.D
DM, g/kg MDCS	635	5
CP	218	1/2
EE	5	0/03
CF	0	0
Ash	285	1/5
Ca	11/5	0/15
P	5	0/04
Na	33/4	0/07
K	65/5	0/22
Cu	3	0/05
GE, Kcal/Kg DM	3653	20
ME, Kcal/Kg DM	2576	25

Tab 2: Final weight, FCR and ADG of lambs

Treatments / Parameter	control	A	B	C	SE
Initial weight, kg	31/8	32/17	32/11	32/15	0.384
Final weight, kg	47/09	47/44	47/01	46/5	0.42
FCR g DMI/g ADG	9/22	9/38	9/31	9.61	0.186
ADG, g/d	166/93	163.58	165/51	159/49	0.042

Discussion

The result about effect of MDCS on animal final weight and ADG lay in the range of the study of Ham and Stock [3]. A significant effect of MDCS on nutrient digestibility (DM, CP, NDF, and ADF) of basal diet can be obtained from chemical composition and a high digestibility of MDCS. In this trial MDCS had no effect on DMI and this agree with the earlier finding of Hatch et al [4]. FCR was not significantly different between treatments in this research as like as the finding of lodge et al [6].

Conclusion

A high mineral content of MDCS can be obtained from molasses and soil pollution. Neutralization with NAOH could be reduced potassium concentration. It can be concluded that MDCS can be mixed with hay and could be safely used as a feed ingredient up to 15 percent of fattening lambs ration without any clinical signs. With regard to environmental pollution problem by discharging of MDCS into the environment, MDCS can be used as a very cheap feed ingredient in animal nutrition.

Acknowledgement

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Survey on the Ensiling of the Rumen Contents and Wheat Straw with Molasses

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Summary

In this study ensiling the rumen contents and wheat straw was performed with four levels of molasses (0-5-10 and 15 percent of DM) and three levels of durability period (30-60 and 90 days). Samples were analyzed to evaluate dry matter, crude protein, pH and ammonia nitrogen (N-NH3). Also, the most desirable method of ensiling was introduced on the basis of ammonia nitrogen contents and visual evaluation. Factorial experiment was used in a complete randomized design with four repetitions to analyze the data. The results showed that there was a significant difference between the dry matter of first treatment and the others ($p>0.05$). Also, a significant difference between crude protein of all treatments and the fourth one ($p>0.05$), showed the highest levels of crude protein in the last treatment. The more the molasses levels increased, the more pH decreased with a significant difference ($p>0.05$). There was a significant difference between the ammonia nitrogen levels in all treatments ($p>0.05$).

Introduction

As feedstuffs, especially roughages are fermented in the rumen relatively slow, there will be many unfermented and semi-fermented feedstuffs in the rumen after slaughter, which may have nutritive value. The nutritive value is the result of numerous bacteria and protozoa as well the feedstuffs in the rumen. Weight of rumen contents can be changed due to age, season, animal spices etc. [10,14,15]. According to the data gathered from slaughterhouses in Iran, the amounts of rumen contents taken from cows and sheep are respectively, 28 (14-40) and 4.5 (2-7) kilograms. Thus, ensiling the rumen contents is one of the simplest methods to use them [5,7,8,11,12].

To ensile the feedstuffs, it is necessary that they have 30-35 percents of dry matter and 6-8 percents carbohydrate, based on dry matter [3,4]. If the dry matter of a feedstuff (to ensile) is lower than above, other feedstuffs (e.g. wheat straw, cereals or hays) can be added to increase the dry matter. Also, enriching carbohydrate sources could be done by adding molasses or cereals. Although there are several methods to evaluate silages, the practical ways are visual and analytical methods. Visual evaluation can be carried out through odor, color and tissue of silages and analytical evaluation can be determined by measuring ammonia nitrogen and pH of samples [6].

Animals, Material and Methods

Based on previous studies, wheat straw and molasses was added to increase dry matter and carbohydrate sources of rumen contents [2,13,15,16]. The amount of wheat straw and

molasses were calculated based on the dry matter (Tab. 1) and added to the rumen contents.

Tab. 1: Dry matter of ingredients of mixtures

Ingredient	Dry matter (%)
Rumen contents	12.43
Wheat straw	90
Beet molasses	78

The experimental design was a complete random with a two factorial arrangement of treatments in three periods (30, 60 and 90 days) [1]. The experimental treatments were described in tab. 2. Mixtures were maintained in barrels and samplings were performed on mentioned periods. Samples were analyzed chemically in Animal Research Centre Laboratory to determine ammonia nitrogen, pH, dry matter, crude protein and visual evaluation [9,13].

Tab. 2: Ingredients of treatment

Treatments	Rumen contents (%)	Wheat straw (%)	Beet molasses (%)	Total dry mater (%)
1	80	20	0	27.94
2	79	16	5	28.12
3	78	12	10	28.3
4	78	7	15	27.7

Results

Based on SAS analysis, the results are shown in Tab. 3.

Tab. 3: Effect of different levels of molasses and durability on the quality of rumen contents silage. Significant effect 0.05 (*), non significant effect(ns)

	Molasses (%)					Duration (day)			
	0	5	10	15	Treatment effect	30	60	90	Treatment effect
DM (%)	31.51^{a}	27.7^{b}	26.63^{b}	26.47^{b}	*	28.78	27.7	27.69	ns
CP (%)	7.14^{c}	7.86^{bc}	8.59^{b}	9.59^{a}	*	8.58	8.11	8.19	ns
Silage pH	5.27^{a}	5.29^{a}	5.06^{b}	4.54^{c}	*	5.02^{b}	5.1^{b}	4.98^{a}	*
Ammonia nitrogen (ml/lit)	168.65^{a}	122.34^{b}	91.79^{c}	45.57^{d}	*	105.72	110.23	105.22	ns
Visual evaluation	10.52^{d}	11.78^{c}	14.83^{b}	15.38^{a}	*	12.8^{b}	11^{c}	15.02^{a}	*

Discussion

Dry matter. Dry matter of treatments were significantly differed regarding to various levels of molasses, but there was no significant difference among dry matters in various periods ($p>0.05$). The results showed that wheat straw could increase dry matter of treatments.

Crude protein. The results showed that CP was increased parallel to the increasing of molasses, probably due to the crude protein of molasses. There was no significant difference between treatments in various periods ($p<0.05$).

pH. Previous studies showed that the pH of ensiled feedstuffs is 4-4.5 [8]. In this study pH of treatments was decreased as level of molasses was increased.

Ammonia nitrogen. It was shown that in desirable silages, levels of ammonia nitrogen are low [2,13]. The results of this survey showed a decrease in ammonia nitrogen as molasses increased ($p>0.05$), but there is no significant difference between various periods ($p<0.05$).

Visual evaluation. Based on the average scores of visual evaluations, there was significant difference between treatments ($p>0.05$). The silage quality promotes as levels of molasses increased that confirms the results of ammonia nitrogen evaluation.

Conclusion

In this study, wheat straw and molasses were used to ensile of rumen contents. Different levels of molasses can be used for ensiling, but the best results were achieved at the level of 10%, with the least waste material. Also several feed additives or other feedstuffs are available to ensile the rumen contents, such as beet pulp, barley straw and etc.

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Dairy cattle mature manure added to corn silage and its use by cattle

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Summary

Non linear rumen parameters were estimated in dairy cattle fed with corn silage and with different concentrations of dairy cattle mature manure. Treatments were: T1 (corn), T2 (corn + 15% of mature manure) and T3 (corn + 25% of mature manure) on different periods of incubation (36, 24, 12, 8, 4 and 2 h). Variables estimated were: dry matter solubility (DMS) with the exponential equation: p=a+b(e^{-ct}), were: p=disappearance velocity of nutrients in a period of time, a=intercept of the extracted portion at the beginning of the incubation periods (hour 0), b= potentially degradable fraction in the rumen, c h^{-1}=velocity or rate of degradability of the b fraction, and t=time of incubation. We obtained the potential degradability (dp)= a+b and effective degradability (de)= a+b*c/(c+0.003) assuming a 3% rate of passage. T2 was the highest (P<0.05) on effective degradability with 79.89^{a}, followed by T3 (62.39^{ab}) and T1 (55.69^{b}) respectively. T3 showed the highest velocity on the rate of degradability (c h^{-1}) with 12.59%, it was much better than the rest of treatments (T1 =0.020% and T2 =0.013%). Mature manure addition in 15% concentration increase the DMS but it retarded its passage though the rumen, in contrast with the 25% concentration that rushed the passage amount in a smaller time and it did not affect (P>0.05) the DMS in comparison with T2. Our conclusion is that if we use 25% concentrations of dairy cows mature manure as a corn silage ingredient it can provide an increase in food intake and therefore a better animal production, besides recycling an animal waste.

Introduction

Corn silage is a high energetic product but at the same time has low protein content; on the contrary, sudan grass silage has a lower quality and therefore is less consumed by cattle [12, 8]. Both are commonly used to feed cattle in Sinaloa, Mexico, being a great percentage of the total cattle's diet. Cattle manure can be considered a problematic waste, causing damage to environment when burnt or accumulate, producing leacheates and no profits. Now its use is being investigated, although more research is needed about its manage and the many uses it can have, including composts elaboration and animal feeding [16] as is said by Smith and Wheeler [15] in relation to the increase in some animal feeding during the last decades. These and other authors [5, 6, 7] also consider that the incorporation of this by-product and molasses improves the energetic and proteic quality of grass silages. The

feeding value of a forage is defined as the capacity to promote animal production as a result of nutrients availability and its consume by the animal [4] and cattle manure can help to improve grasses feeding characteristics and its consumption. The objective of this research was to estimate the effect of molasses and mature manure addition to corn and sudan grass silage at different periods of ruminal incubation on in situ dry matter digestibility and its use by cattle

Animals, Material and Methods

This research took place at "La Posta Zootecnica" of La Facultad de Agronomia of the Universidad Autonoma de Sinaloa, in Culiacan, Sinaloa, Mexico. Six grass silages were evaluated (Table 1), elaborated according to Archilla [3]. Samples were oven dried at 60^0C for 48 h and later analyzed its chemical components such as: Crude Protein (CP), Hemicelluloses (Hemi), Cell Content (CC), Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) [10, 2]. Dry matter degradability was estimated at different periods of incubation (48, 24, 12, 8, 6, and 2 h). Two 300 kg Cebu X Holstein rumen cannulated males were used, introducing a total of 105 10X20 cm nylon bags, pore size 50+/-15 mu and exposition area of 18 mg cm^{2-1} (ANKOM), each with a 5 g of a milled sample, identified by animal and period. Bags were washed for 5 min and later to obtain the in situ Dry Matter degradability we use the Schneider and Flatt [14] formula. Non linear rumen parameters (PNLR) of DMS and NDF were estimated [11] with the exponential equation: $p=a+b(e^{-ct})$, where: p=disappearance velocity of the nutrients in a period of time, a=intercept of the extracted portion at the beginning of the incubation (hour 0), b= potentially degradable fraction in the rumen, c h^{-1}=velocity or rate of degradability of the b fraction, and t=time of incubation. We obtained the potential degradability (dp)=a+b and effective degradability (de)=a+b*c/(c+0.003) assuming a 3% rate of passage [11]. Data were analyzed with PROC NLIN of the statistical program SAS [13].

Results

Non linear rumen parameters are shown on Table 1, T2 was the highest (P<0.05) on effective degradability with 79.89^a, followed by T3 (62.39^{ab}) and T1 (55.69^b) respectively. T3 showed the highest velocity on the rate of degradability (c h^{-1}) with 12.59%, it was much better than the rest of treatments (T1 =0.020% and T2 =0.013%). T2 was the highest (P<0.05) on effective degradability with 79.89^a, followed by T3 (62.39^{ab}) and T1 (55.69^b) respectively. T3 showed the highest velocity on the rate of degradability (c h^{-1}) with 12.59%, it was much better than the rest of treatments (T1 =0.020% and T2 =0.013%).

Discussion

Mature manure addition in 15% concentration increase the DMS but it retarded its passage though the rumen, in contrast with the 25% concentration that rushed the passage amount in a smaller time and it did not affect (P>0.05) the DMS in comparison with T2. The silage nutritious value was estimated by analyzing its chemical composition [2]. The using of manure on silages mixed with easily fermentable forages increases the Crude Protein (CP) contains but also the ashes and ADF content, increasing the tampon capacity and having a negative effect over the fermentation [1, 11, 9].

Table 1: Non linear rumen degradability parameters In situ.
(DM Values on the same row with different letter are statistically different (P<0.01))

Silage	DM				
Treatment	A	B	c (h^{-1})	dp (a+b)	De
T1	53.13	38.65	0.020	91.78	55.69 [b]
T2	84.52	11.82	0.013	96.34	79.89 [a]
T3	88.97	11.03	12.59	100	62.39 [ab]

Conclusion

Our conclusion is that if we use 25% concentrations of dairy cows mature manure as a corn silage ingredient it can provide an increase in food intake and therefore a better animal production, besides recycling an animal waste and decreasing pollution.

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Comparative Zoohygiene Assessment of Two Technologies for Cattle Manure Processing

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Summary

The aim of the present work is to research the possibilities to use two products from manure processing allowed by Regulation No 22 namely: bioslime/which is a product of methane fermentation/ and compost.
In this research the criteria are the status of the raw material regarding the quantity of the main biogenic elements and their quantity in the two products: bioslime and compost.

Introduction

Organic production is the main direction of agricultural development in Bulgaria. Regulation 22/2004 of MAF allows the use of organic manure processed by means of fermentation or composting for the increase of soil fertility.
The reason for manure processing is that it is dangerous for the biocoenosis due to its content of pathogenic microorganisms, eggs of helminthes, viruses and fungi. The regulation states 2 possibilities: methane fermentation or composting. During the last few years research on the technologies for manure processing in biogas installations was increased.
Biogas is produced in a lot of places in natural conditions - in glaciers, hot thermal springs, swamps, manure pits etc. The perfect 'bioreactor' for biogas is the rumen of ruminants. Special conditions are formed for biogas production. They allow high activity of a biocoenosis of microorganisms. Each group carries out certain chemical reactions and the common name of the microorganisms producing methane in anaerobic conditions is methanogenic bacteria. The process of conversion of organic matter into methane without oxygen is called methane fermentation.
Another method for cattle manure deactivation is composting. Composting is an exothermic process of biological oxidation in which the organic substrate undergoes aerobic degradation as a result of the effect of a mixed population of microorganisms in high temperature(65^0-70^0) conditions and humidity. The undergoing processes change the physical and chemical indexes of manure and lead to the formation of a stabilized humificated end product. This product is valuable for agriculture as a source of biogenic elements and as means of soil structure improvement.

Material and Methods

Cattle manure substrate assessment according to the physicochemical criteria as means of raw material and technological assessment of the possibilities for biogas production from this substrate. According to Regulation 22/2004 manure from a farm for extensive breeding was used. The breeding of cows- brown American cow breed was carried out without litter. The buildings have a cleaning system with chain plank transporter. Samples are taken at the end of the transporter, kept in thermos at 0^oC and transported to the laboratories up to 8h. after sampling.
The cattle manure was treated with water and homogenized to obtaining a suspension with 7% dry matter content. The following analyses were carried out on the inputted in the system organic matter:

- using electronic -meter - 211/1 radelkis - Budapest.
- Determination of the total dry residue -total residue after ignition and loss at ignition - BDS 17.1.4.04-80 (58). Environment protection. Hydrosphere. Water quality indexes. Method for determination of total dry residue content, non-dissolved and dissolved substances,1981
- Ammonium nitrogen determination -visual colorimetric method of Nesler - BDS 17.1.4.10-79 (58). Environment protection. Hydrosphere. Water quality indexes. Methods for determination of ammonium, 1980.
- Determination of methane and CO_2 concentrations using electronic gas analyzer of the Draeger company.

Experimental research at sequence-batch process of methane fermentation have been carried out in a bioreactor with 2 L capacity and working volume of 1 L designed in the Institute of Microbiology in BAS. Methane fermentation was carried out at temperature $32+/-1^0$C, 7.2 at homogenization of the substrate for 1min. every hour.

The experimental setting of the composting system(aerobic processing) for cattle manure was constructed from a 7L volume plastic container closed at the top with a lid. Active aeration was used to ensure the needed aerobic conditions. For this purpose a hole was put at the bottom of the container.

4 kg cattle manure were used for loading the system. The manure was distributed in two layers from the total compost material, namely:

1st layer - straw, 5cm, covering the bottom surface of the container

2nd layer - cattle manure, 8cm, on top of the 1st layer

3rd layer - straw, 2cm, on top of the 2nd layer

4th layer - cattle manure, 7cm, on top of the starw

5th layer - straw, 5cm, it's the last layer

Quality assessment of the products from methane fermentation and composting according to physicochemical indexes, including biogenic macro- and micro-elements quantity and possibilities for the use for increasing soil fertility. The same analyses were carried out on the processed organic matter as on the input substrate. A validated intra-laboratory method RESID/02/04 and intra-laboratory methods BDS-EN 13342, BDS EN-13346 were used.

- pH, organic matter and dry matter - ILM;
-Nitrogen determination - BDS-EN 13342
-Determination of P_2K_5, K_2O, CaO, MgO - RESID/02/04
- Determination of N-NH_4, N-NO_3, P_2O_5, K_2O, S-SO_4 - ILM.

Results

Quality assessment of the product from methane fermentation(bioslime) according to biogenic macro- and micro-elements quantity and possibilities for the use for increasing soil fertility. After methane fermentation the ration methane and CO_2 is 70:30.

The biogenic macro- and micro-elements quantity data is presented in Fig. 1 to 4.

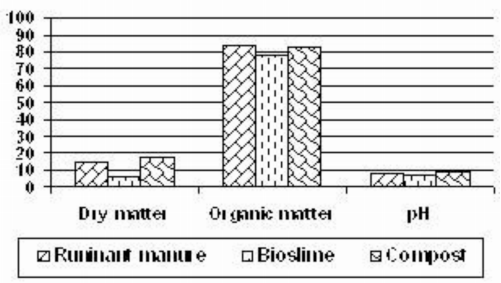


Fig.1: Dry and organic matter content

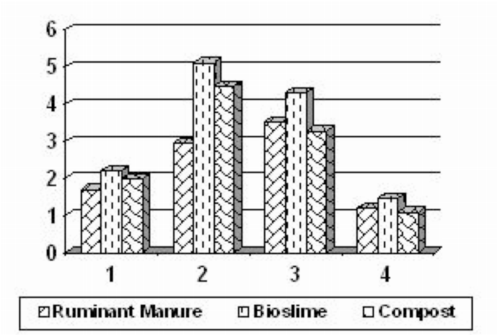


Fig.2: Content of P_2O_5(1), K<$_2$O(2), CaO(3), MgO(4)

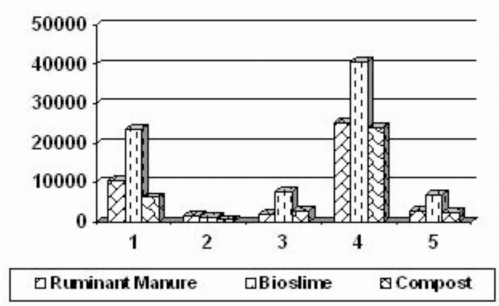


Fig.3: Water soluble fractions content: $N\text{-}NH_4$(1);$N\text{-}NO_3$;(2);P_2O_5(3);K_2O(4);$S\text{-}SO_4$(5)

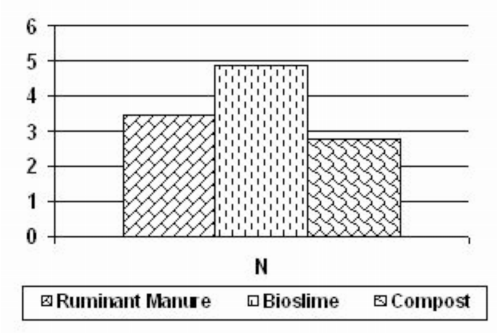


Fig.4: Nitrogen content

At a sequence batch of anaerobic digestion of cattle manure the total quantities of macroelements calculated regarding the dry matter content - N, P_2O_5, K_2O, CaO increase in different degrees in bioslime in comparison with the input material. N 41,56% - 3,44+/-0,14 % ( ) 4,87+/-0,19 % .

P_2O_5 increases with 30,76% - in bioslime 2,21+/-0,06 % and 1,69+/-0,04 % in input material .

K_2O from 2,94+/-0,74 % increases with 73,46% to 5,10+/-0,51 % in bioslime.

CaO increases with 22,85% in bioslime - from 3,50+/-0,35 % to 4,30+/-0,12 %.

MgO concentration increases with 23,52%in bioslime (from 1,19+/-0,12 % to 1,47+/-0,05 %)

The results are analogical regarding the water soluble fractions of macroelements, calculated considering dry matter content. For example $N\text{-}NH_4$ from 10414 mg/kg in input material, increases to 23456 mg/kg,(125%)in bioslime, and $N\text{-}NO_3$ decreases with 17,95%in bioslime - 1289 mg/kg in comparison with the input material - 1571mg/kg. The quantity of P_2O_5 from 2000mg/kg increases with 279,8% in bioslime - 7596mg/kg. The quantity of $_2$ from 25298mg/kg increases with 60,88% in bioslime - 40701mg/kg.The quantity of $S\text{-}SO_4$ from 2694mg/kg increases with 150,03% in bioslime - 6736mg/kg.

Quality assessment of the product from composting(compost) according to biogenic macro- and micro-elements quantity and possibilities for the use for increasing soil fertility. Composting is an aerobic process in natural or artificial aeration. It is divided in four stages: mesophilic, thermophilic, cooling and maturation. The content of compost varies in wide ranges(in % from dry matter): organic substances - 25-80 %; - 8-50 %; N - 0,4-3,5 %; - 0,1-1,6 %; - 0,4-1,6 %; - 0,7-1,5 %. All the pathogenic and sporogenic microorganisms are destroyed for a short time in composting.

The total quantities of macroelements calculated considering dry matter - N, P_2O_5, K_2O, CaO change in different degrees in compost in comparison with the input material. For example nitrogen decreases with 19,76% from 3,44+/-0,14 % (input) to 2,76+/-0,11 % in

compost. P_2O_5 increases with 17,75% in compost - from 1,69+/-0,04 % to 1,99+/-0,03 %. K_2O from 2,94+/-0,74 % increases with 52,04% to 4,47+/-0,46 % in compost. CaO decreases with 6,57% in compost from 3,50+/-0,35 % to 3,27+/-0,33 %. MgO concentration decreases with 8,40% in compost (from 1,19+/-0,12 % to 1,09+/-0,11 %)

The results are not the same for the water soluble fractions of macroelements. For example $N\text{-}NH_4$ from 10414 mg/kg decreases with 41,56% in compost - 6085 mg/kg and $N\text{-}NO_3$ decreases with 74,72% in compost - 397mg/kg in comparison with the input material- 1571mg/kg. Quantity of P_2O_5 from 2000mg/kg increases with 27,55% in compost to 2551mg/kg. Quantity of $_2$ from 25298mg/kg decreases with 5,02% in compost to 2429mg/kg.Quantity of $S\text{-}SO_4$ from 2694mg/kg decreases with 19,04% in compost to 2181mg/kg.

Discussion

Comparing bioslime and compost we notice that the total quantities of macroelements calculated considering dry matter - N, P_2O_5, K_2O, CaO are higher in bioslime than in compost.

Nitrogen in bioslime is 4,87+/-0,19 % which is 76,44% higher compared to compost, where it is 2,76+/-0,11 %. P_2O_5 in bioslime is 2,21+/-0,06 % which is 11,05% higher compared to compost, where it is 1,99+/-0,03 %. K_2O in bioslime is 5,10+/-0,51 % which is 14,09% higher compared to compost, where it is 4,47+/-0,46 %. CaO in bioslime is 4,30+/-0,12 % which is 31,49% higher compared to compost, where it is 3,27+/-0,33 %. MgO in bioslime is 1,47+/-0,05 % which is 34,86% higher compared to compost, where it is 1,09+/-0,11 %.

Conclusion

1. It is determined that the manure from breeding of cows without litter is a suitable substrate for biogas production.
2. The bioslime has higher quantity of the water soluble fractions of biogenic macroelements, which is due to the partial mineralization of the substrate.
3. Methane fermentation allows higher preservation of the basic biogenic elements in comparison with composting.

Acknowledgement

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Comprative Assessment of Three Technologies for Processing of Litter from Birds for the Increase of Soil Fertility. Agroecological assessment of bioslime

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Summary

Comparative research is carried out on three technologies for broiler litter processing in order to apply the obtained products in increasing soil fertility in organic agriculture. Sequence batch methane fermentation /25, 45 days, change of 28+ of substrate on the 46th day/ shows the highest level of mineralization of organic matter and the highest content of the 4 water soluble fractions and main biogenic elements: nitrogen, phosphorus, potassium, magnesium. In composting there is extreme loss of biogenic elements and lower level of mineralization. When stabilizing organic matter with CaO there is no loss of biogenic elements, but mineralization is missing. This makes it impossible for the plants to utilize the product when it's introduced in soil therefore a soil biocoenosis of decomposer organisms can't be formed.

Introduction

Organic production is the main direction of agricultural development in Bulgaria. Regulation 22/2004 of MAF allows the use of organic manure processed by means of fermentation or composting for the increase of soil fertility.
The regulation states 2 possibilities: methane fermentation or composting. During the last few years research on the technologies for manure processing in biogas installations was increased.
Another method for deactivation of manure is composting. Composting is an exothermic process of biological oxidation in which the organic substrate undergoes aerobic degradation as a result of the effect of a mixed population of microorganisms in high temperature(65^0–70^0) conditions and humidity.
The third technology is a modification of composting – stabilizing the organic matter of litter with CaO, which is developed through the Oxalor methodology.

Material and Methods

Research has been carried out with litter from the breeding of four-linear hybrid broilers on straw – 10 cm height; in production houses with surface of $2000m^2$. Litter is taken right after the 45th day of the production cycle and has been stored at $0\text{-}4^2$.
Experiments have been carried out under the following conditions:

1. Sequence-batch process of anaerobic degradation of liquid manure - water solution of broiler litter (7% dry matter and 6,2) in laboratory bioreactor with 6 L capacity and working volume of 4 L. Fermentation continues for 45 days at mesophilic temperature regime 25 1,0. At the 46th day 2/3 of the bioslime is removed and fresh substrate is inserted.
2. Composting. Version A - Laboratory composting installation with additional aeration which is a large scale copy of an OCENE company installation. Composting time is 30 days as temperature increases to 70^0C. Version B -. Laboratory installation for organic matter stabilization according to the Oxalor technology as litter, CaO and water are mixed together in a laboratory homogenizer. After homogenization the temperature is risen to 108 for 120-180min.

Results

The results are presented in table 1. The quantity of organic matter in litter is 90%. In bioslime it is reduced to 51,35% or the process of mineralization is 43%, whereas in composting it's 78,78% or the reduction is 13,145%. The Oxalor technology reduces the organic matter to 48,12% but this is due to the input of inorganic substrate CaO in considerable quantities – 20%.
86% increase of Nitrogen quantity is detected in litter compared to fodder. The quantity of this macroelement in bioslime is high – 206% in comparison with litter. In compost the quantity of nitrogen is slightly higher compared to litter, whereas with the Oxalor technology there is reduction to 49,3%. The phosphorus data is analogical – the quantity is higher in litter than in fodder, five times increase in bioslime and double in compost with reduction in the Oxalor technology (OT) product. One of the essential macroelements for plants is potassium which is increased two times in litter. Its quantity is 321,8% higher in bioslime compared to litter and 149.8% comared to compost.
The reason for the 51,8% higher calcium quantity is due to the structure of litter, which is a complex product consisting of straw, fodder and life products of microbial biocoenosis. The quantity of this macroelement in bioslime is 591% higher than in litter, 269,2% in compost. There is ten times higher Ca quantity in the TO product which is logical keeping in mind the processing technology of organic matter. The same regularity is noticed regarding magnesium – 313% higher quantity in litter compared to fodder; 330% and 176,5% in bioslime and compost respectively compared to litter. In the OT product the difference regarding litter is inconsiderable.
The same regularity is determined regarding the water soluble fractions. Research on the water soluble fractions of nitrogen, phosphorus, potassium and sulfur shows that their highest quantity is in bioslime compared to litter, followed by compost. Their lowest quantity is in the OT product. <beginpage/>

Discussion

Our previous research[1,2,3] shows that it is expedient to optimize the methane fermentation. We accepted this to happen through mathematical modeling using the models of Chen and Hashimoto[4] and Chen and Varel [5]. Our previous research was done on laying hen's manure and the optimal parameters of the methane fermentation were determined where 47% of organic matter degradation was achieved. In the present research we applied a new technology of continuous methane fermentation for the first time – sequence batch regime the root of which is the maintenance of a mesophilic temperature regime/25 in this experiments/ and long stay /45 days/ after that 2/3 of bioslime is removed and new substrate is inserted. The essence is to preserve the microbe biocoenosis carrying out the methane fermentation. The ratio above was deduced after 38 experiments where the mineralization

Tab.1: Basic agrochemical indexes

Indexes	Fodder	Litter	Bioslime	Compost	Oxalor Technology
Dry matter	87,70	43,80	2, 43	45,75	92, 30
Organic matter	94,23	90,00	51, 35	78,18	48,12
Nitrogen	3,50 ± 0,14	6,51 ± 0,26	13,43 ± 0,54	6,60 ± 0.26	3,21 ± 0,13
P_2O_5	1,35 ± 0,13	1,94 ± 0,12	10,6 ± 0,3	4,70 ± 0,12	1,60 ± 0,16
K_2O	1,06 ± 0,11	2,61 ± 0,19	8,40 ± 1,02	3,91 ± 0,15	2,01 ± 0,20
CaO	1,37 ± 0,14	2,08 ± 0,10	12,3 ± 0,2	5,60 ± 0,57	22,3 ± 2,2
MgO	0,258 ± 0,03	0,81 ± 0,05	2,68 ± 0,08	1, 43 ± 0,14	0,737 ± 0,074
Water Soluble Fractions					
$N\text{-}NH_4$		8854 ± 26	59341 ± 28	17993 ± 24	353 ± 9
$N\text{-}NO_3$		559 ± 11	3946 ± 31	1698 ± 11	41,7 ± 6
P_2O_5		13539 ± 21	21687 ± 24	8655 ± 19	46,6 ± 3
K_2O		21183 ± 29	74362 ± 38	32660 ± 36	14360 ± 21
$S\text{-}SO_4$		877 ± 10	3160 ± 12	881 ± 21	-

effect was taken into account. The chosen temperature regime was suitable for the climate in Bulgaria in particular the possibility to economize energy, as relatively low temperature in the bioreactor is maintained, was sought. 43% mineralization of organic matter was reached, which is the highest percent in a number of laboratory experiments for methane fermentation modeling of litter – a substrate which is distinguished by its high content of cellulose.

This substrate was processed in a biogas installation for the first time in Bulgaria and the aim was to do an agrochemical assessment of the bioslime in comparison with the aerobic method for treatment – composting. The reason for this assessment is the regulation for organic production in agriculture/ Regulation 22/ which allows the use of compost or methane fermentation products for the increase of soil fertility whereas the use of mineral fertilizers is forbidden flatly. The comparative research shows that in the closed system for biogas production the quantity of the main biogenic macroelements is higher in comparison with composting /aerobic process where loss of biogenic elements in the form of volatile compounds is determined/ - 2,03 times higher nitrogen content, 2,25 for phosphorus, 2,15 for potassium, 2,20 for calcium and 1,87 for magnesium. The results for the assessment of the water soluble fractions of nitrogen, phosphorus, potassium and sulphur are analogical. This index is very important since the water soluble fractions are the source of biogenic elements for autotrophic organisms. The agroecological assessment of OT is that it preserves the biogenic elements but the level of mineralization is low because the input of CaO and the temperature increase are the reason for the termination of mineralization. That leads to insertion of stabilized organic matter which will degradate gradually in soil. This means that it is inaccessible as a nutrition resource for plants from the beginning.

Conclusion

The new continuous methane fermentation technology – sequence batch regime - of broiler production litter, allows high level of mineralization of organic matter as the researched biogenic elements are preserved – nitrogen, phosphorus, potassium, calcium and magnesium. In composting/the other recommended technology for organic production/ the level of mineralization is lower and the quantity of the biogenic elements is 2 to 6 times lower. The highest quantity of the water soluble fractions of these biogenic elements is in bioslime, too. This gives reason to appoint methane fermentation as the most efficient method for increasing soil fertility in organic agriculture.

Acknowledgement

The research is entirely financed by the Ministry of Education and Science, Scientific Research Fund – contract D-01-376/16.06.2006 "Research on the Qualities of Compost as a Natural Substitute of High-Enegry Consuming Fertilizers".

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Following of the Surviving of Pathogenic Microorganisms in Poultry Litter in Sequensis Batch Process of Methane Fermentation and Comparative Assesmeny in Relation to the Decontamination in other Methods of Bioprocessing

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Summary

Microbiological investigations, connected with tracing of the changes in the quantities of the bacteria in bioreactor with sequensic batch regime of anaerobic digestion (SBRAD) of water solution of litter from broiler chickens (LBC) during 45 days have been carried out. Test-strains (TS) of *E. coli*, *P. aeruginosa* and *S. aureus*, poly-resistant to amphenicols and tetracyclines, have been introduced in the bioreactor, each by 10^7CFU/g of all its content. After the fourth week from the beginning of the SBRAD the tested staphylococci did not been established in the end product. The tested *E. coli* disappeared after the fifth week, but *P. aeruginosa* have been revealed in minimal values till the end of the process.

Moreover comparative investigations of the efficacy of two methods for treatment of LBC and of cattle manure (CM) have been performed with a view to their decontamination (DC): ordinary composting (OC) and after treatment with CaO. For that purpose the same TS have been introduced at the initial stages of treatment in the composts of LBC - each TS by 10^5CFU/g of all content and in the compost of CM - in double smaller concentrations. The results confirmed the high efficacy of the treatment with CaO in order to fully DC of the LBC in short time (2 - 3 weeks). OC was efficacy in this respect too, but in longer period of time (4 weeks). The last product from the SBRAD was free from pathogenic bacteria too, except of single cells of *P. aeruginosa*, which could not be able to provoke infections.

Introduction

Organic wastes from the animal husbandry often contain pathogenic microorganisms (MO). Their delaying in the nature without previous treatment in order to their safety is forbidden by the legislation of Europe Union. Exact recommendations for the most appropriate methods for the purpose are missing till now [2]. Particularly perspective contemporary method in this direction is the anaerobe digestion. End products from this digestion are methane, used like cheap fuel and suitable for manure material. Tivchev et al. [6] reported about vastly decreasing of quantity of the MO in the end product in mesophile SBRAD. Other wide used method for this purpose is the composting for period of time, sufficient for dying of the pathogenic MO and the eggs of the helmints [3, 6]. Many investigations were accomplished in this direction, but the results are not synonymous.

The purposes of the present study were:

1. Following of the quantitative changes of TS, introduced in bioreactor with SBRAD in order

to evaluate of possibilities for decontamination of LBC. 2. Following of the surviving of TS, introduced in LBC, composted by two different methods, as well as in composted CM in order to evaluate of possibilities for their DC.

Material and Methods

Microorganisms. Pure cultures of three pathogenic TS, poly-resistant to amphenicols and tetracyclines, were used: *Escherichia coli* 6-21, *Pseudomonas aeruginosa* 33 and *Staphylococcus aureus* 230.

Nutrient media. elective media (Antisel, Sharlau Chemie S. A., Spain) with added together doxycycline (50μg/mL) and chloramphenicol (16μg/mL) were used, respectively Eosin Methylene Blue agar for *E. coli*, Cetrimide gar for *P. aeruginosa* and Chapman Stone agar for *S. aureus*.

Quantitative determination. of MO was carried out using the classical method in serial 10 times increasing dilutions of the analyzed materials in a sterile physiological solution. Cultures are prepared from these dilutions, three for each medium and dilution. After incubation at 37' for 24-48 hours, the colony forming units (CFU) were calculated in 1 mL or 1 g of the initial material.

Experimental equipments. 1. *SBRAD* of water solution of LBC (7 % dry substance, 6,2) in a laboratory bioreactor with capacity of 6 L and working volume 4 L [5] was carried out for 45 days at a mesophilic temperature regime -25 +/- 0,5'. The three TS were introduced in the bioreactor each in concentration 10^7 CFU/mL from its total quantity. Samples from the outlet are taken at intervals of 3 - 4 days for determination of the quantity of TS.
2. *Composts*
Variant A: 500 g LBC homogenized with CaO in proportion 5:1 - oxalor technology (OT).
Variant B: 2 kg LBC with daily aeration.
Variant C: 4,5 kg CM with daily aeration.
The three TS were introduced in the three composts each in concentration 8.10^4CFU/g (Variant A), 10^5CFU/g (Variant B) and $4{,}5.10^4$CFU/g (Variant C) from total quantity of the respective compost. Samples from each compost were taken at intervals of 7 days for determination of the quantity of TS.
The statistical analysis of the results is done using one-way anal. of variance (ANOVA) and Dunnett post-hoc test.

Results

The generalized data of the results from SBRAD (figure 1) show that quantities of TS gradually decreased and to the end disappeared. However in the last days small quantities of *P. aeruginosa* were revealed.

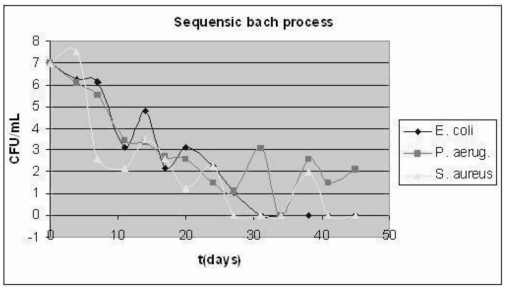


Fig. 1. Dynamics of TS in SBRAD of LBC.

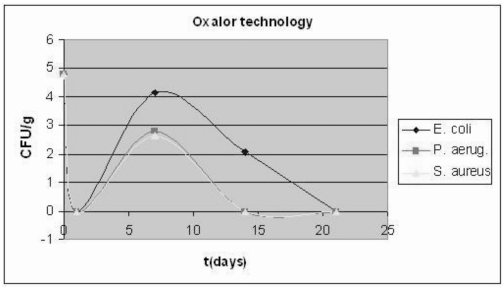


Fig. 2. Dynamics of TS in OT of LBC.

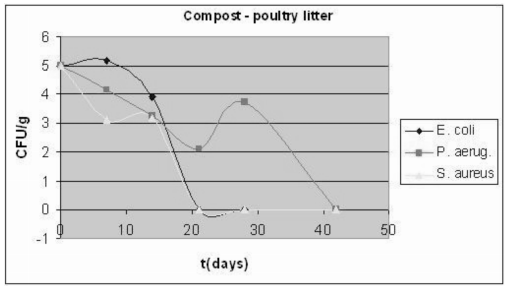


Fig. 3. Dynamics of TS in LBC-compost.

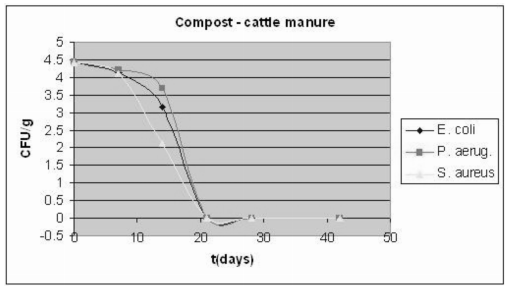


Fig. 4. Dynamics of TS in CM-compost.

In figure 2 are presented the results from OT. In 7-th day TS showed diminution compared to the initial values, which in the 14-th day already was vastly (<0,001) and disappeared through to the third week.

The dynamics of TS in the ordinary composts from LBC and CM are presented in figures 3 and 4. In 14-th day was established vastly diminution of TS in LBC (<0,001). After 21-th day TS were not uncovered in the two composts, except of *P. aeruginosa*, which was established in LBC up to the 4-th week.

Discussion

Analysis of the results shows that the destruction of TS in the bioreactor is connected with unfavourable conditions for them. The methanogenic MO detach acids, alcohols, gases etc. Their concentrations change during the different biochemical stages of hydrolysis, fermentation, acetogenesis and methanogenesis [1]. The present results suggest that mainly these products prove inhibitory action on the TS. Highest sensitivity to them showed the staphylococci, probably because of the lack of external lipoprotein envelop of their cells in contrast to the Gram-negative bacteria. The most resistant to these conditions proved *P. aeruginosa*.

After treatment with full decontamination is achieved in period of 3 weeks. Probably have not fast bactericidal effect on all microbial cells. Some of them keep viability in a few days. However in 14-th day were revealed only single *E. coli* and in 21-th day completely disappeared because of the chemical effect and very low humidity. In OCs TS die in 2 - 3 weeks in CM and in 3 - 6 weeks in LBC. The different microflora in CM seems to have significance for its faster decontamination. Our previous investigations with the same TS but in smaller quantities with about 1 log showed slower decontamination [4]. The present results indicate that with larger quantities of the composted materials can be achieve shorter decontamination, probably because of the raising of the local temperature too. Good aeration

is one of the factors which help the inhibition of MO by diminution of the humidity and giving possibilities for development of fungi, which detach antibiotics. We observed opposite dependence between the number of fungal colonies (mainly from the genera *Penicillium, Fusarium, Aspergillus* etc.) and these of TS.

Conclusion

1. The SBRAD of LBC during 45 days provides good decontamination of the end product except single cells of *P. aeruginosa*, which could not provoke infections after introducing in the soil.
2. he treatment of LBC with CaO leads to better and faster decontaminating effect than the ordinary aerobic composting.

Acknowledgement

The research is entirely financed by the Ministry of Education and Science, Scientific Research Fund - contract No D-01-376/16.06.2006 "Research on the Qualities of Compost as a Natural Substitute of High-Enegry Consuming Fertilizers".

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Hygienic aspects of poultry manure composting

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Summary

Land application of animal residues containing large numbers of viable pathogenic microorganisms is not an acceptable practice.
One of the more important quality aspects of biosolids composts concerns pathogens and/or indicator organisms.
The experiment showed that test salmonellae in the composted substrate decreased by 5 orders compared to initial level but were not completely devitalised even after exposure to temperatures higher than 55 °C persisting for 11 days. Plate counts of other investigated bacteria (psychrophilic mesophilic, coliform and faecal coliform bacteria and faecal streptococci) also decreased in the course of composting.

Introduction

Animals are one of the most frequent sources of environmental contamination related to penetration of nutrients into soil and ground water but also presence of animal and human pathogens. Proper treatment and disposal/utilisation of manure is therefore an inevitable part of correct agricultural practice.
During the storage of animal manure some of the organic substances undergo decomposition and the product obtained is less odorous and also safer from the hygienic point of view. In dependence on the conditions of storage (temperature, aeration, humidity, pH) organic matter is decomposed at various speeds in several temperature stages at which specific microorganisms play a dominant role. [4] suggested that temperatures higher than 55 °C maximized sanitation, those between 45 and 55 °C maximized the biodegradation rates, and between 35 and 40 °C maximized microbial diversity in the composting process. According to this source only the thermophilic range of 55 °C is sufficient to destroy pathogens.

Microbial dynamics: A large variety of mesophilic and thermophilic microorganisms was isolated from compost sampled at different periods during the composting process. These microorganisms can grow at temperatures varying between 10 and 70 °C. Under aerobic conditions, temperature is a major factor determining microbiological variety and intensity of metabolic activities [2] Numerous studies were conducted regarding levels of pathogens in composted substrates and in final compost products. Most of these studies have focused on indicator organisms (faecal coliforms) and salmonellae [1].
The objective of this study was to investigate changes in microbial survival during composting of poultry manure.

Material and Methods

The experiment investigated survival of *Salmonella senftenberg* test strain in poultry manure during composting in the winter period. Poultry manure was mixed with straw at aratio of 1:1.63. The volume of the composted pile was 3 m^3. During the composting process we recorded temperature in the core of the substrate (T1) and 0.1 m below the surface (T2) and relevant microbiological and physical-chemical parameters. Microbial examination included determination of counts of psychrophilic, mesophilic, coliform and faecal coliform bacteria and faecal streptococci.

Results and Discussion

The most important factors affecting the composting process are temperature and water content [5].

The initial dry matter content of the experimental mixture was 32.6%. During the composting the dry matter content ranged between 31.9% and 34.9%.

The most important parameter for pathogen destruction is of course temperature. Adequate temperature can be reached but this is dependent on proper facility design and operation. The most important considerations are good initial mix preparation, and the maintenance of aerobic conditions. This in turn is a function of mix properties, moisture control, aeration, and C:N ratio. These factors impact the composting process, and will in turn affect pathogen destruction.

The elevated temperature in the substrate affected not only survival of the test bacteria but also plate counts of mesophilic, coliform and faecal coliform bacteria and faecal streptococci. We observed a gradual decrease in plate counts of the observed micro-organisms. On day 7 of composting we were not able to recover the test strain *Salmonella senftenberg*. In parallel we observed a decrease in faecal streptococci by one order of magnitude compared to the initial level and decrease in coliform and faecal coliform bacteria by two orders. However, by day 9 of composting the core temperature decreased and bacterial counts increased again. In this stage we were able to recover again the test salmonellae, although at lower level compared to the initial one (10^4 compared to the initial 10^9, i.e. decrease by 5 orders of magnitude).

The initial T1 and T2 temperatures were 26.8 °C and 24.3 °C, respectively.

Recording of temperature in the core of the substrate (T1) showed that temperatures increased above 55 °C on December 1st and persisted above this level for 11 days and then started to decrease.

Temperature measured 0.1 m below the surface also showed an increase to level higher than 55 °C on December 1st but persisted there only for 22 hours and began to decrease.

On the first day of the experiment, after construction of the pile the following bacterial counts were determined: psychrophilic bacteria $1.8.10^8$ CFU per g, mesophilic bacteria $3.2.10^9$ CFU per g, coliforms $8.5.10^8$ CFU per g, faecal coliforms $8.3.10^8$ CFU per g, faecal streptococci $3.0.10^5$ CFU per g, and salmonella $3.8 .10^9$ CFU per g.

The significant increase in temperature detected on the third day in the core of the substrate (64.5 °C) affected not only survival of the test bacteria but also plate counts of mesophilic, coliform and faecal coliform bacteria and faecal streptococci. Increasing temperature resulted in a gradual decrease in plate counts of observed micro-organisms.

On the third day (T1 increased to 64.5 °C) mesophilic, coliform and faecal coliform bacteria decreased by one order and salmonellae by 2 orders.

On day 9 when the pile was turned manually the temperatures persisted in the thermophilic range (T1 was 62.3 and T2 reached 56.6).

The highest temperature T1, recorded during the experiment, was 66.4.on day 11 from constructing the pile and from then on it started to decrease.

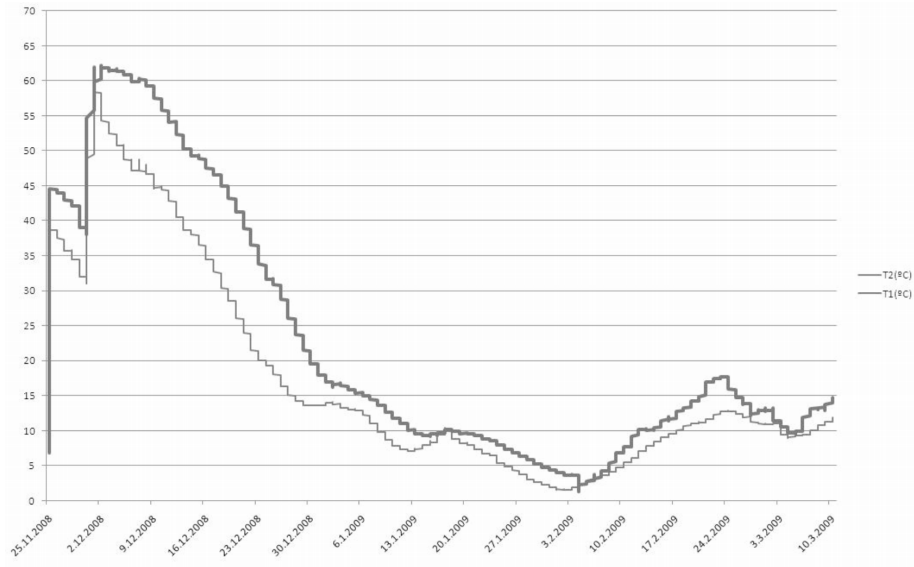


Fig. 1.

On day 63 of composting the following bacterial counts were recorded:
Psychrophilic bacteria $1.3.10^7$ CFU per g, mesophilic bacteria $1.8.10^7$ CFU per g, coliforms $4.0.10^5$ CFU per g, faecal coliforms $1.0.10^5$ CFU per g, faecal streptococci $4.0.10^3$ CFU per g and salmonellae $3.0 .10^4$ CFU per g.
The highest decrease was recorded with salmonella (by 5 orders), coliforms and faecal coliforms decreased by 3 orders, mesophilic bacteria and faecal streptococci by 2 orders and psychrophilic bacteria by 1 order. Our results showed that temperatures in the thermophilic zone persisting for the specified time were not sufficient to devitalized the test salmonellae.
Moisture content is one of the factors that plays a major role in compost stability and consequently in potential regrowth of microorganismsl. It is possible that the compost becomes too dry during the composting, slowing down the process or even stopping microbial activity. The consequence of this practice can result in an incomplete degradation of organic matter [3].
In our experiment the initial total organic matter content was 96.7% and decreased slowly up to the end of the process when the loss in organic matter was the highest (90.2%).
Another factor of extreme importance is the microbial diversity of the compost. The antagonistic effect of many different microorganisms in the compost system has been reported extensively in the literature [3]. It is expected that greater diversity increases competition among populations for substrate. In addition, there are some species, especially some fungi and actinomycetes, that excrete substances which suppress other species, amplifying these effects. A compost which reaches low moisture content during the cooling down phase or during maturation, becomes a unfavourable environment for repopulation of the natural flora. It is expected that these compost products have low microbial diversities, and are therefore vulnerable to pathogen regrowth. Some previous results in our laboratory have shown that the level of microbial diversity can suppress pathogen regrowth even in samples considered unstable in terms of carbon availability.

Pathogenic bacteria during composting cycle. One of the purposes of composting was to eliminate the pathogenic microorganisms and to avoid later

recontamination of waste. Indeed, some authors have used tests of recontamination by inoculating *Salmonella* or *E. coli* in compost pile to find the optimal conditions of pasteurization [2]. They noticed that the final compost water content must be lower than 20% and the ratio C/N ratio must be lower than 15. Composting must suppress all pathogenic bacteria. This occurred when the temperature reached 55 °C. *Salmonella* species are regarded a problem related to the hygienic quality of the compost, probably due to the fact that these bacteria are ubiquitous and capable of rapid growth.

Conclusion

While biosolids may be properly disinfected by composting, there is the possibility of repopulation (or regrowth) of organisms in the compost. Repopulation is also addressed in the new regulations.

Recovery of the investigated bacteria after the initial period of composting indicates shortcomings in the composting process that may be related to failure to observe the correct composting practice, particularly the frequency and way of turning of the composted pile.

Acknowledgement

This study was supported by the Science Grant Agency (VEGA) project No. 1/0630/09.

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Changes in chemical parameters during composting of poultry manure

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Summary

The study was undertaken to investigate the effects of carbon to nitrogen (C:N) ratio and turning frequency (TF) on the loss of total nitrogen (TN) during composting of poultry manure and sawdust mixture in order to produce good quality compost.

During 63 days of composting we recorded temperature in the core of the substrate and its influence on changes in pH, dry matter, NH_4^+, total N, C:N ratio and total phosphorus (Pt). The processes of aerobic composting were most intensive in the initial period as the core temperature reached 62.7C within 48 hours. The temperature in the core of the substrate exceeded 55 °C and remained above this level for 11days up to day 23 from the beginning of composting. The values of pH increased as well as DM by the end of the experiment. In the initial period the concentration of $NH_4^+$and total N decreased due to nitrogen losses along with increasing temperature

The final C/N ratio was 22:1 which indicated maturity of the final product (range 20:1 – 25:1 is considered appropriate) and its suitability for application to soil.

Introduction

Composting is a biological oxidative decomposition of organic wastes during which they are stabilized and converted into a relatively stable humus. It is a process that can turn animal wastes into very valuable and safe substrate that can be applied onto agricultural soil.

Poultry litter is composted to reduce odour and pathogens and to improve its quality as a soil amendment. Organic material rich in carbon, e.g., straw, is added to increase the C:N ratio to achieve optimum degradation of organic C and retention of N through formation of microbial biomass [2]. The microorganisms metabolize organic compounds and produce CO_2, cause increase in biomass and thus support retention of N. Poultry manure is one of the richest sources of N. The N availability from the manure is affected by losses through volatilization, denitrification, immobilization, mineralization and leaching. Mineralization occurs rapidly immediately after the application of poultry manure [1].

Composting, or the controlled biological decomposition of organic waste, has been investigated as amethod of stabilising poultry manure prior to land application. This process produces amaterial with several advantages with respect to handling, e.g. reduced volume, mass of dry matter and odours and, at the same time, decreases hygiene risk related to the content of pathogenic micro-organisms and parasites in poultry droppings. Composting process immobolizes N in the manure and produces humus that can be used as source of organic materials and slows down release of nutrients.

The aim of the present study was to investigate poultry litter mixed with sawdust with regard to biodegradability of the mixture and

the properties of the final products from the point of view of its hygienic quality and suitability for application to agricultural soil.

Material and Methods

Poultry manure and straw used in the experiment were examined for the basic physical and chemical properties (Tab. 1).

Excrements collected from a layer house on poultry farm were produced in one day and total quantity used in the experiment amounted to 300 m^3. The experiment was conducted in winter in a pilot-plant open composting facility under a roof.

The substrate for composting was prepared from poultry manure and cut straw mixed at a ratio of 1:1.63 by volume. During 63 days of composting we recorded temperature in the core of the substrate and 0.1 m below the surface. Samples were taken from the core of the substrate to determine the relevant chemical parameters, namely pH, dry matter DM, organic matter, NH_4^+, total nitrogen N_t, total phosphorus P_t and C:N ratio. The methods used corresponded to the STN 465735. The C content was calculated according to the content of organic matter by the method of [5]. Results are reported per dry weight.

Tab. 1: Physical and chemical properties of the basic materials

	Straw	Solid Poultry manure
pH	7.01	7.02
DM (%)	95	28.6
OM (%)	96.2	91.1
IM (%)	3.8	8.9
NH_4^+ (g.kg^{-1} DM)	0.1	1.96
N_t (g.kg^{-1} DM)	4.2	45.5
C:N ratio	117:1	10:1

Results and Discussion

In the present study, quantitative changes in physical, and chemical, properties of poultry manure and straw were studied in order to gain better understanding of the composting process and to evaluate the suitability of the final product as a soil amendment. The poultry litter was composted in forced-aeration piles and the substrate was turned manually on day 9 of composting.

During composting of animal manures some organic substances undergo decomposition and the product obtained is less odorous and also safer from the hygienic point of view.

In our experiment we observed that on day three of composting the temperature in the core of the substrate increased to 64.5 °C while at the depth of 0.1 m it reached only 54.8 °C but continued to increase..

Overall, the temperature in the core of the substrate exceeded 55 °C and remained above this level for 11days up to day 23 of composting The highest temperature was recorded on day 11 of composting (66.4 °C) after which it started to decrease.

Temperature recorded 0.1 m below the surface increased above 55 °C by day 3 but remained above this level only for three days. The highest temperature reached was 65.2 °C. The processes were most intensive in the initial period as the core temperature reached 62.7 °C within 48 hours. This was reflected also in pH which increased from the initial level of 8.3 to 9.23 by 48 hours of composting. At the end of the decomposition process, the pH was 8.61 (Fig. 1).

The pH increased with the progress of decomposition process [1].

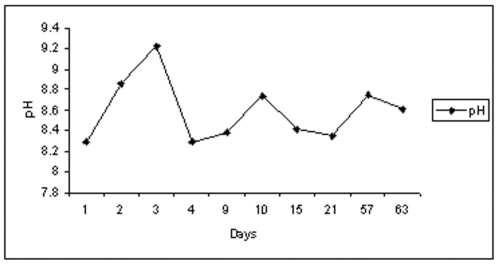


Fig. 1 The course of pH during 63-day composting of poultry manure mixed with straw

The initial moisture content in the composted substrate was 67.4%. During the composting the moisture was in the range 68.1 to 65.1%.

In our experiment the initial total organic matter content was 96.7% and decreased slowly. By the end of the process the loss in organic matter was the largest (90.2%).

Due to microbial degradation processes the initial content of NH_4^+ (13.3 g. kg^{-1}) increased to 22.9 g.kg^{-1} by day 9 of composting. At the end of the experiment, on day 63 of composting, the content of NH_4^+ was 21.7 g.kg^{-1}.

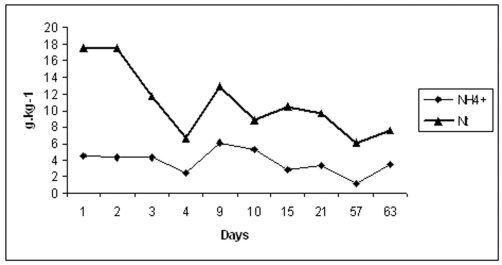


Fig. 2 The course of NH_4^+ and N_t during 63-day composting of poultry manure mixed with straw

The N content in the manure declined linearly in the presence of straw under aerobic decomposition and the reduction was slow. The loss of Nt could largely be attributed to volatilization of ammonia (Fig. 2). Other authors [3] also observed losses of N in poultry waste resulting in initial increase in C:N ratio which reached 10:1 at the beginning of the experiment.

Organic material, e.g., sawdust, is added to increase the C:N ratio to achieve optimum degradation of organic C and retention of N through microbial biomass formation [2]. In the mentioned study, bench-scale compost reactors were used to determine the relative contributions of poultry litter and of the amendment (sawdust) to the biodegradability of a compost mix. Approximately 29% of the volatiles lost from the poultry litter mix came from the sawdust.

In general, phosphorus content increased slightly during the intermediate phases irrespective of the methods of aerobic decomposition. Mixing straw had least effect on the total P content of this manure.Similar result was reported in [1] who observed an increase in total P content during aerobic decomposition.

The data suggested that adding straw to poultry manure was beneficial in terms of reducing the loss of N regardless of the method of composting.

Conclusion

After 63 days of composting the chemical parameters in the composted substrate underwent considerable changes that corresponded to intensive decomposition. The final C/N ratio was 22:1 which indicated maturity of the final product (range 20:1 – 25:1 is considered appropriate) and its suitability for application to soil.

The experiment suggested that the composting of poultry manure blended with straw would enhance the N, P and carbon status of the manure and thus improve its quality. The study presented allowed us to conclude that incubating poultry manure with straw under aerobic condition is beneficial to the quality of the manure.

Acknowledgement

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Regional transport efforts for animal farm manures

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Summary

A reasonable substitution of often expensive, limited and energy-intensive mineral fertilizers with animal farm manures can be realized by establishing a regional material flow management capable in optimizing manure flows. At the same time, environmental problems caused by manure over-application can be reduced. The general model setup presented in this study can contribute considerably to environment protection.

Introduction

The increasing specialisation in agriculture results in an increasing regional decoupling of crop nutrient demand and nutrient availability from animal farm manures. Utilization of mineral fertilizers implies a high energy input and hence becomes increasingly expensive [1]. Regions with high intensity animal farming often have animal farm manure nutrients in excess to what can be used sensibly as fertilizers on the local agricultural land. This development can bear the risk of over-fertilization on the one hand and use of mineral fertilizer as the only nutrient source on the other hand [11]. A combination of both mineral and organic fertilization can realize a high nutrient use efficiency [3]. A common means to prevent environmental harm and farm-level nutrient surpluses is the export of manures to farms with lower animal densities. Transportation is costly, especially for manures with high water contents. A regional material flow management capable in combining both the nutrient supply and demand areas is appropriate. Most analyses concerning manure management systems are farm level studies [9,13]. Some regional models were also developed, partially considering actual distances [12].

To avoid over-application of manures in surplus areas and at the same time make use of the fertilizer value in areas with lower animal densities, a model is developed as a means of a regional material flow management. The model input is a most realistic estimate of annual quality and quantity of the manures produced and nutrient demand of the crops and grass land in distinct areas of a region. The model determines an optimal distribution of the various animal farm manures at the lowest transportation effort.

Material and Methods

Study area. Lower Saxony, a federal state in north-west Germany, serves as a study area for the modelling. It comprises 45 districts with a total of 2.6 million hectares of agricultural land. The western part of Lower Saxony is dominated by animal farming while arable farming prevails in the east.

Determination of input parameters. The relevant parameters impacting on animal farm manure transports and their costs are quantity and quality of the manures produced and the capacity of land use systems to utilize manure as fertilizer.

The 29 animal farm manure classes considered in the model were determined by using district level data on number of farm animals sorted by species, use, age, and weight [4,10]. These were combined with the general reference values on nitrogen (N) and phosphorous (P) excretion of farm animals and average amount of manure produced by the animals [7]. A simplifying assumption was made for the type of manure produced: all pig and all cattle manure was considered being slurry, all poultry manure was considered being solid manure.

The N and P demand of a total of 16 land use classes was derived by the combination of agro-statistics [5] with average yield levels [6] and fertilizing advice [8].

Modelling. To optimize the distribution of manure nutrients at lowest transportation costs a linear optimization model was developed [2]. Assumptions for the modelling are that each district can provide nutrients in animal farm manures and that each district comprises areas of various land uses with a specific nutrient demand. The extent to which the manures are transported depends on local manure production, scenario constraints on manure nutrient application and manure availability after transportation between districts as depending on the constraints. The modelling language is GAMS (General Algebraic Modelling System). The model is linked to an Excel template for data input and result visualization. Its output is the optimal manure distribution to the agricultural land and the minimal manure transports between all districts at lowest overall transportation effort. Hence, manure nutrient deficient or surplus districts are also defined. For this study, constraints are that a maximum of 80% of the N and P demand of the individual crop classes may be covered by animal farm manure N and P.

Results

Restricting animal farm manure nutrient application to an equivalent amount of 80% of the crop nutrient demand results in extensive manure transportation activities between the districts of Lower Saxony (Fig. 1a). In the model, most western districts with comparatively high animal densities become manure surplus districts and have to export manure. A total of 3 million t of manure is exported from 19 surplus districts. Nevertheless, most of the manure is used locally in the districts of origin. The liquid slurries are predominantly used locally to cover the local crop nutrient demand or, if districts are surplus districts, are exported into districts close by. A detailed view into the manure exports from Cloppenburg (CLP) district shows how the model gives preference to the transportation of water rich slurries to the closest surrounding districts (Fig. 1b). In comparison to the slurries, poultry dung is transported to the more distant districts because of its low water and high nutrient content.

Discussion

Regional examinations are important for numerous purposes, e.g. designing policy measures for the protection of environmental goods and services [11]. Modelling approaches such as presented in this and other studies can be used as helpful tools in analysing what impacts most on the regional level [12]. The modelling

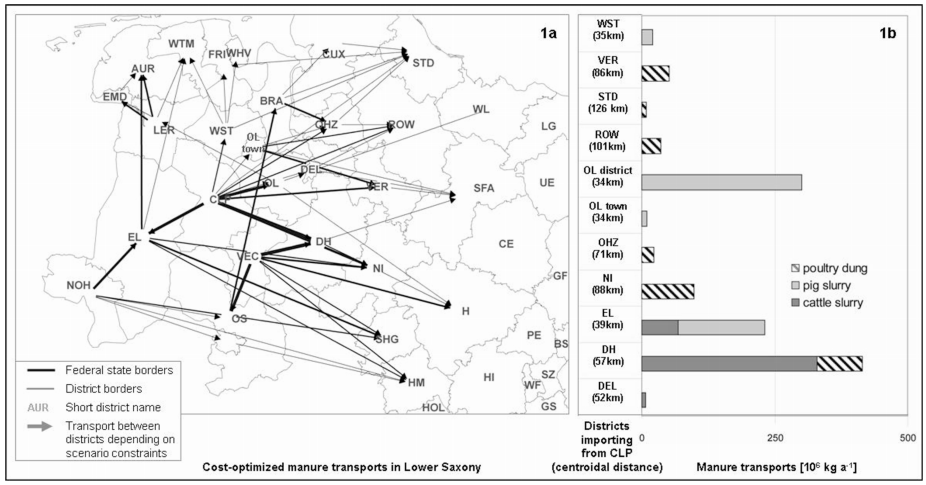


Fig. 1: Modelling results for the scenario constraints that allow for a coverage of 80% of the crop nutrient demand with animal farm manures. Fig. 1a depicts the least possible transport effort of manures between the districts in Lower Saxony. Bold arrows indicate large manure flows, slim arrows indicate small manure flows. Fig. 1b gives an example of how the model distributes the different types of manure from a surplus district (Cloppenburg, CLP) to 11 receiving districts. The cumulative terms of poultry dung, pig slurry and cattle slurry contain the 29 manure classes of the model.

results show that manure exports from surplus regions are considerably high even in the optimized model case and even though the constraints were set to a low limit. For this reason, additional management means other than export have to be considered in a future setup of the model. Such measures could include manure separation, biogas production or digestate processing steps.

Conclusion

A regional material flow management for farm manures based on this model will enable a reasonable substitution of often expensive, limited and energy-intensive mineral fertilizers with animal farm manures in districts with little animal farming. At the same time, environmental problems caused by manure over-application can be reduced. Hence, the basic model setup can contribute considerably to environmental protection.

Acknowledgement

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Hygienisation of organic waste during the dynamic aerobic composting

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Summary

The survival of pathogenic bacteria (*Salmonella* spp., *Escherichia coli*, *Enterococcus* spp.) during short-time-composting of kitchen waste in a dynamic forced-aerated in-vessel composting system working with a heating jacket was studied. In order to control the reliability of composting techniques on eliminating pathogens, *Salmonella enterica ssp. enterica serovar* Senftenberg was used as indicator. Where the composting with the aid of a heating jacket was proceeding (temperature into whole composting material $> 70\,^{\circ}C$), the input indicator bacteria were eliminated in every case. In contrast, composting without heating jacket resulted high numbers of pathogens and the presence of *Salmonella* Senftenberg.

Introduction

Pathogenic bacteria in kitchen waste have comparable risks like in slaughterhouse wastes for man and/or animals [1]. The EU regulation (EC) No. 208/2006 defines the treatment of animal by-products. In composting plants category 3 materials must be sanitized for 1 hour at 70 °C. A sufficient strategy for prevention of microbiological risks in dynamic aerobic composting plant is the guaranteed hygienisation of the biowaste and an adequate technical equipment [2]. In this study the hygienisation of kitchen waste in a dynamic forced-aerated in-vessel system (400 l) (Fig. 1) working with a heating jacket was studied. An optimum composting method to inactivate both indicator (*Salmonella enterica ssp. enterica serovar* Senftenberg) and pathogenic (*Salmonella* spp., *Escherichia coli*, *Enterococcus* spp.) microorganisms during the dynamic aerobic composting was investigated.

Material and Methods

The natural degradation process should be utilised for heating to reduce the energy input into the process. The feedstocks (kitchen waste, wood shavings and paperboard) have been decomposed in ca. one month under aerobic conditions and regular mechanical turning. Temperature was monitored as an indicator of the efficiency of the composting experiments. 7 batches was investigated, there from were 6 hygienisated by the heating jacket and 5 supplementary tested with test bodies. *Salmonella enterica ssp. enterica serovar* Senftenberg as indicator was brought into the reactor composting process and investigated on it's decline. The samples were obtained for microbial analysis from various locations during the entire composting process (Fig. 2). Reductions of the number of indicator (inoculated in test bodies, process control) and pathogenic (produce control) microorganisms were measured by the most-probable-number method.



Fig. 1: BioSal®-dynamic composting plant (BioSal Anlagenbau GmbH, Bad Lausick, Germany)

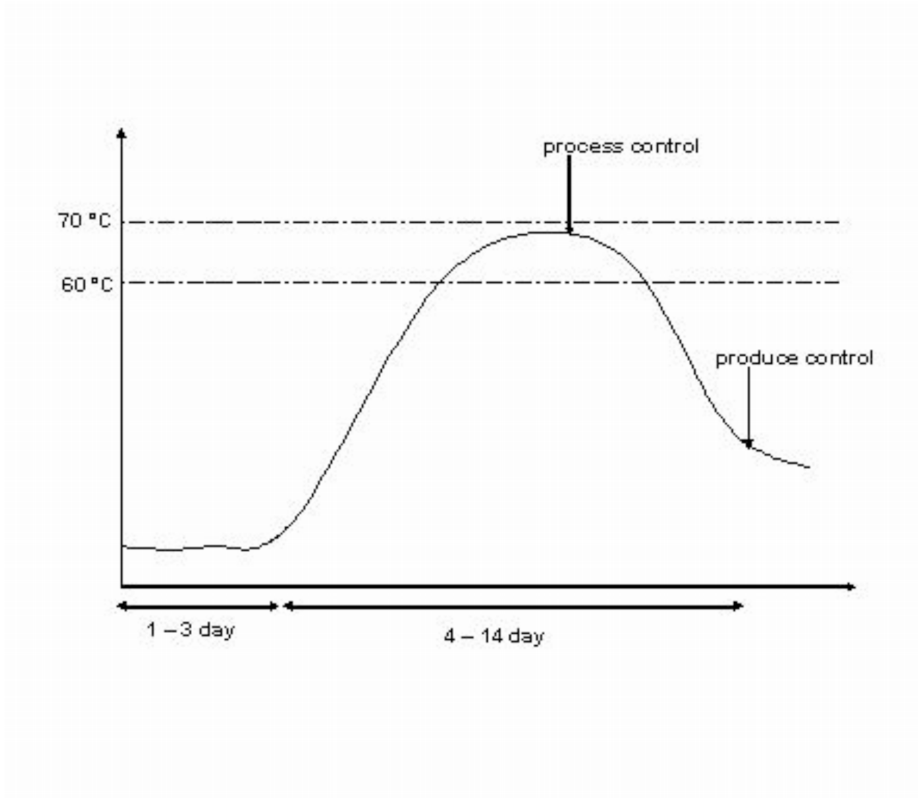


Fig. 2: temperature profile during composting and points of time of process and produce control

Results

Valid observations where taken at 7 batches (Tab. 1). In the majority of the investigated samples the process controls (*S.* Senftenberg) were tested as negative (exception batch No. 18). In the outputs *Enterococcus* spp. were detected in all samples (batch No. 18 with high number of 10^6 CfU/g), whereas three samples (batch No. 14, No. 18 and No. 19) were positive for *Escherichia coli* with low numbers (10^2 CfU/g and 10^4CfU/g), each. In one case (batch No. 18) the heating jacket was defect, consequently the hygienisation could not proceed. Therefore, in this batch *Salmonella* spp., *Escherichia coli*, *Enterococcus* spp. were detected in high amounts in the output material and the process control with *S.* Senftenberg was positive as well.

Table 1: Microbiological results for composting experiments

Batch No.	Total count (in Cfu/g of dry compost)	*Salmonella spp.* (in 50 g of dry compost)	*E. coli* (Cfu/g of dry compost)	*Enterococcus spp.* (Cfu/g of dry compost)	Process control with *Salmonella* Senftenberg	Composting procedure
11	$1{,}0 \times 10^9$	not detected	not detected	$>1{,}1 \times 10^4$ *(Enterococcus durans)*	not analysed	with heating jacket
13	$7{,}8 \times 10^6$	not detected	not detected	$1{,}1 \times 10^3$ *(Enterococcus durans)*	not analysed	with heating jacket
14	$8{,}1 \times 10^6$	not detected	$2{,}3 \times 10^2$ *(E. coli 1,2)*	$4{,}3 \times 10^4$ *(Enterococcus faecium)*	not analysed	with heating jacket
15	$4{,}0 \times 10^6$	not detected	not detected	$2{,}4 \times 10^2$	not detected	with heating jacket
17	$1{,}5 \times 10^9$	not detected	not detected	$2{,}4 \times 10^2$ *(Enterococcus faecalis 3)*	not detected	with heating jacket
18	$1{,}3 \times 10^8$	detected	$2{,}9 \times 10^2$ *(E.coli 1)*	$> 1{,}1 \times 10^6$	detected	without heating jacket
19	$1{,}3 \times 10^8$	not detected	$1{,}5 \times 10^4$	$3{,}8 \times 10^3$ *(Enterococcus faecium)*	not detected	with heating jacket

Discussion

Our study shows the important role of heating jacket for the hygienisation of kitchen waste in the short-time-composting. In one case the heating jacket was defect and thereby the hygienisation for 1 hour at 70 °C could not proceed. Consequential in this batch the pathogenic and the indicator microorganisms were not inactivated completely because of the low temperatures achieved in the composting reactor. The survival of the *Enterococcus* spp. in each batch indicated clearly that this species are more resistant to higher temperatures than the other organisms tested in this study and is similar to findings of Cekmecelioglu at al. [3]. Summarizing, our results show that 1 of 7 (batch No. 18) batches was not sanitized according to the EU regulation.

Conclusion

In conclusion, the composting process must be evaluated by monitoring both temperature profiles and survival of pathogenic microorganisms. Sophisticated in-vessel methods and adequate technical equipment provide the most process control; therefore, they produce a safety and the

most uniform and predictable product [4]. A composting system working with a heating jacket can the hygienisation of kitchen waste in the short-time-composting guaranteed. This is particularly important if the final product is to be used for growing vegetables or for gardens. To confirm these results further analysis of the input and output material related to the pathogenic microorganisms and pH-values will be conducted.

Acknowledgement

Special thanks to Karin Fiedler for her laboratory assistance.

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Upgrading food processing waste from animal origine to consumable ingredients: Fishproduction as an example

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Summary

In the growing scarcity of natural resources, it is crucial to increase the effectiveness of the use of the resources. By looking at the amount and kind of waste and by-products in a process, this can be upgraded to for instance biodiesel or can even be used as an ingredient for human consumption after special treatments. In our process especially waste from the fatter animals like pigs and chicken and also fish can be renderred and provide a valuable resource. Mechanical Deboned Meat (MDM) from chicken can result in low fat (30% reduction) basis for animal feed, the fat/oil is a valuable basis for the soup industry.

Introduction

In several industries a high amount of waste is produced. Especially in the food processing industry this can cause problems. The problems can vary from high costs for destruction and transport to pollution of the direct environment. This pollution can cause human and animal risks and inconvenience by risk of bacterial growth and massive outbreak of infectious diseases or environmental pollution. The goal should be to reduce the amount of waste by using as much waste as possible for other purposes like human or animal food or as a ingredient for energy winning (feed for biomethanisation or biodiesel). This paper will deal with the description and results of a new methode to convert waste in a usefull products for animal or human consumption.

Animals, Material and Methods

As example we take the fish producing industry. From a standard fish (catch or production) only about 34% is for consumption of fish meat. So 66% of the production is waste. The composition of this waste is as follows:
In fishcatching this majority is normally dumped in the sea , where it is clearly a pollution, expressed by Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD). In farm fishproduction and fishprocessing the waste has to be carried of for destruction .
Due to the possible fast growth of bacteria under the normally present conditions (see Table 1), any processing of the waste should be on-site.
Therefore we developed a rendering plant with a typical lay out (Fig. 1) who realises in the system a typical retention time of 4-5 minutes. After this system the oil/fat flow can be hygienised by UV treatment and should be cooled down in order to prevent bacterial growth.

Table 1: temperature range for growth of pathogens [1]

	Temperature °C		
Pathogen:	Min.	Optimal	Max
Salmonella	5	35-37	47
Campylobacter	30	42	47
E.coli	10	37	48
S.aurus	6,5	37-40	48
C.botulinum	10		50
B.cereus	4	30-35	48-50

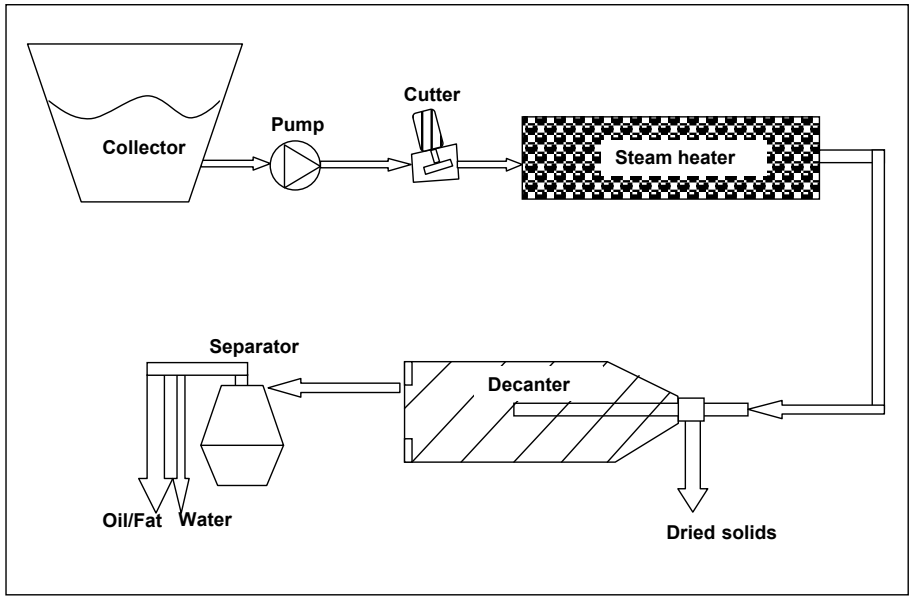


Fig. 1: Typical lay out of the rendering plant

Results

In our system the fish waste is collected in collector and the cooked, crushed and separated. Because the influent has been heated by steam, in general all disease germs have been killed before starting the decantation. This process results in two flows:

1) The sediment, which contains between 35-40% Dissolved Solids (DS) is also called fish meal. This sediment now is a high grade product for the fish industry itself or other animal feed.

2) The effluent. This contains 10% water, 90% fish oil and a low amount of fines (<0,5%). The effluent is further cleaned by a disc stack separator: liquid-liquid separation by means of G-forces. The amount of G-forces used is 9000G. The oil and water is then separated. The fish oil is fit for human consumption. It contains high levels of Ohmega 3 and Omega 6 (ratio 7:1).

The water can then flow in the sewage to be treated further for BOD/COD reduction or can be dumped in the sea directly.

For the chicken industry, the process is the same except for the temperature: this should be kept below 35 °C in order to protect the

proteins in the sediment. The typical values for chicken skin is that it contains >45% fat, after the process it contains <25%. This low fat meat is then suitable as cattle feed.
After cleaning the fat it can be used as a basis four soups and sauces. This process should be in inline with the deboning process, in order to keep the bacterial (especially the Salmonella) growth as low as possible (see table 1). Flash freezing is recommended after this step

In catlle and pig slaughterhouses the system can be used for blood treatment. All the blood is collected and injected with steam. This makes the blood to coagulate and is separable with a decanter. The plasma (65%) and red/white bloodcells (including platelets: 35%) are separated. The bloodcells can beused as an animal feed for young animals to increase the resistance and performance.

Discussion

This paper described a method to treat waste from animal processing plants to convert it to profitable products for animal and human consumption.
The amount of energy that is put into the rendering business strongly depends on the economic situation. At high energy costs one is looking for alternatives and higher efficiency. There is anyway more and more pressure to take care of a sustainable animal production and to protect the environment from unnecessary pollution. The described sytem can contribute to increased efficiency and reduced pollution.
Great precaution has to be taken when commercial interrest prevales above safety. Product integrety and safety should always be first.
It was difficut to get valid information about the results of the process because the involved food industry considered the measurement data as confidential and are therefore not easily available. In order to get more transparancy, this should be changed.

Conclusion

Using decantation in a rendering system waste can be upgraded to (very) valuable elements, what can decrease the amount of waste significantly. This is also a commercial benefit on the long run.

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Hygienic safety of slacked lime treatment in the tanning process

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Summary

The results of two experiments showed that the standard slaked lime treatment procedure is capable of reducing superficial test bacteria and intracutaneously injected bacteria by 8 log or more within 8 to 36 hours. Mechanical influences alone reduced the test bacteria by 2 log.

Introduction

Since European animal by-product regulations require the safe and validated treatment of category 3 materials, the question whether the traditional lime treatment as the first step in the tanning procedure provides sufficient hygienic safety for the product had to be answered by experimental process validation (EFSA 2005). This was done under practical conditions in two different large scale liming vat. The aim of the investigations was to ascertain whether the factors in the tanning process (high alkaline pH, constant turning and walking the hides, 8 - 36-hour exposure to highly alkaline lye) reduce or kill the test bacteria to ensure hygienic safety before further treatment of the material.

Material and Methods

For the studies, fresh hides from cattle were contaminated superficially with a microbial suspension. The microbial suspension was also injected into the tissue. The prepared skins were exposed to the normal liming process together with other hides to ensure the standard procedure in two independent runs.
Laboratory tests were carried out first to clarify methodic issues, particularly the establishment of a method for the introduction of the test strain superficially and by injection and to determine recovery as well as for the selection of a suitable disinhibition medium (for neutralizing the slacked lime).
The recovery and detection of the test strain from the marked areas of contaminated material was carried out by standard bacteriological methods.



Fig.1: Intracutaneous injection of bacteria in the marked areas of a fresh hide from cattle

Results

The results in table 1 show that the superficial salmonella and enterococci as well as the intracutaneously injected bacteria could not be detected quantitatively or qualitatively after the contact time of 36-hour in the tanning process.

Table 1: Qualitative and quantitative detection and recovery of test strains from hides of cattle superficially contaminated or injected with salmonella and enterococci after a contact-time of 36 hours in the tanning process (cfu/ml)

Salmonella pc + ic	qual.	Quant.	enterococci pc + ic	qual.	quant.
1	-	-	1	-	-
2	-	-	2	-	-
3	-	-	3	-	-
4	-	-	4	-	-
5	-	-	5	-	-
6	-	-	6	-	-
7	-	-	7	-	-
8	-	-	8	-	-
9	-	-	9	-	-
10	-	-	10	-	-
11	-	-	11	-	-
12	-	-	12	-	-

pc = percutaneous (superficially) ic = intracutaneous
qual. = qualitative detection of salmonella and enterococci
quant: = quantitative detection of salmonella und enterococci
+ detection possible - no detection possible

The tests were carried out in a second company with a smaller liming vat. Table 2 shows, that after a contact-time of 8 hours in the liming vat, the superficial salmonella and enterococci as well as the intracutaneously injected bacteria could not be detected qualitatively or quantitatively (with two exceptions for enterococci).

Discussion

The experiments show that in the tanning process of the first company after a contact time of 36-hours the superficial salmonella and enterococci as well as the intracutaneously injected bacteria could not be detected quantitatively or qualitatively.

Because of the highly alkaline liquor and the various additional chemicals in combination with the mechanical influences through the walking, turning, and the enormous pressure created by the weight of the hides, the test strains were safely inactivated in the tanning process. The process influences obviously created the possibility for the slacked lime to go through the skin tissue and also to inactivate the strains injected intracutaneously.

In the experiments in the tanning process of a second company after a contact time of 8-hours the superficial salmonella and enterococci as well as the intracutaneously injected bacteria

Table 2: Qualitative and quantitative detection and recovery of test strains from hides of cattle superficially contaminated or injected with salmonella and enterococci after a contact-time of 8 hours in the tanning process (cfu/ml)

Salmonellen pc + ic	qual.	Quant.	Enterokokken pc. + ic	qual.	quant.
1	-	-	1	-	-
2	-	-	2	-	-
3	-	-	3	+[1)]	-
4	-	-	4	-	-
5	-	-	5	-	-
6	-	-	6	-	-
7	-	-	7	-	-
8	-	-	8	+[1)]	-
9	-	-	9	-	-

pc = percutaneous (superficially) ic = intracutaneous
qual. = qualitative detection of salmonella and enterococci
quant: = quantitative detection of salmonella und enterococci
[1)] = positive intracutaneous samples
\+ detection possible - no detection possible

were not detectable either quantitatively or qualitatively.
In two samples, a qualitative detection of enterococci was still possible. The two positive samples were from the skin area which was contaminated intracutaneously by injection with enterococci. Although quantitative validation conditions have been met here, the results are an indication that after eight hours the lower limit has been reached and so thus a margin of safety can't be compromised.
It should be noted that the tanning process (drum) in the second company has smaller proportions than the drum in the first company. Therefore, the mechanical effects of walking, turning and pressure by the hides of the many other cattle did not have have an equal influence to that in the process of the first company.

Conclusion

The validation experiments ("worst case szenario") hides contaminated by bacterial test strains superficically and by intracutaneously injection have shown that the slacked lime treatment in the tanning processes are able to reduce the test strains (*Salmonella* Senftenberg W 775 and *Enterococcus faecalis*) by more than 8 log within eight and 36 hours.
The same applies for the inactivation of pathogens with similar resistance.
In the sense of the validation process in accordance with the requirements for animal by-products (category III-material), the experiments with injection of the microbial suspension into the tissue (spread of pathogens in the septicaemic phase of infection) proved a reduction more than 6 log. Regarding superficial pathogens
(accidenticial superficial contamination), it must also be assumed that in addition to the inactivation of pathogens on the material, a mechanical removal of bacteria is also possible. The inactivation of the bacteria in the fleet is much faster than on the surface.
It must be noted that in practice, skins with different weights and thicknesses, depending on the age and breed of the animals are found the contact time of the hides in the slacked lime treatment while the tanning process is in no case allowed to exceed the time of eight hours.
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Part XII

Horse session

Chapter 33

Block 8A

The importance of diet and feeding practices in the development of common health issues in the horse

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Overview

Within the field of equine health, the link between diet and disease strengthens with ongoing research. Not surprisingly, the makeup of the diet and feeding practices can have an impact on digestive health, but also perhaps less intuitively on other areas such as respiratory, muscular and locomotory function. This review will seek to discuss the relevance of dietary factors and feeding practices to the development and management of a number of commonly encountered health issues in the horse.

Laminitis. Laminitis is a prevalent condition that has been estimated to affect 7-13% of the equine population. Many of the feeding strategies employed for horses or ponies at risk from laminitis target key stages in the current proposed mechanism of the disease:

Limit intake of rapidly fermentable carbohydrate–. Restriction of grazing is of paramount importance due to its unpredictable content of water soluble carbohydrates. Limitation of the available grazing area is also important as ponies can consume nearly 40% of their daily dry matter intake in just 3 hours (Harris et al. 2007).

Optimise pre-caecal starch digestibility -. This can be achieved by feeding small meals and using cooked cereals as this increases the pre-caecal digestibility of starch significantly (Harris et al. 2007). The use of additives with amylase enzyme activity may also offer further advantage in maximising pre-caecal starch digestibility (Richards et al. 2004).

Maintenance of the hindgut environment and microbial population –. Live yeasts, such as Saccharomyces cerevisiae (SC) have been reported to reach the hindgut (Gobert et al. 2006) successfully and have been demonstrated to ameliorate the negative effects of a high starch meal on the hindgut environment (Medina et al. 2002). Buffering agents such as bicarbonate which have been used in dairy cattle to relieve rumen acidosis may offer a further strategy against laminitis, with the advent of encapsulation technology to deliver such alkalising agents to the hindgut.

Improve sensitivity to insulin -. Glucose has been shown to be essential for maintenance of lamellar integrity and disrupted glucose homeostasis has been suggested as a trigger for laminitis. Research suggests that there are significant differences in insulin sensitivity between normal horses, ponies, and ponies with a history of laminitis (Treiber et al. 2007) and that this is affected by both diet and exercise (Treiber et al. 2006).

Nutritional ingredients including chromium-enriched yeast, magnesium, vitamin E, co-enzyme Q10, chromium-enriched yeast, Silybum marianum (milk thistle) and Cinnamomium (cinnamon) are reputed to improve the sensitivity to insulin, although research in this area in horses is very limited.

Antioxidant support -. Ischemia, reperfusion, inflammation and insulin insensitivity are characteristics of laminitis that are reported to increase the production of reactive oxygen species (ROS) and free radicals. Dietary antioxidants and associated co-factors including vitamin E, beta-carotene, vitamin C, copper, zinc, manganese and selenium are therefore important.

Respiratory Health. Nutritional factors in equine respiratory health and disease, particularly in recurrent airway obstruction (RAO) or inflammatory airway disease (IAD) remain poorly understood. Feed contaminants such as moulds and associated mycotoxins (secondary mould metabolites) are implicated in the aetiology of respiratory disease. Contaminated forage and grain present significant respiratory challenges to horses (Buckley 2007) with organisms such as Aspergillus fumigatus, Thermoactinomyces vulgaris and Micropolyspora faeni being especially deleterious. For example, inhaled mycotoxins have been specifically linked with the development of RAO in horses.

Conversely, feed ingredients such as antioxidants (and co-factors), omega-3 fatty acids and mycotoxin binders have received attention recently for potential therapeutic efficacy. Polymeric glucomannan mycotoxin binders have been reported to alleviate clinical indicators of mycotoxicosis (Fusarium sp) including suppressed appetite and serum gamma-glutamyltransferase activity (Raymond et al. 2003).

Endogenous and dietary antioxidants are believed to play a central role in respiratory health, for example, an association has been made between oxidative stress and exercise induced pulmonary haemorrhage (EIPH) and RAO. The oxygen-rich lung tissue is susceptible to damage from ROS thus strong antioxidant defences from ascorbate and glutathione, as well as selenium or copper which function as co-factors for the antioxidant enzymes, glutathione peroxidase (GPx) and superoxide dismutase (SOD) are vital.

In RAO affected horses, both with and without current airway inflammation, the total ascorbic acid concentration was reduced and the redox ratio (ratio of oxidised ascorbic acid to total ascorbic acid) was increased in pulmonary epithelial lining fluid. Furthermore, the extent of airway inflammation was correlated with the magnitude of this change (Deaton et al. 2004). In addition, (Kirschvink et al. 2002) has previously reported that exercise tolerance and endoscopic inflammation score are improved in RAO affected horses in remission following 4 weeks supplementation with vitamins E and C and selenium. However, absorption of ascorbic acid in horses is poor but supplementation with both ascorbyl palmitate and calcium ascorbyl-2-monophosphate (CAP) can increase ascorbic acid concentration in lung lining fluid.

There are studies to support a protective role for omega-3 fatty acids in human asthma, a related condition to RAO. However, supplementation of RAO affected horses with long chain omega-3 fatty acids in horses did not significantly alter clinical indicators of pulmonary function despite some changes to the omega-3:omega-6 ratio in plasma and leukocyte plasma membranes (Khol-Parisini et al. 2007).

Gastrointestinal Health. The anatomical evolution of the horse's gastrointestinal (GI) tract, coupled with our modern feeding practices make horses susceptible to a number of GI problems.

The microbial community within the equine GI tract is dynamic and is very sensitive to certain dietary factors including sudden dietary

change, as well as the starch and fibre composition of the diet (Medina et al. 2002). Veterinary medications such as antibiotics (Fey et al. 1997) and possibly anthelmintics (Goachet et al. 2004) and management factors such as environmental change and transportation are all reported to impact on the microbial balance of the GI tract, which may contribute to the development of GI issues in some instances. Opportunistic growth of pathogenic bacteria such as Salmonella sp., Clostridium sp., E. coli sp. and other organisms including Cryptosporidium are implicated in colic, colitis, laminitis and diarrhoea.

Probiotic, prebiotic and other nutraceutical ingredients are of interest due to their reputed ability to modify the hindgut environment and microbial balance therein.

Prebiotics. Short chain fructooligosaccharide (ScFOS), typically derived from sugar beet or chicory, have been reported to reduce the incidence of colic (Volter 1999), but the degree of efficacy may relate to the quantity of prebiotic administered.

Mannanoligosaccharides (MOS), which are prebiotics derived from yeast cell walls reputedly bind pathogenic bacteria within the GI tract, thus reducing their ability to adhere to the gut wall and initiate disease. There are numerous studies to support this action in other species and (Ott 2005) reported that the incidence of diarrhoea, where treatment was justified, was significantly reduced in the foals of mares supplemented with MOS prior to and following foaling.

Live Yeasts. The live yeast SC is one of the more extensively studied probiotic ingredients available, with the majority of the published work in horses to date having used the strain NCYC 1026. There is growing evidence to suggest that live yeast ameliorates the potentially detrimental changes to the hindgut population and environment that are associated with feeding high starch rations (Julliand 2006).

A direct effect of live yeast on GI issues such as colic, laminitis and diarrhoea has not been established, although Saccharomyces boulardii was suggested to significantly decrease the severity and duration of acute enterocolitis in a group of hospitalised horses (Desrochers et al. 2005) .

Bacterial Probiotics. The effect of bacterial probiotic species in horses is unclear, with contradictory research being published. This is probably because efficacy is likely to be strain specific and therefore beneficial results for a particular strain should not be extrapolated to others.

Psyllium. Consumption of sand and dirt by horses can contribute to colic, weight loss and chronic diarrhoea through irritation and obstruction of the GI tract. Psyllium husk has been used historically as a preventative for sand colic and there is some recent evidence that supports its efficacy both in isolation (1000g) (Hotwagner et al. 2008) and in combination with SC, lactobacillus acidophilus and enterococcus faecium (200g) (Landes et al. 2008).

Mycotoxin Binders. Although not unequivocal, mycotoxin ingestion has been proposed as a factor in the development of colic (Barnett et al. 1995). Whilst avoidance of mycotoxin contamination is desirable, mycotoxin binders can be used to facilitate their removal from the gut.

Equine Gastric Ulcer Syndrome (EGUS)

Numerous studies conducted in the 1980s and '90s with the advent of gastroscopy identified EGUS as a widespread problem for horses within a variety of different sporting disciplines (58-90%). In contrast, the incidence in grazed horses appears to be considerably lower

(see review by Bell et al 2007).

In EGUS the damage caused to the lining of the stomach arises from prolonged exposure of the gastric mucosa to gastric acid and associated proteolytic enzymes, in the absence of sufficient protection, such as from the buffering effect of saliva.

Horses produce gastric acid continuously, and high starch diets tend to prolong postprandial acid secretion. In addition, large meals can lead to inadequate mixing in the stomach and localised starch fermentation, which may contribute to gastric damage (Nadeau et al. 2003).

During feeding, chewing normally stimulates the production of saliva, which contains a natural buffer 'bicarbonate', which proffers some protection to the gastric mucosa. The amount of saliva produced in response to forage is about twice that produced for the same dry weight of concentrate feed, as proportionally more chewing is involved. Feeding multiple forms of forage can increase overall forage intake (Thorne et al. 2005). Horses fed alfalfa have shown a lower incidence of gastric ulcers compared to those fed grass hay (Nadeau et al. 2000).

During periods when the horse has no access to feed or forage, the protective affect of saliva is reduced. Unfed horses have a significantly lower gastric pH, compared to those with free access to hay (Murray et al. 1993). .

Repeated electrolyte or salt administration may also exacerbate gastric ulceration (Holbrook et al. 2005). Conversely, the addition of oil to the diet may offer some protection against gastric ulceration by improving the endogenous protection offered by the gastric mucosa (Cargile et al. 2004).

Supplements fed to help maintain gastric mucosal health are unlikely to be as effective as proven veterinary drug treatments such as omeprazole. However, they can be used as a useful adjunct to veterinary therapy where efficacy is present. Ingredients with antacid properties such as aluminium hydroxide and magnesium hydroxide are typically used, as are others that have the ability to reduce inflammation or to form a protective coating on the gastric mucosa. .

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Natural solutions for horse nutrition

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Summary

Equine nutrition has come a long way in the last decade. Maintenance of normal gut function is down to a combination of management and animal factors; natural products may form part of that process. Saccharomyces cerevisiae strains are used in animal nutrition to enhance fibre digestion and the feeding of mannanoligosaccharides (MOS) has been shown, in many species, to reduce colonisation of pathogenic bacteria. Interest in organic mineral supplementation has increased over the years and the potential advantages in digestion, absorption and transfer to tissues examined. It is generally accepted that the bioavailability of dietary components is greater for organic rather than inorganic forms of minerals. The threat of mycotoxins, which are implicated in the aetiology of various health issues has led to interest in the inclusion of adsorbents in feed. One such adsorbent, glucomannan oligosaccharides, has been shown to effectively bind many different types of mycotoxins.

Introduction

Laminitis, colic, respiratory health and muscle dysfunctions are common causes of distress and suffering, as well as economical loss. The link between these problems and nutrition is well established, however, relationships are not always clear and often multifactoral. Much equine-related nutritional research has focused on solutions to these and similar issues. Over the years, organisations have developed and marketed numerous products to address them, such as live and dead yeasts, various mineral supplements and prebiotics. This paper will attempt to discuss the key natural solutions currently available for horse diets.

Maintaining optimal gut function

Maintenance of normal gut function is down to a combination of management, as well as animal factors, and supplemental products may form part of that combination. The horse has evolved to require a minimum amount of dietary fibre as a food source for micro-organisms located in the caecum and large intestine [1]. In addition, horses can digest very little starch at any one time and, consequently, the small intestine struggles to deal with concentrate meal containing more than 3-4g starch per kilogram of body weight per meal [16]. Disruption of normal hindgut function by reduced dietary fibre or increased dietary starch can result in reduced buffering capacity and decreased caecal and blood pH leading to acidosis. Additionally, many caecal microbes cannot survive this low pH and die, releasing bacterial endotoxins into the blood stream. Acidosis actually relates to a lowering of blood alkali reserves and many horses suffer from a sub-clinical form, particularly those in hard work coupled with limited access to forage. In this situation it may be prudent to consider a supplement/additive to help beneficially modify the hindgut environment. Live strains of the yeast, Saccharomyces cerevisiae are used in animal nutrition to enhance fibre digestion in both ruminants and non-ruminants due to their ability to metabolise nutrients in an anaerobic environment and promote the growth of beneficial bacterial [14]. One particular strain, 1026, is effective in its

stimulation of fibre-digesting and lactic acid-utilising bacteria [13], which can create a more stable gut environment, reducing the risk of gut disturbances and, potentially, insulin resistance. Studies have demonstrated improved fibre digestibility [9] and increased caecal pH [12][10] in horses on high starch diets receiving a live yeast culture (Yea-Sacc®^{1026}, Alltech Inc., Nicholasville, KY)) compared with unsupplemented horses. This higher pH stabilises the caecal environment, which is conducive to improved feed efficiency and prevention of gut disturbances. Increased fibre digestion was also noted by Jouany et al. [6] irrespective of whether the diet was high in fibre or starch. The impact of live yeast appears to go beyond simply increasing fibre digestion in some cases. Increased utilisation of phosphorous in horses supplemented with S. cerevisiae cultures, such as Yea-Sacc®^{1026} has been described [2], which may be of interest when feeding the aged horse [5]. Beneficial effects have also been seen in mares during gestation with regard to milk quantity and quality, resulting in faster growing foals [3].

One digestive problem that frequently occurs in young animals is diarrhoea, resulting in poor hydration status and ill thrift. As the feeding of antibiotics as a prophylactic measure is restricted in some countries, research has been conducted into more natural solutions. The feeding of mannanoligosaccharides (MOS) to reduce the insult from pathogenic bacteria has attracted much attention in both horses and livestock alike. MOS is derived from the outer cell wall of yeast and is known to have the capacity to bind pathogenic bacteria, preventing their adherence to, and colonisation of, the gut lining. Coupled with this, the strain on the immune system is much reduced and more energy can be partitioned to growth. It has been demonstrated that supplementing the mare with MOS (Bio-Mos®, Alltech Inc., Nicholasville, KY) resulted in higher immunoglobulin concentrations in the colostrum [20] corresponding to an increased average daily gain of the foals from supplemented dams (unpublished data). Stimulation of the non-specific immune response by MOS has been demonstrated in rodents [24], as well as stimulation of humoral immunity, which is of importance with regard to vaccine efficacy.

Maintaining optimal antioxidant function

Oxygen is an essential part of mammalian life, however, there are detrimental effects of generating cellular energy from oxygen i.e. the production of free radicals. Despite this, the body has developed mechanisms to minimise this, including the utilisation of antioxidants. Thus, the overall effect of free radicals depends on the scale of their production coupled with the efficiency of the animal's antioxidant response [21]. This first line of defence involves mineral-containing enzymes, including superoxide dismutase, glutathione peroxidise and catalase, to name but a few. Minerals, such as zinc, manganese and copper also play key roles in the implementation of this defence.

Selenium is a component of around 25 proteins, including glutathione peroxidises, thioredoxin reductase and iodothyronine deiodinases. These selenoproteins have major roles in metabolism and rely on the availability of Se in the body and/or diet. Se also plays a role in immunocompetence, gene expression, thyroid metabolism and male fertility. Therefore, adequate Se status in the horse is vital for optimum health and performance. Many areas of Europe are denoted as having Se-deficient soils (especially Scandinavia) and it is common place to fortify feed with inorganic Se, usually as sodium selenite (NaSe). However, there are marked differences in the efficacy of absorption and metabolism of supplemental inorganic vs organic Se [23]. Selenium that is absorbed from organic sources, such as selenium yeast can be incorporated into either selenoenzymes or general proteins as storage for future use [22]. This enables the animal to build Se reserves for times of physiological stress, such as parturition, illness and aerobic exercise. Exercise may be of particular importance with regards to Exertional Rhabdomyolysis. Many studies

have shown greater incorporation of Se as selenoproteins/amino acids from selenium yeast into body tissues compared with NaSe [15][8] [18][7]. Aside from the physiological benefits of organic over inorganic Se sources, there is also the issue of toxicity. Sodium selenite is toxic to mammals in certain concentrations, in contrast to Sel-Plex®(Alltech Inc., Nicholasville, KY) for example, for which no LD50 has been found.

Zinc, copper and manganese act as co-factors for antioxidant enzymes, thus availability in the diet impacts on overall antioxidant status. As with Se, the body requires adequate levels that can be called upon in times of physiological stress. As discussed previously, the form in which the mineral is supplied in the diet is important and should mirror the form that animals have evolved to utilise. It is generally accepted that the bioavailability of dietary components is greater when attached to organic rather than inorganic ligands [4]. Minerals chelated with amino acids are stable, water-soluble [4] and are actively absorbed across the intestinal membrane. Inorganic minerals are subject to interaction with other compounds that may reduce the availability of the mineral. Mixtures of chelated minerals, such as Bio-Plex minerals (Alltech Inc., Nicholasville, KY) have demonstrated increased mineral retention in yearlings [11].

The mycotoxin threat

Mycotoxins are secondary fungal metabolites, which can contaminate feeds resulting in poor condition, lethargy, depression, lameness [19] and sometimes death. For horses, important mycotoxins include aflatoxins, zearalenone and fumonisins. The increased feeding of haylage has highlighted the threat of mycotoxins, which are implicated in the aetiology of health issues, such as colic and respiratory diseases. Avoiding contamination is difficult and testing for mycotoxins is expensive, therefore, one solution is to include a mycotoxin adsorbent in the ration. Effective mycotoxin adsorbents should have a low effective inclusion rate, be stable over a wide pH range, high capacity and affinity for mycotoxins and be able to affirm the chemical interaction with the mycotoxin. Many adsorbents are based on clays, however, a natural adsorbent in the form of glucomannan oligosaccharide has been shown to effectively bind many different types of mycotoxins. Raymond et al. [17] noted decreased reduction in feed intake of, and decreased toxin absorption from, contaminated grains in mares. Routine inclusion of effective, natural adsorbents at times when contamination risk is high may be prudent to minimise the possibility of adverse health and performance effects.

Conclusion

Maintaining gut health, as well as providing the horse with an antioxidant reserve minimises the risk of gut disturbances, respiratory and performance problems. Natural solutions include digestive enhancers in the form of live yeasts, antioxidant minerals as organic chelates and mycotoxin adsorbents. There is no doubt that a holistic approach to feeding is conducive to maximising gut function.

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How can Bedding and Forage influence Pulmonary Function?

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Introduction

It is well-known that several equine respiratory disorders are directly caused by or exacerbated by inhalation of airborne dust [1], with the most widely recognised example of this being recurrent airway obstruction RAO (previously termed chronic obstructive pulmonary disease, COPD) or Heaves. Of all organs, the respiratory tract presents the largest and most delicate surface of contact with potentially deleterious airborne material. Breathing exposes the lungs to the adverse effects of a complex mixture of gaseous and particulate pollutants [10]. Heaves is caused by inhalation of agents in airborne organic dust including moulds and bacterial endotoxins. Inflammatory airway disease, another chronic pulmonary disease mainly encountered in young race horses subjected to strenuous exercise, may also be associated with inhalation of airborne pollutants including micro-organisms and organic dusts [2]. Acute respiratory diseases, including those caused by infectious agents, may be exacerbated and prolonged by concomitant dust inhalation. Furthermore, it has been proposed that heaves may develop after a respiratory viral infection, and that reduction of airborne dust is thus an important consideration in the management of horses with viral infection to prevent the potential subsequent development of heaves [11]. According to the definition of the term used in human medicine "occupational disease", as any chronic ailment that occurs as a result of work or occupational activity, RAO may be seen as an occupational disease of the horse. In fact the link between the management and hygiene of the horse kept for sport or for pleasure and their pulmonary function is very close. In this paper we will expose the major survey-based consolidate findings on different horse management systems and their effects on pulmonary function and health of the horse.

Material and Methods

Methods of quantification for respirable airborne organic dust and aeroallergens. As there are different types of dust present in horse barns, boxes and other stabling systems it is necessary to differentiate between those particles which penetrate the respiratory system causing respiratory reactions and those who do not. Respirable dust refers to dust particles of sufficiently small aerodynamic size ($< 5\mu$m) to allow deposition within the lower respiratory tract. This respirable fraction is considered as a reliable index of the respiratory hazard associated with dust inhalation [3, 7]. Traditionally different samplers have been used for measuring *airborne dust concentration (ADC)* by collecting the dust onto pre-weighed filters permitting the calculation of mean, not maximum ADC. Woods et al. used a Marple Personal Cascade Impactor attached to the halter of the pony with a genesis air pump to operate the sampler attached to a surcingle to the ponies back. Having the sampling heads attached on either side of the head collar, 15 cm from the nostrils allowed

collecting airborne dust present in the ponys breathing zone. This provided a more accurate indication of the amount of dust entering the respiratory tract than with sole remote sampling [14, 26]. Sampling of the airborne dust concentrations in the general area of the stall were conducted with other types of particulate samplers (8-stage cascade Impactor).
Measurements may be also done on samples of dust collected on forage and bedding samples giving an objective assessment of a given forage and bedding [23]. As in the before named procedures, here too, it must be carefully standardised to obtain reliable information. Results are usually given as the number of particles/litre of air.
Recently though real-time continuous particle monitors have shown their advantages in identifying short duration elevations in ADC which may be detrimental to equine respiratory health in the face of a relatively low mean ADC [4].
For this purpose a compact portable laser photometer that measures and records ADC in real-time revealed itself as practicable. It is generally used to measure aerosols in a wide variety of environments, from offices to industrial workplaces. In order to ensure exclusive sampling of the respirable dust fraction, the aerosol stream is initially drawn through a cyclone pre-classifier [4].
The microflora of hay which is baled with high moisture content is dominated by thermotolerant actinomyces and fungi, including *Aspergillus fumigatus*, *Faenia rectivurgula* (previously termed *Micropolyspora faeni*) and *Thermoactinomyces vulgaris*. These moulds are considered to be largely responsible for induction of pulmonary inflammation in heaves affected horses [7, 13, 15]. These mould spores pose special problems for identification because most of the spores of fungi and actinomyces cannot be identified by microscopic examination alone [9]. Therefore, identification may require culture on Sabouraud's media at 46 to 56°C [23].

Results

Environmental prevention of pulmonary dysfunction. The primary sources of airborne dust in equine stables are the feed and bedding, and the highest respirable dust exposure results from mould-contaminated forage and/or bedding. Dust levels at mucking out may reach 10-15 mg/m^3, comprising 20-60% respirable particles. This equates to 3000 respirable particles/ml or 12,000,000 inhaled particles/breath [1]. It is thus clear that many stables contain concentrations of total dust which exceed the suggested provisional air hygiene standard for biologically active dust exposure in cotton mill workers of < 2.5 mg/m^3. Thankfully, effective hygiene measures may reduce dust exposure by up to 90% [8].
When examining the effects of stable design and ventilation it results important to guarantee a ventilation rate of at least five air changes per hour. Webster et al. Notice that in stables with a clean, well-manage bedding this ventilation rate is obtained without mechanical intervention provided the top half of the stable door is kept open at all times [25].
In a study on airborne dust and aeroallergen concentration in a stable under two different management systems, Woods et al. [26] measured mean total and respirable ADC of 17,51 mg/m^3 and 9,28 mg/m^3, respectively, in the breathing zone of a horse managed in a conventional hay and straw system. Reducing the horse's exposure to respirable dust in the breathing zone resulted as critical. Mean breathing zone total and respirable ADC of 0,52 mg/m^3 and 0,3 mg/m^3, respectively, were achieved by utilizing feed and bedding with low dust concentration (wood shaving as bedding and pelleted feed) [26].
These findings where confirmed also in a study of Clements and Pirie [4] when using the mentioned more advantageous real-time continuous particle monitors in various management systems. They revealed that both mean and maximum breathing zone respirable dust concentration (RDC) were significantly reduced in equine stables by changing the environment

from hay feed and straw bedding, to haylage feed and wood shavings bedding. Furthermore, comparing the influence on breathing zone RDC of feed and bedding, there was no significant difference in mean RDC between environments with the same feed source, irrespective of the bedding material used. This made the authors conclude that feed had a greater influence on mean and maximum RDC than bedding [4]. The main reason for this finding was likely due to the fact that measurements were made in the region of the horse's breathing zone and more time will be spent with the nostrils in close proximity to *ad libitum* forage compared with bedding. As mentioned in the methods of dust quantification this method is considered important as it more accurately reflects the likely challenge to the respiratory system in common stabling compared with more remote sampling.

It is known that soaking hay has a positive effect on respirable dust concentrations [5, 16]. The experimental procedures state that soaking hay for a period of 30 minutes is sufficient to reduce respirable challenge with minimum nutrient loss (Moore-coyler 1996). Interestingly enough also the environment in a neighbouring stable has an important influence. In stables within a common airspace the mean and maximum background RDC was significantly reduced by implementing management changes (low dust management) in a neighbouring stable [5].

Total and respirable airborne dust endotoxin concentration also was analyzed separately in order to determine its role in causing respiratory exacerbations in predisposed horses. It was firstly determined that endotoxin concentration is significantly higher in conventionally managed stables when compared to low dust stables and pasture management [14]. A few years later the role of inhaled endotoxin on pulmonary and systemic effects was investigated by challenging asymptomatic heaves-susceptible horses and control horses with soluble lipopolysaccharide (LPS). Detectable lung dysfunction was induced in heaves-susceptible horses although clinical score, tracheal secretion and airway reactivity remained the same. LPS also induces a dose-dependent neutrophilic airway inflammatory response in both groups. Comparison of the effects of acute LPS inhalation and hay/straw challenges suggest that inhaled endotoxins is not the only cause of heaves [17]. The interaction of different types of allergens in the respirable airborne dust seems to be necessary to cause inflammatory pulmonary reaction. This was confirmed one year later by the same research group. When fractioning hay dust suspensions and administering the fractions singularly through inhalation challenges, there was a significantly lower reactive airway response than when all fractions were administered together [18]. Also when comparing the response of heaves-affected horses with the response of control horses after challenge with three mould antigens (*Micropolyspore faeni, Aspergillus fumigates* and *Thermoactinomyces vulageris*) there resulted to be a reaction in both groups on *T.vulgaris* while *M.faeni* and *A.fumigates* affected only the heaves-susceptible group. The absence of pulmonary disease in control horses after challenges confirms that heaves is a pulmonary hypersensitivity, rather than a non-specific toxic response [13]. In conclusion there results to be a synergic effect of all components in organic airborne dust in traditionally managed stable environments in affecting pulmonary function.

Conclusion

The most simple way to prevent and treat pulmonary diseases which are caused or exacerbated by inhaled organic dusts, including heaves, is to reduce the level of exposure to airborne dust [20]. Of all management improvements keeping horses permanently outside, with a shelter against inclement weather and no supplementary hay feeding, is the ideal dust-free regime [1]. Reduction of clinical signs und lung dysfunction in most heaves-affected horses occurs after 3-7 days on pasture [6].

Unfortunately this becomes difficult for numerous practical reasons and sport horses often are

stabled indoor. Low dust forage is essential to reduce negative effects on pulmonary function. Besides pelleted diets and soaking hay, silage is currently the best, but quite expensive alternative to hay [1]. Hereby it is important to minimise the possibility of a horse developing botulism from silage feeding by using only airtight bags, by consuming the silage within five days after opening the bag or by vaccinate horses against botulism. Botulism is mainly associated with the use of big bale silage [12, 19].

Low-dust bedding has also shown to be an important source of airborne fungal spores, endotoxins and other pro-inflammatory agents. Therefore wood shavings, paper bedding, peat moss and sawdust are suitable alternatives to straw [21, 22, 24]. However as with straw, they can become a source of respirable dust and endotoxins when used in deep litter systems, or in warm and poor ventilated stables. Moreover, poor quality wood shavings may result in higher airborne dust and fungal spore levels than good quality straw [23].

Finally, surrounding environment and improved stable ventilation contribute in an important way to lowering dust concentration since beneficial effects of careful management in one stable may be totally lost by the proximity of hay or straw storage, which is a potential source of inhaled particles.

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Chapter 34

Block 9A

Assessing practical markers for their suitability in estimating the pain experienced by horses with laminitis.

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Summary

The assessment of pain experienced by horses is complex, often inaccurate, and varies widely among practitioners. During laminitis it is supposed that horses suffer severely from pain. It would be ideal if there were an accurate, reliable and sensitive method of assessing this pain as the condition progresses, and as treatment is applied, to improve the condition of the horse. This work considers various parameters and their suitability as markers to assess the pain experienced by horses undergoing treatment for laminitis. 14 horses were assessed during their treatment period. Heart rate, respiration rate and hoof temperature were all significantly correlated with the Obel grading score for lameness. Other parameters, including body temperature, digital pulse and behavioural attitude were not. The horses improved their lameness grade over the period of the trial. It is concluded that the use of the simple practical measures described may be usefully applied by owners and practitioners as markers to estimate the pain suffered by horses under their care.

The aim of this study was to analyse the amount of pain horses suffer when recovering from laminitis.

Introduction

The purpose of this research project was to analyse the amount of the pain experienced by the horse when recovering from laminitis, pain which has been described as "unrelenting" [9], which would assist the veterinarian, and the owner of the horse, to make an informed decision regarding an indivudual horse's future. Medical assessment of pain experienced by humans is difficult enough, where the patient can respond and verbalise their experience. With animals this is much more complex, and with prey animals this is further complicated as they are likely to express expressions of pain as this might attract the attentions of a predator [1]. As reported [10] there is a wide variation in the the scoring and management of pain in horses among veterinarians themselves A range of assessment tools are used. Previous work looking at before and after treatment with non-steroidal anti-inflammatory drugs has shown that hormone levels were largely unaffected by treatment [13] and hormone levels remained unaffected after painful orhtopaedic surgery [11] and selected behavioural responses, including those of movement of a presumed painful leg, showed no differences. Reliable assessments of pain remain to be determined even in the light of new technological tools such as diagnostic imaging [3]. There remains no gold standard method, technique or measurement that is reliable, repeatable and sensitive [15].

The pathophysiology of laminitis in the horse remains unclear [2] ,and medication remains of limited help if the laminitis is severe. The causes, suffering and partial recovery are varied [7] and as a result horses continue to suffer, not only in one locality, but worldwide. Between 75-80% of laminitis cases identified do not recover [12]. It would be of great assistance to veterinarians and horse owners to have

a better understanding of the pain experienced by equines with this condition, enabling early preventative treatment or early decisions regarding euthanasia of the suffering individual; thereby reducing the overall suffering experienced both by individual horses, and the anxiety and distress of their owners.

The Obel scoring stystem [8] is considered to be an accurate means of assessing the severity of laminitis [6], and it was decided to use this, subjective measure, as the reference measure for other parameters that might be more easily used by the practitioner and owner. Such measures included respiration rate, heart rate, body and hoof temperatures and others indicated in the method section of this paper.

Material and Methods

There were 14 horses sampled in this study from a wide range of sources: warmbloods, Finnhorses, Icelandic horses and ponies. Icelandic horses were included as they are more inclined to obesity and therefore more likely to contract laminitis. Ages ranged from five to 26 years.

The study took place at Hyvinkään equine veterinary hospital in Hyvinkää, southern Finland, in the summer of 2008. The parameters below were taken on a daily basis during the period of their treatment, ranging from 1 day to 11 days. Treatment was with non-steroidal anti-inflammatory drugs (NSAID).

On arrival at the hospital an anamnesis was taken of each horse. The first inspection, as the subsequent daily observations, included measurements of attitude, heart rate, respiratory rate, temperature, digital pulse, temperature of the hoof. Lameness was evaluated with the Obel grading score[8]. This method has been described previously [4]. It is a scale from 0-4. Obel Grade I features frequent shifting of weight between the feet, no discernible lameness at the walk, and bilateral lameness at the trot. Obel Grade II horses do not resist having a foreleg lifted, nor are they reluctant to walk, but they do show lameness at the walk. Obel Grade III horses do resist having a foreleg lifted, and are reluctant to walk. Obel Grade IV horses will walk only if forced. The same assessor estimated lameness using this grade on each occasion throughout the study.

Attitude represented observation of their behaviour. This included: standing position of the horse, appetite; fresh hay was offered and its acceptance or otherwise was noted; position in the box, interest in its environment and signs of pain were recorded. Patients were observed if they were shifting their weight in the box and how much they were laying down.

Heart rate was measured manually, with a stethoscope, and respiration rate by observation by the first author. Temperature was taken rectally with a digital thermometer. The thermometer used was an ADC ADTEMP 422 Veterinary Digital Thermometer. Digital pulse was measured by palpation of the horse's digital arteries, which are on the inside and outside of each leg at the level of the fetlock and pastern. A normal horse should have a pulse that is very slight or difficult to feel.

The temperature of the hoof was assessed in comparison with the temperature of the adjacent hoof, by digital palpation by the first author, using a method previously described [13].

Each of the horses staying overnight in the hospital were housed in individual boxes, with a thin layer of either wood shavings or peat as bedding. The choice of bedding was not related to factors related to laminitis, but simply of availability.

Each horse was offered a diet solely of grass hay, and this was provided ad libitum. No additional feed was provided.

Data were analysed for correlations between parameters measured and Obel scoring for each horse. Obel grade was also correlated with days across all horses, using the Minitab statistical package, version 13.

Results

As the numbers of observations for each horse were highly unbalanced means scores for each parameter were calculated for each horse across days. These parameters were then correlated with the Obel grade.

Significant correlations were observed between Obel grade and: respiration rate (P = 0.008), heart rate (P = 0.005) and hoof temperature (P = 0.026). The most significant of these, the heart rate scores gave an R-squared value of 50.1% suggesting a possible, though not large, predictive value for this parameter.

Using multiple regression analysis, combining heart rate with hoof temperature gave a significant relationship (P = 0.007) with Obel grade, and an R-squared value of 59.4%. Further multiple regression analysis was not analysed, on the basis that the sample size of 14 horses would not support further statistical manipulation than the incorporation of two parameters into the model.

No correlations were found for either rotation, body temperature, digital pulse from either the front hind limbs, or behavioural attitudinal measures.

There was a significant day effect (P<0.001)00, with mean Obel grades negatively correlated (-0.905) with number of days over the study period .

Only one horse was euthanased at the completion of the data collection period, on the grounds of; the data from this horse was included in this analysis.

Discussion and Conclusion

That three of the measured parameters, heart rate, respiration rate and hoof temperature were significantly correlated with the Obel grading of lameness gives hope that a simple measure, of practical and practicable use to owners and veterinary practitioners alike, may be realisable.

Although an increase in heart rate has been identified previously as a measure of pain experienced by animals [14], the finding that heart rate was significantly correlated with Obel grade was not expected, following the findings of [11]. However, these authors compared before and after (up to 72 hours after) surgery measures, and it may be that the current study, over a longer period, identified the response of increased heart rate to pain experienced over a longer period.

It may be that combining each of the significant parameters measured in this study: heart rate, hoof temperature and respiration rate, might give a more reliable index to assess pain than the use of one of these measures in isolation. There is evidence that this is the case for a combination of heart rate and hoof temperature.

Behavioural assessments did not show significant correlations with lameness, which is a finding that is in line with previous observations [11]. Therefore their use, or at least their sole use, cannot be recommended as reliable indicators of pain experienced by the horse in cases of laminitis. Of the other parameters tested and found to show no significant correlation with Obel grade: temperature, digital pulse and rotation, the last is confirmation of other findings [5] who also found no effect on the degree of rotation.

It might be argued that the Obel grade itself could be used to assess levels of pain, as it is already considered a reliable estimate of lameness [6]. However, this is not a sensitive scoring system, using a system of four grading levels, might not reliably assess pain, and involves the horse in moving and trotting to assess the grade, activities that might well be a source of additional pain to the animal.

The finding that Obel grade was significantly negatively correlated with day in treatment is a reassuring one, at least to this practice. Lameness among the horses declined during their treatment at the equine hospital.

While not claiming to have found the ”gold

standard" for accurate and reliable pain assessments in the horse, identified elsewhere [15], this study provides hope that simple, applicable parameters can possibly be used by horse owners and practitioners as a guide to the pain experienced by horses suffering from laminitis. Furthermore, the use of such markers may allow the identification of a problem before the onset of clinical laminitis.

If it can be shown that these findings are repeatable, owners of horses with laminitis, and veterinarians may have practical and applicable markers for pain experienced by the horses under their care. This has applications in the prompt consultation of veterinarians by owners, and efforts to provide analgesia. Recovery from this affliction could also be observed rapidly by owners, and conversely continuing evidence for high levels of pain in individual horses identified, allowing early judgement regarding the advisability of euthanasia which would reduce the length of suffering endured by the horse.

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Effect of different group sizes on activity, lying and social behaviour of young horses

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Summary

It is often discussed that the inactivity of horses causes diseases of their musculoskeletal system. Due to these problems the objective of the investigation was to quantify if the size of a group does has an effect on the behaviour of young horses. Data from 42 horses in the age of one to two years have been involved in the investigation. The data of two small groups were compared with data of one big group with 23 horses. The movement and lying behaviour of 28 horses were measured with ALT pedometers. The social behaviour of 33 yearlings was documented by direct observation. The results show that the median of activity of horses in group A is 82 activity impulses per fifteen minutes. In group B this increases to a median of 101 and group C reach the highest median of 149. The investigations of the lying behaviour show a significant difference between the three groups. International research studies have been shown that keeping of horses in big group husbandry systems is not very common by the owners of horses. In contrast these investigations have shown that horse keeping in big groups has no negative influence on the social behaviour of horses.

Introduction

It is often discussed that the inactivity of horses causes diseases of their musculoskeletal system. Due to these problems the objective of the investigation was to quantify if the size of a group does has an effect on the behaviour of young horses. Foals have to be reared in big pasture areas in herds. This helps to get physical and psychical resilient animals [9, 13]. Recent studies claimed to keep young horses in group husbandry, due to this period's importance for growing impulses and encouragement of normal social behaviour [11]. Co-housing of other well compatible horses could help to bring more activity into the herd [12, 13]. Lack of free movement is not just a problem in the winter months [8]. Thus in spite of increasing group housing systems in young horse husbandry big walking areas are missing [1, 2]. In this investigation the parameters movement activity, lying behaviour and social behaviour have been analysed. The aim of this experiment was to investigate the effect of small horse groups versus big horse groups on behaviour and activity of young horses.

Material and Methods

Data from 42 one to two years old horses have been included in the investigation. All horses were kept in group housing divided into three different group sizes.

Herds of young horses of three national stud farms were included in the experiment. For

the stud farms A and B yearlings born in the year 2007 were used. In farm C two years old mares were available for the study (year of birth 2006).

The data of two small groups A and C (eight and eleven horses) were compared with data of one big group of 23 horses (group B). On farm A 8 horses and on farm B and C 10 horses were used in the test groups. Activity lying temperature pedometers (ALT pedometers) were used to record movement activity and resting time through out 24 hours a day. The movement and lying behaviour of 28 horses were measured with ALT pedometers. Animal data were recorded throughout the complete test period of ten days per group using measuring intervals of 15 minutes. The pedometers are attached to the horse's rear leg to avoid gathering activity impulses from scraping and pawing hooves.

The stored data were red out from the pedometer by radio transmission and were stored in a MS Access database for further processing.

The social behaviour of 33 yearlings was documented by a present observer during two hours after reading out the pedometers. All aggressive interactions were manually recorded and classified in aggressive display (e. g. flat ears) and aggressive interactions (e. g. biting, kicking).

Statistical evaluation of the ALT pedometers was carried out with the software program SAS version 9.1.3. service pack 4 (SAS Institute Inc., Cary, NC). All traits were tested for normality with the Shapiro-Wilk-Test included in the UNIVARITE procedure with the NORMAL option and were found to not follow normal distribution. Thus all hypotheses were tested with the Kruskal-Wallis-Test provided in the NPAR1WAY procedure, using the WILCOXON option. The activity impulses were transformed into minutes by division by 120. Outliers range up to 1000 minutes.

Results and Discussion

As shown in figure 1 movement behaviour increased with group size. A doubled group size leads to twice the recorded activity impulses. The results show that the median activity of horses in group A is 82 activity impulses per fifteen minutes. In group B this increases to a median of 101 and group C reach the highest median of 149. These differences are significant at an error level of $\alpha = 0.05$. The increasing age of the young horses had no influence on the movement activity. International research studies have shown that keeping of horses in big groups is often not accepted and is described as "not natural" [7, 10, 15]. In contrast these investigations have shown that horse keeping in big groups has no negative influence on the social behaviour of horses. Also other authors refer that in wildlife herds with 50 animals are also possible [9]. An increasing of the movement activity was expected, because there is more interaction between the animals. These results correspond well to other studies that have found more voluntarily motion in group housing systems [13, 14]. Additionally living in a social structure helps the growing horses to promote metabolism and strengthen joints and to prevent locomotive and respiratory diseases [2]. Increased activity is better for the horse's health compared to less activity [4]. Joint diseases are the main reason for culling of horses because of inactivity [5, 16].

The investigations of the lying behaviour show a significant difference between all groups which are also found in paired tests between the farms (table 1).

Our hypothesis that a higher number of horses decreased the lying time was not confirmed. All investigated herds had nearly same resting time. In contrast [6] found out, that the lying time in bigger groups is increased, so that higher group sizes should not be used in horse husbandry. The calculated of our experiment data show that animals in group B lie down 10.9 times per day while the small groups lie down 6.6 times per day. There was a lot of interaction between the animals even without getting up. In addition to the movement behaviour the social was recorded. In group A 45 repulsive behaviours had been observed in total. They were divided into 31 (69 %)

aggressive displays and 14 (31 %) aggressive interactions. In group B 149 actions of social behaviours could be observed in the same time interval. There were more aggressive interactions (84, 56 %) than aggressive displays (65, 44 %) altogether. Normally the individual distance was kept by the guideline of (2.5 withers height)^2 [3], but maybe this should be adapted to bigger group sizes.

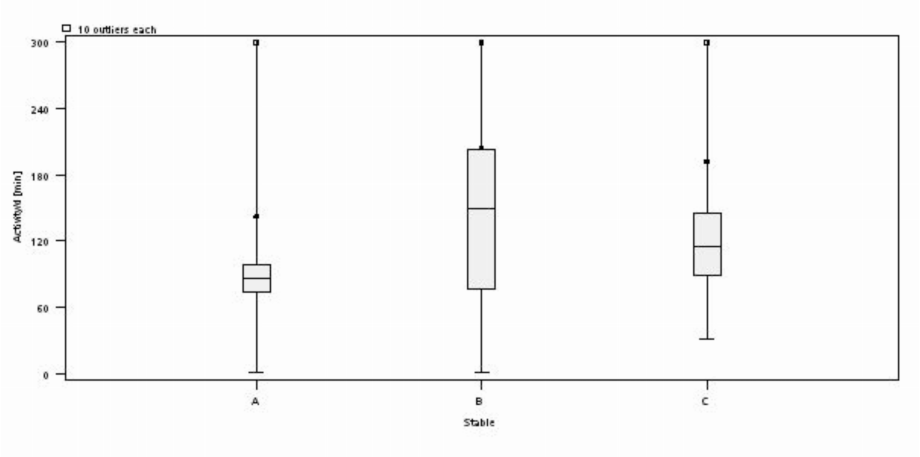


Fig. 1: Activity behaviour

Tab. 1: Results for the resting behaviour

		Measures of location for resting contacts per 15 minutes					Kruskal-Wallis-Test		
Stable	N	Max	Q75	Median	Q25	Min	Chi-Square	DF	Pr > Chi-Square
A	8478	60	30	0	0				
B	4797	60	48	0	0	0	195.3092	2	< .0001
C	6091	60	17	0	0	0			

Conclusion

These investigations support the results of other studies which ascertain that activity of horses is dependent on their space on offer and on the group size. Keeping of horses in bigger groups helps increasing activity and does not cause increasingly more injured horses. Social contact is a necessary component of normal horse behaviour, especially for growing horses. Altogether, if there is a good management, husbandry of young horses in big groups (more than ten animals) is possible.

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A survey on the hygienic standard of feeds for horses and its implication for environmental conditions and animal health

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Summary

Well balanced diets/rations are essential prerequisites for wellbeing, fitness as well as fertility and longevity of horses. Therefore, the energy and nutrient supply is of special interest. It can be assessed by feed intake (amounts and composition of feed) and composition and the analyses of different parameters in body own substrates (blood, urine etc.), respectively. Moreover, the feeding technique has to be considered (relation of roughage to concentrate, the frequency of feeding, amounts per meal). However, the wellbeing and health (especially of the alimentary and the respiratory tract) strongly depend on the hygienic quality of the offered feeds. In the following comments, a diversity of lacks concerning the hygienic status as well as consequences for the horses' health will be presented. The following interpretation is based on 917 feedstuffs sent in for quality check (year 2000 until 2008). Moreover, it has to be emphasised, that these results do not reflect the typical situation in practice, because the evaluated feedstuffs were sent in due to health disorders.

Introduction

Not only a well-balanced diet (energy, nutrients), but also the proper hygienic quality of feeds is essential for a horse's health (e.g. dust development in the stable during feeding, ingestion of undesirable microorganisms etc.). In cases of health disorders, horse owners mainly assume hygienic problems in concentrates. Hence, an in-depth sampling of roughage is usually neglected. But litter material as well as hay or straw belong to the most important sources of (mould) loads of the stable air [1, 4]. The highest concentrations of bacteria and moulds were found in untreated cereals and roughage, whereas processing, like pelleting or purification of feeds (pelleted complete diets for horses) results in reduced counts of microorganisms [3, 6, 4]. Some of the moulds in mildewed feedstuffs are able to produce mycotoxins that affect the immune system [2] and potentially lead to disorders of the respiratory system [8]. Also, conspicuous dusty feedstuffs frequently contain a high contamination by mites [1, 7, 8]. These mites as well as bacteria and moulds, e.g. *Aspergillus*, are able to get into the air in the form of fine and respirable fractions and can lead to respiratory disorders [8]. Therefore, the hygienic quality of feedstuffs does not only represent a direct risk for the horses' health, but also an indirect burden due to a negative influence on environmental hygiene. In order to get an idea of the extent to which feedstuffs are involved in the case history of affected horses, the hygienic status of feed samples (n=917) sent in between 2000 and 2008 was characterized.

Animals, Material and Methods

All feedstuffs were sent in due to health disorders in horses, along with detailed preliminary reports. A sensory examination was carried out for each sample. The evaluated parameters were the grip (texture, moisture), the colour (discolouration due to microbial spoilage), outward appearance (dusty character, massive infestations of pests or mites), the smell (mouldy or yeasty odour etc.) and the microbiological quality (standard analysis methods for bacteria, moulds and yeasts). The results of the microbiological analyses were assessed using benchmarks given by the working committee "feedstuff microbiology" of the VDLUFA in 2007 for the concentrates and by WAGNER et al. [10] for the roughage (hay, straw, silages).

Moreover, the vast majority of the feed samples was also analysed for lipopolysaccharide (LPS) levels (Limulus-Amebocyte-Lysate-Test), which give indirect information about the hygienic standard of the feedstuffs (LPS: part of the cell wall in gram-negative bacteria only). The advantage of this method is that dead bacteria can be detected as well, whereas classical microbiological methods only cover bacteria that live and grow on the different culture media. Therefore, this analysis can also be used for feedstuffs that were processed and hygienised (dried with a high temperature, pelleted or acidified feedstuffs).

Results and Discussion

The sensory control of the feedstuffs showed high variations of the smell in oats and roughage. Mites were mainly found in oats, whereas hay predominantly contains pests like *Lepinotus spp.* (see table 1).

Table 1: Sensory examination of feedstuffs for horses with deficiencies of hygiene

feedstuff			smell	appearance	texture	parasites***
n = 917		n	%	%	%	%
I	oats	237	*39.7*	*62.9*	*9.28*	*23.6*
	barley	17	*23.5*	*47.1*	*0.00*	*0.0*
	corn	25	*16.0*	*0.0*	*4.00*	*4.00*
Σ	cereals	279	36.6	56.3	8.2	20.4
II	muesli	62	*46.8*	*11.3*	*12.9*	*12.9*
	concentrate*	67	*7.46*	*2.99*	*2.99*	*7.46*
	mash	8	*12.5*	*0.00*	*25.0*	*0.00*
Σ	mixed feed**	137	25.5	6.57	8.8	9.49
III	hay	277	*43.7*	*45.5*	*39.0*	*43.0*
	grass silage	93	*47.3*	*43.0*	*0.00*	*2.15*
	straw	131	*62.6*	*85.5*	*29.0*	*8.40*
Σ	roughage	501	49.3	55.5	29.1	26.3

A heavy, dusty character or a mouldy odour of feedstuffs is a typical sign of moulds and was correlated with higher levels of moulds in many cases, whereas a yeasty smell indicated high counts of yeasts. Moreover, a massive load of pests or mites was combined with a lower dry matter content of the feeds, which generally caused higher counts of bacteria. The detected and differentiated bacteria as well as the moulds require a distinction between epiphytes and microorganisms indicating spoilage and a determining of normal counts (n.c.) in

different feeds [5]. Most of the hygienic aberrances were detected in cereals and roughage (see table 2), whereas commercial mixed feeds showed less imperfections.

Table 2: Microbiological status of different feedstuffs for horses

		total	aerobic germs (cfu > n.c.*)		total	moulds (cfu > n.c.)		total	yeasts (cfu > n.c.)	
		n	n	%	n	n	%	n	n	%
I	oats	182	71	*39.0*	175	78	*44.6*	153	54	*35.3*
	barley	12	2	*16.7*	11	2	*18.2*	8	1	*12.5*
	corn	6	2	*33.3*	6	1	*16.7*	5	1	*20.0*
II	muesli	55	19	*34.5*	55	8	*14.5*	46	8	*17.4*
	concentrate**	48	3	*6.3*	48	4	*8.3*	42	1	*2.4*
	mash	6	0	*0.0*	6	0	*0.0*	5	0	*0.0*
III	hay	245	55	*22.4*	240	117	*48.8*	206	15	*7.3*
	grass silage	83	27	*32.5*	94	37	*39.4*	71	36	*50.7*
	straw	129	54	*41.9*	136	63	*46.3*	108	17	*15.7*
I	cereals	200	75	*37.5*	192	81	*42.2*	166	56	*33.7*
II	mixed feed***	109	22	*20.2*	109	12	*11.0*	93	10	*10.8*
III	roughage	457	136	*29.8*	470	217	*46.2*	385	10	*2.6*

For feedstuffs that are processed (drying, high temperature, intensive grinding, high pressure, addition of acids etc.), classical microbiological investigations (cultural proof) do not necessarily give reliable information. In these cases, the assessment of the lipopolysaccharide level (= LPS; part of the cell wall of gram-negative bacteria) gives an indirect information about the hygienic standard of the feedstuffs (see table 3).

Based on the results of the sensory examination and the data derived from the analyses, the assumption of a causal correlation between the intake of the concerned feed and the observed clinical signs, as expressed in the preliminary report, could be verified in many cases.

Conclusion

1. A critical examination of the feedstuffs, from the delivery until feeding, is a fundamental precondition for avoiding health disorders or reduced performance caused by lacks in the hygienic standard.
2. Veterinarians should be able to assess the hygienic quality of the offered feedstuffs (particularly in cases of a high frequency of colics, disorders of the respiratory tract etc.).
3. However, not only the industrially produced mixed feeds/concentrates, but especially the roughage (even if it is only used as bedding material like straw) is worthy of note and should be examined critically (intensive sensory control combined with further analyses).

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Table 3: Lipopolysaccharide levels of different feedstuffs for horses

		total	lipopolysaccharide level (yg/g)					
			< 20		20 - 50		> 50	
		n	n	%	n	%	n	%
I	oats	112	36	*32*	23	*21*	53	*47*
	barley	9	4	*44*	3	*33*	2	*22*
	corn	4	3	*75*	1	*25*	0	---
II	muesli	41	32	*78*	5	*12*	4	*10*
	concentrate*	28	20	*71*	8	*29*	0	---
	mash	5	3	*60*	2	*40*	0	---
III	hay	123	30	*24*	26	*21*	67	*55*
	grass silage	17	5	*29*	7	*41*	5	*29*
	straw	45	3	*7*	9	*20*	33	*73*
I	cereals	125	43	*34*	27	*22*	55	*44*
II	mixed feed**	74	55	*74*	15	*20*	4	*5*
III	roughage	185	38	*21*	42	*23*	105	*57*

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Basic investigation of explorative and flight behaviour in sport horse stallions with regard to the level of schooling

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Summary

The individual explorative and flight behaviour of 35 sport horse stallions was investigated by quantifying the approaching time in three test situations (Novel Object Test, Startling Test, Human Approach Test). Depending on different levels of schooling, the group of the three year old stallions showed more explorative behaviour than the older stallions.

Introduction

The individual behaviour of horses consists of the species-appropriate behaviour (species: horse), the hereditary behaviour (genetic variations: breed, patriline) and the individually acquired behaviour (learning). Horses show individual variations of behaviours from an early age on, as well as of behavioural traits underlying temperament and personality. Temperament is generally described as the "biological" (i.e. genetic) basis on which personality, as a result of environmental influences (i.e. experience), is constructed [1].

Flight behaviour is part of the normal behaviour of horses. Because of the horse's evolutionary development with highly effective locomotor abilities, flight is an efficient method for horses to avoid hazards. Explorative behaviour is essential for locating new resources and obtaining information about the environment and therefore to avoid unnecessary flight. Hence there is a close relationship between explorative and flight behaviour.

This study investigated the influence of the level of schooling on the individual explorative and flight behaviour.

Animals, Material and Methods

The investigation was performed on 35 sport horse stallions that were housed in the national stallion depot Celle (Lower Saxony, Germany). The horses were divided into two age groups. Group 1 consisted of 18 stallions that were three years old. They have been trained for the stallion performance test. The schooling of the horses to become a riding horse started nine months ago. Group 2 was composed of 17 sires aged four to twenty years. The schooling had also been started at a similar age like the horses of group 1. Therefore, in this group the level of schooling varied dependent on their age.

The tests were performed in an indoor riding school. The horses were familiar to the environment from lunging and riding, but they never had the opportunity to stroll around in this area. In the riding school, a testing arena (14,8 x 14,8 m) was separated by a fence. All test situations were filmed by a camcorder and

evaluated after the whole test period was finished. Each horse was investigated separately without visual or physical contact to other horses. The stallions were supposed to show individual coping strategies without any influence of humans. This was accomplished by leaving them lead free in the test arena for the single test situations.

To warm-up the horses before the tests, each stallion was lunged 30 minutes in a predetermined way. This was important to minimize potential injuries and to create comparable initial conditions.

Testsituations:

A novel object (blow-up toy) was placed in the middle of the arena. The time until the horse touched the object with its nose was measured.

Startling Test:

An unknown object (another blow-up toy) was pulled with the speed of 3,65 m/s to the border of the test arena. The taken time from the flight reaction caused by the visual stimulus until the approach to and the striking of the object was analyzed.

Human Approach Test:

An unknown person was standing immobile in the middle of the test arena. The person did not gaze straight at the horse. The time until the horse touched the person with its nose was studied.

For each test, the maximum time allowed was five minutes.

Each horse was tested a second time after seven days. A changing of the main components (objects) was performed in the second test passage to minimize a potential learning effect and to survey the repeatability at any other time. The unknown person remained the same to ensure identic human behaviour.

The approach time in the three test situations were reviewed by examining the video recordings. Analyses were carried out with the statistical software SAS, Version 9.1 (SAS Institute, Cary, NC, USA). The generalized linear models were calculated with the procedures "GENMOD", "COUNTREG" and "LOGISTIC", furthermore the SAS macro "PROBCOUNTS" was used for calculating the model-predicted count-distributions. Error probability less than five percent ($p<0.05$) were considered statistically significant. All data were included into a descriptive analysis and the lognormal distribution was confirmed by visual assessment of normal probability plots of logarithmized values. The data were compared using two-way analysis of variance, time as repeated measurements and age group with two levels as independent measurements with Turkey's post-hoc test for mutiple pairwise comparisons of approaching time parameters. Logarithmic transformation was performed prior to analysis; hence geometric mean and geometric standard deviation was calculated on the original scale.

Results

In the Novel Object Test (fig. 1) eight horses (group 1, week 1: two stallions; group 2, week 2: six sires) did not touch the object within the five minutes. In comparison of the approaching time there was a slight difference in the first week: the younger stallions (group 1) approached faster than the older ones of group 2. This effect was however not significant. In the second week there was no difference, the approaching time in both age groups was similar. Certainly the repetition of the test after seven days influenced the behaviour of the horses: both age groups showed a significant ($p<0.001$) faster approaching in the second test week.

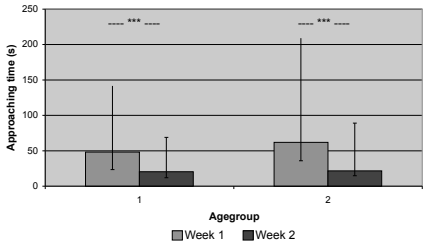


Fig. 1: Approaching time in the Novel Object Test (group 1, week 1: n=16; group 1, week 2: n=18; group 2, week 1: n=17; group 2, week 2: n=11)

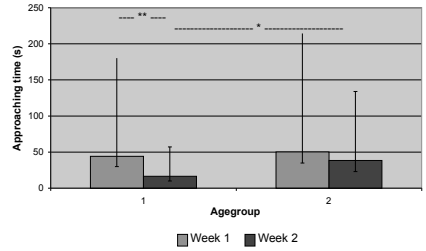


Fig. 2: Approaching time in the Startling Test (group 1, week 1: n=16; group 1, week 2: n=15; group 2, week 1: n=14; group 2, week 2: n=14)

In the Startling Test (fig. 2) all of the horses showed a flight reaction when the object was moved. Nine stallions did not approach in the maximum time (group 1, week 1: 2; group 2, week 1: 2; group 1, week 2: 3; group 2, week 2: 2). Comparing the two age groups the test demonstrated no difference in the approaching time in the first week. In the second week the stallions from group 1 approached much faster than the stallions from group 2; this difference is significant ($p<0.05$). Focussing on the groups by itselves the younger stallions from group 1 approached significantly ($p<0.01$) faster in week 2 compared to week 1. The sires of group 2 showed a slightly faster approach in the second week, but this was without significance.

In the Human Approach Test (fig. 3) two horses did not approach within the five minutes (group 1, week 1: 1; group 2, week 1: 1). In both weeks the stallions from group 1 got in contact with the unknown person about twice as fast as the sires from group 2 ($p<0.01$). Within the groups the comparison of week 1 to week 2 showed no discrepancy.

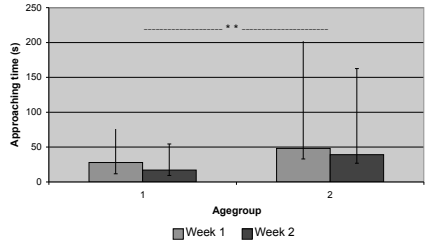


Fig. 3: Approaching time in the Human Approach Test (group 1, week 1: n=17; group 1, week 2: n=18; group 2, week 1: n=16; group 2, week 2: n=17)

Discussion

Hausberger [1] assessed an influence of genetic factors (breed, patriline) on reactions to fear-inducing situations but environmental factors such as conditions of life or the type of work performed also have an influence on horse personality. In our study life-conditions and the type of work were the same for all stallions, but the level of schooling varied. This seems to have an effect on the explorative and flight behaviour. Curiosity and explorative behaviour are more distinctive in foals and younger horses. In this age the learning capacity is at its maximum [4]. Scientific studies indicate that younger horses learn more readily

than mature horses. They likely adapt better to changes in physical and social environment as well [2]. This can be observed in our study as well. The younger stallions (group 1) showed exceeding explorative behaviour in two test situations (Startling test and Human Approach Test) compared to the older stallions (group 2). The behaviour of horses in the human-horse relationship is often based on a combination of their own temperament und previous experiences made in the contact with human. Visser [3] noticed an increase of touching an unfamiliar handler over a training period of five weeks with no significant dependency of the training method (traditional vs. "sympathetic" training method). In our study the horses were all trained the traditional way. They showed no difference of approaching faster to an unknown person after one week, but in both test weeks the younger stallions (group 1) got in contact faster than the older ones (group 2). Depending on the maturity and the level of schooling, horses learn to pay more attention on instructions from their handlers. They are trained to react to signals and not to act independently. A reason why the older horses showed less explorative behaviour could be that the environment of the test area is linked with training since it is always used for this purpose only.The repetition of the tests after seven days showed a decrease of the approaching time in both age groups. This concludes a familiarising of the test-situations (not the objects). More correlations between the different test situations are subject to further evaluations.

Genetic influence can lead to different susceptibilities to the same environmental conditions. This however does not suggest "bad" or "good" horse temperaments but rather implies the presence of different horse types that may require different management or training schedules [1]. The further evaluation of our results may induce advanced possibilities to adjust training methods to individual attributes of horses.

Conclusion

The three tests showed an influence of the level of schooling on the explorative and flight behaviour of horses. Younger horses approached faster to the startling object as well as to an unfamiliar person.

Acknowledgement

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Investigations on Feeding Stalls and Automatic Feeding Systems for Horses kept in Groups with Regard to Animal Welfare

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Introduction

For the individual feeding of hay and concentrate in group housing systems of horses conventional feeding stalls, where each horse has one stall, were recommended by the ministry of agriculture of Germany with regard to animals well-being [1]. In this system the horses can eat simultaneously which is in accordance with the typical behaviour of horses. In contrast to that automatic feeding systems base on a not simultaneous feeding. The advantage of this system is that it is possible to give the horses a lot of small portions over the whole day what is good for the ingestion and furthermore this system stimulates the horses to move, which is important for their health. This research aims to compare threatening and submissive gestures, visiting frequency, stress situations, situations with risk of injury and displacement activities in the area of automatic feeding systems with those at feeding stalls.

Animals, Material and Methods

270 horses living in run-out sheds at 10 stables with feeding stalls and at 11 stables with automatic feeding systems for hay and concentrates were observed. These stables were otherwise similar regarding the keeping of the horses and the management of the facilities, each group consisted of 8 to 21 horses. Every group of horses was observed visually in winter for six sessions, each of 4 hours. These six slices form together 24 hours, a complete day.
To be comparable the different farms had to comply with a lot of criterions (e.g. mares and gelding together in a herd, different ages, straw ad libitum, at least 2 feeding times in feeding stalls and at least 10 feeding times (hay and concentrate) in automatic feeding systems). The observed areas around the feeding equipments were beforehand defined (they nearly had the same size).
In the feeding area all visits by every horse were recorded, as well as all avoidance behaviours, all threatening gestures with risk of injury (biting, rear leg kicking and charging) and all threatening gestures without risk of injury (threatening, biting threats, threatening swinging and rear leg kicking threats), all stress situations all situations with risk of injury and all displacement activities.

Results

Horses in stables with feeding stalls visited the feeding area (waiting area, the station, exit area) 45.5 ± 27.3 times on average in 24 hours, and those in stables with automatic feeders for

hay and concentrates 93.1 ± 53.5 times. This difference was significant.

Altogether, 6297 agonistic behaviour patterns were registered in the feeding area (avoiding behaviour 40.6%, edging out of others 12.8%, biting/rear leg kicking/charging 12.7 % und threatening/biting threats/rear leg kicking threats 33.9 %). 22.5 % of these types of behaviour were recorded in the feeding stations, 77.5 % together in front and behind of these. In the stables with feeding stalls there were 31 % of the observed threatening gestures inside the feeding stations, in the stables with automatic feeders only 21 %. In the 10 stables with feeding stalls together 1635 threatening and submissive gestures were to be observed in the feeding area, in the 11 stables with automatic feeding systems this number was 4662. In the stables with feeding stalls 46 % of the observed threatening and submissive gestures were threatening gestures without risk of injury, 12 % with risk of injury, 5 % edging out of others and 32 % avoidance. In stables with automatic feeding systems 29 % of all actions in the feeding area were threatening gestures without risk of injury, 13 % with risk of injury, 16 % edging out of others and 42 % avoidance. Most of these actions causes no injuries.

In the area in front and behind the feeding equipments threatening behaviour, without risk of injury or with risk of injury, occurred more frequently in stables with automatic feeders (9.1 ± 12.9 times and 3.3 ± 4.8 times respectively per 24 hours and horse) than in stables with feeding stalls (3.4 ± 7.5 times and 0.9 ± 2.8 times respectively per 24 hours and horse). These differences were not significant. Avoidance gestures were observed 4.7 ± 8.3 times per day and horse in stables with feeding stalls and 13.8 ± 17.5 times in stables with automatic feeding systems. This contrast was significant.

Inside the feeding equipment threatening without risk of injury was observed significantly more often in stables with feeding stalls (2.6 times per 24 hours and horse) than in stables with automatic feeding systems (0.5 times per 24 hours and horse). Threatening with risk of injury was observed 0.9 times per 24 hours and horse in stables with automatic feeding systems and edging out of others 5.2 times. In stables with feeding stalls only 0.6 actions of threatening with risk of injury and 0.6 actions of edging out of others were noticed. Only the difference in edging out of others was significant.

The management of the stable, however, proved to have the most important influence on the agonistic behaviour of the horses ($p < 0{,}001$).

Looking at the average value of threatening gestures and avoidance per visit it is to be seen that there was not one threatening gesture and avoidance per visit in both feeding systems. But there were very big individual differences between the horses too.

In stables with feeding stalls there were to be observe within 24 hours per horse only 3.7 ± 13.1 situations that can cause stress and 1.3 ± 4.8 situations that can cause injuries on average. Such situations were to be seen in stables with automatic feeder clearly more often (17.0 ± 33.4 and 8.6 ± 15.3 times respectively per horse and 24 hours).

Displacement activities were observed in stables with feeding stalls 0.3 ± 1.0 times per horse and 24 hours and in stables with automatic feeders for hay and concentrate 12.9 ± 23.3 times. In both feeding systems the standard deviation was very high and in both feeding systems situations with risk of injury were seen least.

Horses in stables with automatic feeders for hay and concentrate visited the feeding area with 93.1 visits per day almost twice often than horses in stables with feeding stalls. Automatic feeders stimulate moving which is necessary for the horses' health.

The more often the feeding area is visited, the more frequently agonistic behaviours occur [4]. Horses have fine tuned threatening and avoiding gestures with the aim to avoid injuries. In the feeding area in stables with automatic feeding systems only 13 % of the agonistic behaviours were threatening gestures with risk of injury and 87 % behaviours without risk of injury (threatening behaviour without risk of injury, avoidance and edging out of others). In

stables with feeding stalls 12 % were threatening behaviour with risk of injury and 88 % agonistic behaviours without risk of injury. That shows clearly that horses try to avoid fights with risk of injury. If you apply the observed interactions not to 24 hours but to the single visit there were no differences between both feeding systems. In both systems the threatening gestures with risk of injury (0.03/per visit in stables with feeding stalls, 0.05/per visit in stables with feeding systems) as well as the threatening without risk of injury (0.13/ per visit in stables with feeding stalls, 0.10/ per visit in stables with automatic feeding systems) and avoiding (0.10/ per visit in stables with feeding stalls, 0.15/ per visit in stables with automatic feeding systems) resulted in less than one agonistic gesture per visit. To sum up this it can be said that there was only one agonistic behaviour every tenth visit and only one threatening gesture with risk of injury every twentieth visit. However there were very big individual differences between the horses too.

The more often the feeding area is visited the more often situations that may result in stress or injuries were observed. Therefore it is to be said that feeding in feedings stalls leads to less risk of stress and injuries compared with feeding by computer controlled systems.

Conclusion

In conclusion: both feeding systems have their advantages and disadvantages. First it can be said the more often the feeding area is visited, the more frequently threatening gestures without and with risk of injury occur. The same goes for the number of situations that may result in stress or injuries. That is why feeding in feedings stalls leads to less risk of stress and injuries around the feeding equipment compared with feeding by computer controlled systems. Horses in stables with automatic feeding stations can eat more undisturbed, however. The great advantage of automatic feeding systems are the small fodder portions and from that stimulating the horses to move, an advantage for the health [2,3]. So this system can be recommended. While assessing this study it should be taken into account that there were very big individual differences between the single horses. A striking result of the statistical evaluation is that the management of the stable has a decisive influence on the observed features. Therefore both feeding systems can be recommended, especially as the number of conflicts in the feeding area in all stables was relatively low and no injuries in connection with the feeding were to be observed in the course of the observations to this study.

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