9th International Congress
in Animal Hygiene

ISAH'97

17 - 21 August 1997
Helsinki, Finland

Proceedings

Volume 2

Editor: H. Saloniemi

Organized by

International Society for Animal Hygiene
and
University of Helsinki, Department of Clinical Veterinary Sciences, Section of Animal Hygiene

Helsinki 1997

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”Healthy Animals, Sound Production Environment, Satisfied Consumer”

ISBN 951-45-8077-X
University of Helsinki, Department of Clinical Veterinary Sciences, Section of Animal Hygiene

Helsinki 1997

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Cold housing and open housing – effects on dairy cattle health, management and production

Cold housing and open housing—effects on health, management and production of dairy cattle.

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Summary

The body of published information about the effects of cold or open housing on health, management and production is scarce. Cold ambient temperatures have little adverse effect on herd milk production and may be beneficial. During periods of cold temperature, dairy cattle increase feed intake. This increased intake and availability of ad libitum feed may compensate for the loss in rumen digestibility of forages commonly associated with cold temperatures. Cold and open barns may be beneficial in controlling environmental mastitis and some foot conditions because the stall and floor surfaces are drier. Dairy farmers lead the way, often adapting building systems to meet their needs. However, there is a need for farm level research to document the effects of housing type and the associated interior equipment with health and performance.

Key Words: dairy cattle, cold housing, health, management, production

Introduction

In 1996, dairy producers could attend grand opening celebrations for one or more new dairy barns every week in Ontario, a Canadian Province with 7800 dairy farms. The new barns put more air into dairying, fresh and often very cold air.

The trend to cold or open housing is not unique to Ontario. Dairy farmers in most of the cold regions of the world are adopting housing technologies that take advantage of cooler ambient temperatures and climatic conditions. Cold housing is of interest to us because of the real or perceived benefits to dairy cattle health and production. Open housing also provides new challenges and opportunities in herd management.

Thermal comfort and production

Thermal comfort is one of four components of a satisfactory environment for farm livestock (Webster 1983). Regional and local environmental conditions vary greatly in temperate regions, leading to adoption of numerous barn types to manage the internal environment for cattle. In temperate areas of North America, two-storey tie-stall barns, one-storey tie-stall barns, loose housing barns, and free stall barns dot the rural landscape. Outdoor yards for exercise or

feeding can be found with all barn types. Often two or more barn types appear on one farm, attesting to the change in housing over time. A recent innovation is cold and open housing, usually with free stalls. Within each barn type, efforts focus on providing an optimum thermal environment to achieve performance and cow health. The effects of thermal environment on milk production of dairy cattle appear in reviews and papers by Ames 1980, Young 1981, Webster 1983, and Johnson 1987.

Some trials used treatment groups alone to study the effects of cold on lactating cows. In Canada, MacDonald exposed six late lactation cows to temperatures ranging from -20°C to 3°C in a loose housing barn. He concluded cold temperatures had no effect on fat corrected milk, total solids, solids-not-fat or percent solids-not-fat. However, crude protein increased as temperature decreased. The experimental cows reacted more to extremes in temperature than to low daily average air temperature. The cows showed signs of stress at -18°C and had reduced milk yield when temperatures dropped below -12°C (MacDonald and Bell 1958).

Christopherson showed the thermoneutral zone for milk production in Holstein cattle included a temperature range from -5°C to 21°C. Milk production declined at temperatures outside these ranges (Christopherson and Young 1986). In Japan, Shijimaya et al. found late lactation cows produced more milk (19.4 vs 19.1 Kg) and milk components when housed in a cold barn (-3.7°C to 3°C) (Shijimaya, Furugouri, and Miyata 1985). The 12 heifers in Brouček's experiment declined in milk production as the ambient temperature dropped and recovered as the temperature increased. The shelter was open, loose housing, and temperatures ranged from -19°C to 3°C (Brouček, Letkovičová, and Kovalčuj 1991).

Using treatment groups only, experiments generally show decreases in milk yield and increases in milk components (e.g., milk fat, protein) following exposure of lactating dairy cattle to cold ambient temperatures. Sudden low extremes in temperature had greater effects than average daily temperatures.

However, trials using treatment and control groups revealed results differing from the individual cow experiments. After using a switchback trial protocol, the Japanese researchers found daily milk, 4% fat or solid corrected milk yield were similar for cows housed in cold and warm barns (Shijimaya, Furugouri, Ando, and Katayama 1986). Brouček (1995) found milk production was greater in Holstein cows kept in an open free stall barn (32.7 Kg per cow per day) than in matched pairs of cows kept in a warm enclosed tie-stall barn during winter (31 Kg per cow per day). His cows in cold housing experienced temperatures 9 to 20 degrees C colder than cows in the control group, and -19.4°C was the coldest temperature (Brouček, Arave, Nakanishi,

Mihina, and Hetényi 1995). In Utah, Arave found milk production (29.5 Kg per cow per day) and composition similar during winter for Holstein cows kept in enclosed or open (overhead roof) free stall housing during three consecutive winters (Arave, Macauly, and Russev 1994).

Thermal comfort and management

The cold temperatures in open barns present new challenges and opportunities to managing a dairy herd. Since 1987, open and cold housing for dairy cows worked successfully in Idaho where temperatures plummet below -34°C (Kearley 1994). Cold ambient temperatures require the use of a tractor and scraper to remove manure. Flush water systems and other mechanical scrappers are not suitable for extremely cold temperatures. Windbreaks and curtains on sidewalls may be needed to buffer winter winds. These can be removed to afford maximum ventilation in summer months.

The adoption of green house structures with transparent roof coverings or sidewalls is a recent innovation in cold housing for dairy cattle. These buildings serve as shelter for rearing calves and as free stall barns and milking parlours for lactating cows. The farm magazines in North America frequently print articles describing the benefits of this barn type to readers. Invariably, owners like the brightness and improved air quality, and they claim the cows are more comfortable. One of the largest farm buildings in Sweden is a dairy barn with a transparent roof (Dolby, Ekelund, and Jeppsson 1994). Researchers report that it provides a favourable indoor climate, and that solar radiation contributes to reduced humidity and drier surfaces. In Ontario, the cost of constructing a green house barn approximates the cost of building a more conventional open barn. Nonetheless, green house barns are being built in Ontario, more for calf housing than for dairy cows at this time.

Michigan researchers found milk production levels influenced the environment of cold barns under winter conditions (Tillotson and Bickert 1994). The increased heat load associated with high producing dairy cows was a ventilation benefit, causing an increase in the air changes in a cold barn with appropriate stack ventilation. The researchers suggest existing farm buildings may need changes to ventilating systems as milk production increases in the herds.

The results of exposure to cold are of practical and economic importance. When exposed to cold temperatures, the digestibility of forages decreases in cattle. This has a negative effect on feed efficiency (Young 1983), (Christopherson 1986). Lactating dairy cattle compensate for cold stress by increasing feed intake. Feed intakes were greater for lactating cows kept in cold or open barns during winter (Brouček 1995 and Shijimaya 1986), and milk production did not suffer. The

practical significance of reduced digestibility of forages during cold ambient temperatures may be unimportant in North American dairy operations. Indeed, the adoption of ad libitum feeding using total mixed rations may be the feeding management practice that contributes to the success of cold barns. In addition, the real or perceived improvement in cow comfort and health from cold or open housing may offset the reduced efficiency of forage digestion.

Thermal comfort and health

Mastitis and lameness are probably the two most common diseases affecting dairy cattle. There are conflicting reports about the effect of cold temperature on clinical mastitis. After applying local cooling to individual quarters, Brown concluded the cooling may increase Somatic Cell Counts in the milk (Brown, Thomas, Cook, Riley, and Booth 1977). The studies on the effects of cold on milk production by Arave, Brouček, MacDonald and Shijimaya do not report udder health information. Because of the significant impact of mastitis on milk production and the outcome of the trials, one would have expected the authors to report on mastitis if it had occurred. Frozen teat ends and subsequent mastitis are probably the greatest risk for cows in cold housing. In two consecutive winters with severe wind chill, Kearley found none of the cows in the open barns had frozen or chapped teats. Frozen teats and the ensuing Staphylococcus mastitis were a problem with cows in the open outdoor lots. One might expect the drier conditions in open barns to be beneficial to controlling mastitis caused by environmental organisms.

The health and productivity of dairy cattle housed in cold and open housing demonstrates their ability to withstand cold stress. In experiments that adjusted ambient temperatures from +15 to -7 and back to +15°C, dairy cattle proved to be very adaptable to thermal alteration of the environment (Nový, Knížková, Jílek, and Kunc 1996). Cold stress should evoke metabolic heat production, mobilisation of free fatty acids from fat tissue, and glucose from glycogen stores in the liver (from studies cited in Brouček 1991). However, Shijimaya (1986) found heat production, plasma glucose and free fatty acid were similar for cows in cold and warm housing.

A literature search on the influence of cold housing on lameness produced no relevant information. However, there is a growing body of knowledge showing associations of lameness with specific types of construction in free stall housing. A study in France showed an increased risk of laminitis with a high step (>15 cm) at the entrance to the milking parlour, a step in front of the feed area, or with short cubicles (< 2.25 m) (Philipot, Pluvinage, Cimarosti, Sulpice, and Bugnard 1994). In addition, one needs to consider diseases of feed delivery and acidosis as predisposing causes of lameness (Nordlund 1995). The drier surface conditions in cold, open barns might be beneficial in controlling strawberry footrot.

The characteristics of stalls, floors, feeders, bedding material, stall dividers and other equipment in contact with the cows probably exert a greater impact on cow health than the choice of a cold building. The role of housing and management in the prevention of bovine diseases is the subject of the keynote address by O. Østerås at this conference, and by other speakers in that session. When considering expansion of a dairy farm, owners should seriously consider the impact of new facilities, feeding practices and biosecurity measures on cow health and survival. Minnesota dairy producers who expanded their herds described herd health as the greatest challenge in the transition period (Conlin 1996). The choice of barn type (e.g., cold, open, warm) may be important in the control of emerging diseases that are often epidemic in nature. Multiple-resistant Salmonella typhimurium DT104 of cattle is an example (Evans and Davies 1996). The authors identified biosecurity and sanitation practices as risk factors for the disease. Although the survival of pathogens varies with environmental conditions, one would suspect that the drier conditions in open barns would be a benefit to disease control.

Conclusions

There is evidence of no detrimental effect and, some benefit, on milk production for lactating cows in cold or open housing compared to cows in enclosed or warm housing. Management of feeding, manure handling and wind protection assure success in the cold barns. The reduced humidity and drier interiors of open barns is a benefit to cattle health. Cold or open housing per se has no effect on mastitis or lameness of cattle. However, the body of published information about the effects of cold or open housing is scarce. The dairy industry would benefit from additional research using treatment and control farms. Nonetheless, dairy producers continue to lead the way by building cold or open housing, and by putting fresh air back into dairying. Personal experience, testimonials, or the desire to decrease capital and operating costs may be influencing the choice more than research results.

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A field study of the effects of cold loose housing systems in Finland on the health and welfare of dairy cows and calves - a questionnaire to farmers and veterinarians combined with farm visits

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Summary

Cold loose housings systems differed significantly between different farms in this study. The welfare of the animals on most of the farms was very well in spite of simple, uninsulated buildings. Most of the farmers were very satisfied with their cold loose housing system, the veterinarians were more critical. Coldness was not so much a problem in itself - the right attitude, the skills of the farmers and the functional planning of the loose housing systems were the most important factors in determining the welfare of the animals and the farmers. Cold loose housing systems for dairy cows need to be planned and designed well and the farmers have to understand the effects of coldness to be able to change their working habits as needed.

Key words: Cold loose housing, dairy cattle, welfare

Introduction

In Finland it has been very uncommon to build uninsulated barns to dairy cows and there are a lot of prejudice and doubt about them because of the long, cold winter. However, in the last few years many farmers have become interested in them because of their lower building costs. Also many organic farmers are interested in cold loose housing systems because they offer animals more natural surroundings than insulated barns.

The purpose of the study was to get a general view of how the cold loose housing systems built so far in Finland affect the health and welfare of dairy cows and calves and what kind of problems have arisen.

Material and methods

In September 1995 a questionnaire was sent to 21 farmers, who had a cold loose housing system for dairy cows, 13 farmers answered. A questionnaire was also sent to veterinarians, who had some cold loose housing systems in the area, 5 veterinarians answered. 13 farms were

also visited in different parts of the country. The farm visits were done between the winter 1995 and the fall 1996.

Results

Cold loose housing systems differ significantly between different farms. Some farmers have built totally new buildings, some have used old stanchion barns as much as possible. Many farmers have built new milking parlours, some milk the cows in the old tie stall barn. Some farms have a very open building with only three walls, most buildings are closed with four walls. In the study most farms had cubicle systems, only a few had deep bedding and one a sloped floor system. Straw or peat were used as bedding in cubicles in most cases, on a couple of farms sand. The base of cubicles was usually soil, not concrete. All but one farm had small calves, milking fascilities, calving and handling pens in an insulated building. The one farm situated in south of Finland also planned to build an insulated place for calves and milking. The other farmers moved the calves from the insulated building to the uninsulated one mostly when calves were 2-6 months old, some after the insemination and the pregnancy testing of heifers. Concentrates were delivered during milking or by an electronically controlled dispenser. Silage and hay were served either inside the same building as the lying area of cows was in or outside. The feeding table usually had a roof which reduced snowfall on the feed. During the summer, most of the cows were out on pasture, some had a possibility to go out all year around.

On most of the farms the welfare of animals was very well in spite of simple, uninsulated buildings. The coldness was not so much a problem in itself - the right attitude, the skills of the farmer and the functional planning of the loose housing systems were the most important factors in determining the welfare of the animals and the farmer.

Most of the farmers were very satisfied with their cold loose housing system. Their previous cow housing system had usually been unpractical, old stanchion barns with poor ventilation and much manual work. The farmers usually said the work had become easier and lighter, because more work had now become mechanized. One farmer was very unsatisfied and complained his work load having increased much since the change to a cold loose housing system.

All the farmers said the cows were more peaceful and frendlier, in better condition and seemed to enjoy themselves. The welfare and the health status of cows had improved. The appetite was good. The calvings had been easier. It was easy to notice when the cows were in heat. The farmers felt that the cows had been healthier than before. Mastitis hadn´t usually been any problem. Warm summer time had been more problematic than the cold winter. According to

the farmers claw and feet problems, ketosis and respiratory diseases were uncommon and fertility was better than before.

The coldness during winter time wasn´t a big problem. The temperature between −5° and −10°C was optimal. Freezing cold hadn´t affected the health and the productivity of the cows. No one of the farmers had noticed frostbites on the teats or the udders. When it was very cold the cows lay down more. Peat, sawdust and sand bedding in cubicles froze more easily than straw. People working in the cold loose housing systems had to take coldness into consideration for example by using suitable clothing and avoiding the use of much water in cleaning the udder and drying the udder and the teats well. No one used teat dip. Some used teat ointments, which were waterless. During the winter time udders were usually very clean and so they didn´t need much cleaning. Usually the farmers told that the cows were cleaner than before.

The standing/dunging passages were scraped one to seven times a week by tractor depending on the farm and the weather. Most of the farmers used some bedding on the passage, either straw or peat. The farmers didn´t feel that the freezing of the manure was a problem, because the frozen manure didn´t make cows dirty. When the manure froze they didn´t scrape the passage until it melted so much it loosened from the floor. The outside yard needed sometimes some sand on it not to become slippery and icy.

According to the farmers the milk yield hadn´t changed much in the cold loose housing system. On many farms the milk yield had increased slightly.

In general, there hadn´t been any bigger problems. Most of the cows had learnt to use cubicles. Some farms had had problems with birds. One farmer was very unsatisfied and complained that nothing went well: the cows didn´t use cubicles, the milking parlour and the calves were situated in an uninsulated building which didn´t work and was very unpleasant to the caretaker. Most of the farmers would build a cold loose housing system for the dairy cows again, even if it were not cheaper than an insulated building. Most of them would also recommend it to other farmers who understand the responsibility associated with coldness and simple buildings. Most of the farmers complained it was very difficult to get information about the cold loose housing systems. More studies and material in Finnish would be needed.

The veterinarians were more critical than the farmers. They were not worried about the coldness in itself, but they were worried about the management in general. It is very important that the caretaker is skilled and has a sense of responsibility. The health and the welfare of animals is good if the management and the accommondations are satisfactory. The problems don´t usually differ from the other types of dairy cattle housing systems. Simple, uninsulated buildings, coldness and rationalization of work can aggravate problems. The biggest problems

were in the feeding, quality of feedstuffs, manure removal, care of lying areas and design of animal treating and handling fascilities. Each cow should have a clean, comfortable bed, reasonable shelter from the weather, sufficient access to clean, wholesome food and water, sufficient space to move around without difficulty or interference from other cows, and sufficient space and opportunity to express essential patterns of behaviour. It should also be possible to direct cows without difficulty to the milking parlour, calving pens and perform veterinary treatments and do artificial inseminations.

Conclusions

A cold loose housing system for dairy cows in not free of problems but worth of consideration also in Finland. Each housing system has it´s pros and cons. Coldness is not so much a problem in itself. A well functioning cold loose housing system offers both cows and caretaker pleasant and agreeable surroundings. A cold loose housing system requires careful planning and a farmer with animal-oriented attitude and professional skills in dairy cattle management. He should be interested in the welfare of his animals and ready to change his working habits according to the weather and season.

Farmers have difficulties to compare their own systems to others and see possible weaknesses and problems. More research need to be done on the effects of cold loose housing systems on cattle health, welfare, fertility and productivity. It is also important to study how systems and management should be designed to achieve the best possible compromise between the needs of the cows and the farmers for buildings and structures.

Effects of cold weather on dairy cows kept in open barn

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Summary

Thirty Holstein cows paired by age and current milk production were randomly assigned to two housing treatment groups. One group (outside) was housed in an open free-stall facility, the other group (inside) in a warm enclosed tie-stall barn. The experiment lasted twelve weeks. The average daily feed consumption of the cows from an open barn was 35.8 kg during the all experiment which is 123.1 % of the intake of the dairy cows kept in a warm barn. The average production during the all period of the experiment (32.7 kg and 30.96 kg) was significantly different. A significant increasing of milk fat and protein content was recorded on the sixth week. Contents of lactose and solids-non-fat had a similar trend with a rapid decrease on the sixth week. Feeding behaviour appeared to be most affected by type of housing. Outside cows were observed ruminating slightly longer than inside cows.

Key Words: dairy cows, cold, open barn, milk yield , milk composition, feed intake

Introduction

Temperatures of the environment are regarded as very important factors, which may negatively affect on dairy cows. Although high temperatures are assessed as more harmful, in some climatic areas it is very low temperatures that are crucial (Brouèek et al. 1991). The effect on production depends on temperatures of the environment, breed, adaptation to local climate and on feeding intensity (Kovalèiková et al. 1984; Mossberg et al. 1993). In lactating cows, cold weather reduces milk yield and increase milk fat percentages (MacDonald and Bell, 1958). Arave et al. (1994) did not find significant changes of milk composition in the mild winter weather during 1990-92 years. The cows did not appear stressed by cold temperatures.

Methods

Thirty Holstein cows were in trial from December 9 to March 2 (twelve weeks). They were then randomly assigned to housing treatment group.The first group (outside) was kept in open free stall barn and the second group (inside) in warm enclosed tie stall barn. Feed consumption

was recorded individually by Calan doors in the open free stall unit and by separation between cows in a warm tie stall barn. Total mixing ration per cow and day consisted from 11.3 kg alfalfa haylage, 4.5 kg corn silage, 4.8 kg alfalfa hay and 16.7 kg concentrate mix. Milk yield was measured twice daily by computer. Milk composition was determined on composite AM-PM samples. Body weights were taken twice monthly. Temperature and humidity were measured in both units.

Results

Temperature regime

All temperature differences between an open and warm barn were significant. Average daily temperatures during all experiment in an open barn where the first group was housed were below -2 °C. The temperature were lowest in sixth and eighth week. An average of minimum temperatures and the lowest minimum were in the open barn during the eighth week (-19.4 °C and -24.7 °C). During seven out of twelve weeks of the experiment the averages below -10 °C were recorded.

Food consumption

Feed intake was during the whole experiment higher in dairy cows kept in an open barn. The average daily feed consumption in the animals from an open barn during the all experiment was 35.8 kg which is 123.1 % of the intake of the dairy cows kept in a warm barn. During the all period of experiment a higher consumption of feed needed for l kg of milk was recorded in a first group. During the all experiment, the feed consumption needed for l kg of milk in dairy cows from an open barn was higher by l6.8 % compared with the control group from a warm barn.

Body weight

Cows from the second group housed in a warm barn showed a higher body weight during the whole experiment compared to a first group. The differences, however, were not significant. In both groups the body weight increased since the second to the twelfth week.

Milk production

No significant differences in milk yield of the cows from an open and warm barns were find during any week. In all cases the milk production was higher in a first group. The average production during the all period of the experiment (32.7 kg and 30.96 kg was significantly different.

Milk composition

In both groups, fat and protein content had a similar trend. A significant increasing was find on the sixth und eighth week, when the lowest temperature was recorded. This elevation was statistically significant in the outside group in relationship to the inside group. The mean fat yield in dairy cows kept in the environment with lower temperatures was 0.117 kg higher than in the other type of housing. This was probably caused by the cold stress when depot fat and proteins are mobilized or it is a genetic inserted protective mechanism of the mother cow directed to give its calf the most proper food. Contents of lactose and solids-non-fat had a similar trend with a rapid decrease on the sixth week. Somatic cell count was no significant higher during the all period of observation of the outside group. The greatest differences were recorded on the sixth and eighth weeks. Although this might be due to a higher number of cows with mastitis, it is also possible that it is caused by hypothermal stress.

Hormone levels

No significant differences in the levels of cortisol between the outside and inside groups were found. During the fifth week the concentration of cortisol level in cows kept in the environment with extremely low temperatures was increased to 0.47 μg/dl but during the eleventh week it returned to the initial level. In the group from the warm barn, a slight increase in cortisol levels occurred on 8th week (to 0.38 μg/dl). Average levels of cortisol were slightly higher in the inside group (0.235 μg/dl vs**.** 0.252 μg/dl). The average noradrenaline (1290 pg/ml vs. 981 pg/ml) and dopamine levels (708 pg/ml vs. 510 pg/ml) in the cows from the open barn were slightly higher than in the cows from the warm barn. Adrenaline levels showed an opposite tendency, they were higher in the cows from the warm barn (562 pg/ml vs. 883 pg/ml).

Health Condition

More incidents of mastitis and leg diseases appeared in an outside group. On the contrary, more cases of reproductive diseases occurred in an inside group.

Behaviour

During three 24 h observation periods cows from both groups spent longer lying on left than on their right side. During the coldest temperature outside cows were eating significantly longer than inside cows (15.5 % vs. 8.5 % of total time). Outside cows were observed ruminating slightly longer than inside cows. In an average of the three observations the length of rumination was in the open group 32.6 % and in the warm group 30.6 %). In both groups there were more bouts of the lying on the left side than on the right side. Greater differences between left and right laterality were recorded in the trial group. Time standing as well as lying did not differ between groups. Feeding behaviour appeared to be most affected by type of housing.

Conclusions

Based on this experiment it is concluded that the open conditions in a free-stall housing at the temperatures approaching -23 °C are not stressful to dairy cows which are optimally fed and were not demonstrated by any significant changes in decrease in live body weight and milk yield. Temperatures below critical temperatures of lactating cattle have effect on milk composition although causes may be different.

This publication is based on work sponsored by the U.S.- Slovak Science and Technology Joint Fund in cooperation with Agency for International Scientific and Technological Cooperation under Project Number 010-95.

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Group and cold rearing of dairy calves: feeding behaviour and growth. Results from two pilot experiments.

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Summary

Two experiments were carried out in autumn 1995 and in spring 1996. The calves were reared inside the barn in a group pen (Group 1) and in individual boxes (Group 2) as well as outside in a group pen (Group 3), where the calves could choose either heated or unheated shelter. There were six calves in each group including both female and male calves of Ayrshire and Friesian breeds. The calves came to the 7 week experiments at the age of 8-16 days on average.

The growth of the calves (798, 793 and 844 g/day in the 1st experiment and 723, 722 and 847 g/day in the 2nd experiment in different groups, respectively) did not differ significantly although there was a slight trend that the calves of the outside groups grew better. Total dry matter intake was nearly the same in different groups, but the calves of the outside groups ate more concentrates. At the beginning of the experiments the calves ruminated daily on average about 1 h and in the end about 6 h, and more during nights than daytime. Daily rhythm was almost the same in different groups, calves being most active during milk-feeding times.

Key words: calves, rearing methods, eating, ruminating, temperature

Introduction

Calves in Finland are normally reared in heated buildings in single boxes up to two months of age and bucket fed twice a day. This method does not fullfil the physiological and behavioural needs of the calves. There is now an increasing interest to house dairy cows in simple, unheated buildings. Calves can tolerate relatively low temperatures, the lower critical temperature of dairy calves being about 8-10 ^{o}C from 3 to 56 days of age (Webster et al. 1978). In many experiments the daily gain of the calves has been the same in cold housing compared with warm housing (e.g.

Jorgenson et al. 1970, McKnight 1978, Hansen 1984). However, in some of the experiments the food intake of calves in cold housing has been higher (McKnight 1978, Kunz & Montandon 1983).

Group rearing of calves in a changing climate has not been widely studied. The aim of these experiments was to compare the performance of young calves in warm and cold group housing systems in a Nordic climate, and to develop practical ways for rearing calves in these conditions with respect to their physiological and behavioural needs.

Material and methods

The experiments were made in autumn 1995 and in spring 1996. The calves were reared inside the barn in a group pen (Group 1) and in individual boxes (Group 2) as well as outside in a group pen (Group 3), where the calves could choose either heated or unheated shelter. The individual boxes were 120 cm long and 99 cm wide with a wooden slatted floor. The wooden group pen was 3.5 m x 3.07 m and also had a slatted floor. Heated and unheated shelters were both 12 m^2, bedded with straw, and the yard connecting them was 40 m^2, covered with bark. The number of calves in each group was six, including both female and male calves of Ayrshire and Friesian breeds. The calves came to the 7 week experiments at the age of 8-16 days on average.

The calves were given 2 litres of whole milk three times per day, at 07.00, 14.00 and 20.00 using teat-buckets. Hay, a barley-oats-mixture (5 % minerals) and water were offered *ad libitum*. Feed consumption was measured weekly. The calves were weighed every week, too. The environmental conditions, temperature and humidity, were registered automatically.

The behaviour of calves was studied by direct 48 hour observation every second week. The observation interval was 2 minutes. Parameters included in the feeding behaviour were the number of observations of eating hay or concentrates and ruminating.

The feeding and behavioural data were not analyzed statistically, because the calves were reared in groups and the number of groups (=n) was only two. However, the weights and growth of calves in both experiments were analyzed separately using the least squares analysis according to the following model: Y(ijkl)= Mμ + group(i) + sex(j) + group x sex(ij) +race(k) + error(ijk). In these tests individual animals within groups were used as a statistical unit, hence, the indication of significant differences between treatments has to be interpreted with caution.

Results

During the two experiments the temperature varied inside the barn between 15..20°C (12..22 °C), in the heated shelter between 9..21 °C (13..25 °C), in the unheated shelter between -9..25°C (-2..25 °C) and outside in the yard between -8..25°C (-1..29 °C), for the experiments 1 and (2), respectively.

The growth of the calves did not differ significantly between groups in these experiments, although the calves in the outside groups grew slightly better, and in the second experiment the difference was nearly significant (p=0.057). There was not a big difference in the total dry matter intake, but calves in the outside groups ate a little more concentrates. In the 1st experiment the feeding efficiency did not differ greatly between the groups, but in the 2nd experiment it seemed to be better in the outside group (**Table 1**).

In the beginning of the experiments the calves ruminated about one hour per day on average, in the 1st exp. most of ruminating occuring in group 3 and in the 2nd exp. in group 2, probably due to older calves in these groups. During the last observation (at about 7-8 weeks of age) the calves ruminated on average 6 hours (5 h 14 min - 6 h 49 min in different groups) (**Table 2**).

Conclusions

The calves managed very well both inside and outside the barn, and the growth of the calves was good also compared with other experiments. The growth differences between the groups were not statistically significant, though there was a trend that calves grew better in the outside groups. The calves had free access to food, but the calves in the outside groups did not eat more than the calves inside the barn. Eating periods were short and occured mainly during daytime. The calves ruminated more during nights than daytime.

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Table 1. Growth, feed intake and feeding efficiency of the calves reared inside the barn in a group box (G1) and in individual boxes (G2) and outside in a group pen (G3).

Age 1-8 weeks	Experiment 1				Experiment 2			
	Group 1	Group 2	Group 3		Group 1	Group 2	Group 3	
	Mean	Mean	Mean	s.e.m.	Mean	Mean	Mean	s.e.m.
No. of calves(ó / ♀)	6(4 /2)	6(4/2)	6(3/3)		6(3/3)	6(3/3)	6(2/4)	
Age at start (days)	7.8	8.7	11.7		11.3	15.5	11.3	
Initial weight (kg)	47.2	49.1	50.3	2.41	54.1	53.0	52.7	1.06
Final weight (kg)	86.3	88.0	91.6	3.88	89.5	88.4	94.2	1.69
Daily gain (g/day)	798	793	844	37.8	723	722	847	34.0
Intake/day								
Total DM, kg	1.11	1.12	1.14		1.16	1.12	1.19	
Hay DM, kg	0.098	0.119	0.096		0.117	0.127	0.083	
Concentrates DM, kg	0.226	0.223	0.270		0.250	0.217	0.336	
ME, MJ (MAFF)	19.46	19.61	19.79		21.17	20.43	21.24	
Growth, g/kg DM	725	708	740		623	645	712	

s.e.m. = standard error of mean, DM=dry matter, ME=metabolizable energy

Table 2. Eating behaviour of the calves, % of observations.

	1st observation			2nd observation			3rd observation			4th observation		
1st Exp.	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3
Eats conc.	0.7	0.6	0.7	1.8	1.2	1.7	1.9	2.0	2.4	3.4	2.6	3.0
Eats hay	2.1	2.0	1.9	3.1	4.0	2.1	5.1	5.3	4.3	6.8	5.5	5.4
Ruminates	3.7	3.3	6.8	16.8	15.8	26.2	29.2	25.6	25.3	28.4	25.4	21.8
2nd Exp.												
Eats conc.	0.3	0.3	0.1	0.5	0.5	0.8	0.8	1.1	1.1	1.7	1.5	2.4
Eats hay	3.1	5.1	0.3	4.9	4.4	3.8	7.3	4.9	3.6	5.0	5.0	4.0
Ruminates	3.5	8.9	6.1	20.2	16.2	25.9	19.9	20.4	27.3	21.8	22.7	26.0

The influence of low temperatures on health state of calves

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Summary

Observations were carried out on a cattle farm in Čaňa, SR. The health state of newborn calves housed in hutches was evaluated in the period of 5 years.

Key words: hutches, calf rearing

Introduction

High concentration of animals in a limited space and utilization of industrial elements of production has been characteristic of cattle rearing in Slovakia in the recent period. This was reflected in a negative influence on health of animals as well as in higher death rates that occurred despite observation of nutritional principles as a result of unsuitable microclimate conditions. The large-capacity rearing of farm animals increases demands on management of animals in all its complexity. The shortcomings in this direction, together with neglecting the veterinary-hygiene measures, result in insufficient utilisation of the genofund of animals and in decreased economy of rearing. One of the important factors that affect the health and performance of farm animals is the system of housing which, especially for young animals, has its own specifities.

The paper presents practical experience and results from calf rearing over the years 1992-1997, obtained under the conditions of cold rearing.

Material and methods

The influence of external conditions on the health of calves housed in hutches from calving up to the weaning (3-4 months) was investigated in years 1992-1997 on a cattle Čaňa-Gyňov rearing cows of the Holstein breed. This farm operates with a closed turnover of the herd. Calves were reared outside in hutches at external temperatures from -20^{o}C in winter to +38^{o}C in summer. After the calving, the calves received colostrum and were transferred to hutches where they were fed milk acidified with formic acid and were also provided hay and feed mixture.

Results and discussion

The housing of dairy cows on the farm investigated is of stanchion type on litter. Before 1992, the newborn calves were kept in a profylactorium up to the age of 10-12 days and then they were transferred to houses to the section of milk nutrition. High requirements on providing the acceptable microclimate as well as high losses of calves exceeding 10% resulted in the change in the housing system of calves by placing them outside into hutches. This decision was made because of unsatisfactory microclimate in the profylactorium and also during the group housing in the milk nutrition section, mainly from the point of view of high humidity. It is generally known that such an environment results in increased microbial counts and negative influences on the health and development of calves. This phenomenon, referred to as growth depression, puts an excessive load on animals housed and decreases the effectiveness of the immune system.

After the calving, proper treatment and acceptance of colostrum, the calves are transferred to wooden hutches with some run area in front of them. The front part of hutches is 1.2 m high with a 0.5 m overhang, protecting the calves from an unfavourable climate, and the hight of hutches decreases to 1.05 m in the back. They are 1.2 m wide and 1.45 m long. The run area in front of the hutch is 1.6 m long and 1.2 m wide and a bucket for milk feeding is placed on the right at its front distant end. On the right side of the run area a hay rack and trough for feed mixture are located. The entrance to the enclosure is on the left at the front distant end. The ground below hutches has 3% slope. Similar construction of calf hutches is described by Brouček et al. (1994). Straw bedding is provided in hutches and added daily. After the weaning period the hutches are removed, cleaned and disinfected.

In the period of 1992-93, the calves were milk fed 3-times a day. In the first year of the experiment the animals received maximum care which was reflected in high weight gains and low losses, however, in 1993 the level of care decreased.

Since 1994 the calves have been fed only twice a day receiving a dose of 4-6 litres of milk. This way of feeding is recommended by several authors. Results presented in the Table as well as other data obtained during our investigations show that the care of the personnel affects the effectiveness of calf rearing. The access of calves to the forage has evidently a positive effect on the development of the digestive system. A mobile equipment was used for warming and distribution of milk. Drinking water was also warmed up during winter. The calves were kept in the hutches for 3-4 months until they reached the weight of 100-140 kg and then they were transferred to cold houses with runs.

While the losses of calves in calf houses in Slovakia amount to 13.4% (Murgaš, 1994, Kubina, 1994), Bates (1994) reports losses as high as 20%. The losses are ascribed to unsuitable microclimate and resulting pneumonias and gastrointestinal diseases. The advantages of cold housing of calves are pointed by Brouček (1994), Ballasch and Tamási (1988) according to whom the morbidity and mortality of calves kept in hutches is considerably lower in comparison with that of calves reared in calf houses.

Table: Some parameters of cold rearing of calves

Year	No. of calves	Ave. daily WG in g	No. weaned per year	No. died	Death rate in %	Level of care
1992	19	736	95	1	0,8	excellent
1993	28	545	106	2	1,49	acceptable
1994	43	683	130	3	1,7	very good
1995	41	662	109	5	3,3	very good
1996	37	599	87	3	2,4	good
1997/3m	42	704	31	1	1,3	very good

WG - weight gain

Conclusion

Cold housing is one of the possible ways of calf rearing. This system of rearing corresponds to the physiological requirements of calves. According to our observations as well as those of other authors, independent on outer climate conditions, this way of rearing is successful.

The results presented in this paper were obtained within the scope of solving the project No.95/5195/575.

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Zoohygienic estimation of incliviclual variants of calves growing of half open housing

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Summary. The most defy-distributed technologies of calves and younger animals growing, nucluding their growing in individual cages on the open air and calf-sheds of the half-open type, have been continuese, out since 1960. It was stated that low air temperatures, being the nominating factor for such conolitious, lead to initial loading on main organs and organism systems, increase their adaptational abilities, accelerate the establishment of physical thermoregulativ, improve resistance, growth, development and significantly decvase the mordefility of calfes. Some indexes of thermobalance, heat lossess of organism by main ways, the value of energetic over-expluditure compensation, value of adrenal functional state AKTG-testing for the evaluation of organism adaptation abilities were defined more precisely. Optimum technological variants were selected.

Key words. Cages; calf-sheds; system of rear (reise); "cold" method; functional state of organs; thermoregulation; nature resistance, adaptation.

Introduction. Intensificator of cattle-breeding expacts through study and wide introduction of optimum, zoohygienically based technologies of calfes and younger animals in addition to selection and full-value nutrition (Shukanov A., Onegov A., Nikitin I. 1986; Kunz P., 1985). In this connection a wide study must be provided of separate environmental factors and a complex of factors influence on the organism, its etimologic, physiologic and biothermistry reactions-answer and on that summary results, which is received from the system of maintanence (Demchuk M., Gavrilec E., Jankowski, 1988). Namely science lyes in the basis of single-minded improvement of young animals growing, their adapted for longterm intensive use; mastering of calves prophylaxis methods by the improvement of zoohygienic and veterinary-sanitary measures directed at the increase of dynamic abilities, adaptional abilities and natural resistance of growing organism (Batin, 1984; Rafai P., Kovacs F., Ballash A., 1985).

Besides some achievments scientily exploved an still questions dealing with the mechanisms and dynamics of postnatal stabilizing and functioning of cardiac-vascular, hypotalam-hypophis-adrenocortical system (HHACS) and hypotalamo-hypophisis-tieroid

(HHTS) systems, that gives scientific approaches to the regulations of adaptation processes in the calves organism and influence mechanisms on these processes of brading (Kostin A., 1970).

Materials and methods. On dairy farmes of South-Western region of Ukraine a number of experiment were held on zoohygienic estimation of the most videly speed variants of calves growing during prophylaxis dispansery and milking periods and for young animals up to 6 and 9 months age. The animals from research group were equated with the analogue in a control group. Calves form control group were growing in cages of the coker-type calf-sheds and the animals from research group in individual or group cages in the open air or half-coker-type calf-sheds. Number of animals most be not less than 6.

Feeding of calves on milk and beesting from drinwing bowes or pails was made by hands or using individual or group inflow of milk cows. Microclimate in calves-sheds and cages was investigated by accepted in zoohygiene methods estimating it by number system (Yu. M. Markov, 1983). Zootechnic, clinical factors, including haemodynamic and electrocardiogram were evaluated according "Methodological recomendations VASGNIL" (1980). Temperature skin reaction was determined by electrothermometer TTC-1 in 5 conditionaly pointed zones of a body (I. I. Khusainov, T. A. Mumuladze, 1975). Taking into account a percent relation of the surface of separate body part to its general surface a mean skin temperature was calculated as well as the intensity of heat expence by radiation, convection and diffusion of water vapour against the background of specific microclimate conditions with the help of biophysical modelling method.

Different hematologic and biochemical investigations were made using the blood from jugular veins: investigations of erythrocytes number and hemoglobin concentration (Ya. L. Germanyuk et al., 1966); a number of leucocytes - calculations in Horyaev camera; a number of T-lymphocytes (M. Joutal et al., 1972 using modification of P. D. Zuev et al., 19778); B-lymphocytes (H. F. Koromyslov et al.,1980); bacteriocytic activity of blood serum (O. V. Smirnova, T. A. Kuz'mina, 1966); its lysosome activity (H. F. Dorofeichuk, 1968); concentration of tyroxine and threejodinetyronine - by radioimmuniologic method with RIO-T_4PG and RIO-T_3PG reactives from the Institute of Bioorganic Chemistry of Byelorussian Academy of Sciences; cortisol concentration before and after loading by corticotropine (AKTG doze is equal to 0,25 units/kg of wass) wich was determined also radioimmunologically with STERON-K125 reactive from the mentioned institute.

All the received data were proccesed statistically and some of them by the method of regressive analysis.

Results. Experiments on calves "cold" method growing showed that normal uterine development (the living weight by born), corresponding preparation of the animals and housings (cages), possibility of motion and rational feeding, the durability of period for adaptation to low temperatures decreases to 10-15 days.

Calves, which grow in external cages, loose heat mainly (40-48%) convectionaly, and less (24-30%) by radiation and only 6-9% with the diffusion of water vapour. Intensity of organism heat lossess, for such calves is for 15% higher than for analogue, which were growing in cover-type calf-sheds under the temperature equal to 7-15 °C. This allows the organism to use 63,5% of "pure" energy for the maintanence of vital activity and to receive of daily accretion of living weight on the level of 690 g. Motor activity of such calves increses for 16,3%. Adaptation process to low air temperatures excites in the organism of new born calf considerable intensification of heart-vascular organs activity and breathing. Level of glucose and ATPH in blood decreases (for 24% and 33%), concentration of milk acid decreases (for 41%). But all these changes were valid only along 3-4 weak after calf birth, after this period symptoms of stress dissappear. "Cold" growing also effects positively on the formation of immoral and cell immunity in the calves organisms. The number of leucocytes in blood increased for 6,4%, lymphocytes - for 7,4%, T- and B-lymphocytes for 10,9% and 12,4% correspondingly. Bacteriocyte and lysocytic activities of blood serum was higher- for 6,5% and 6,3%.

Mentioned above indexes of immunity remained better and for young animals (6 months of rege), which grew in calves-sheds of half-open-type and undertly conditions of animals moving.

Calves growing in individual cages on open grounds only up to 3 months age favoure the animals organisms tempering, accelerates the functional formation and increase of reserve abilities of adrenal cortex (for 54% and 61% correspondingly in summer and winter) in comparison with calves growing in group cages of cover-type calf-sheds. Given positive tendension preserves also after the transition of calves from experimental group to general cover-type calves-sheds. Constant maintanence of calves in group cages in cover-type housings leads to expressed stresses of adrenal cortex function (cortisol concentration in blood reaches 90,6 n/mole/1). All this festiques the exhaustion of adrenal cortex functional abilities and the decrease of adaptational capacity of organism. And as a result we have the increase of morfidity in such groups of calves (initial - upto 60% and repeated - up to 26,7%) and the decrease of calves safety up to 92%. These results of AKTG-testing for calves, growing in group cages of cover-type calf-sheds were defined more exactly by the method of regressive analysis.

Under the working conditions of cattle farmes, producing milk calf-sheds of halfopen-type, as a part of change multisectional dispensary, proved themselves positively. Such calf-sheds allow to decrease for 2,6% the calves morfidity, to preserve the whole live-stock and to increase for 21% accretion of living weight.

For the success adaptation of new born calves to low temperatures (-14 °C and lower) it is nessessary to inlarge the daily norm of calves watering for 26,7-30% in order to provide their planned accretion.

Combination of calves growing in half-open-type calf-sheds with the inflow method of feeding corresponds zoohygienic demands, which are layed down to technologies of such type and physiological peculiarities of animals organisms during the milk period of growing. All this confirmed by a high functional activity of thyroid gland, certain reserve capacity and functional level of adrenal cortex, rate of calves growth and development.

Conclusions. On the cattle farms of air regions it is usefall to introduce such technologies which foresee the connection of milking inflow method and growing of calves first in individual and than (beginning from 20th day) in group half-open-type cages.

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Group and cold rearing of dairy calves: lying behavior. Results from two pilot experiments.

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Summary

The dairy calves were reared for 7 weeks in three groups: inside a barn in a group box (group1) and in individual boxes (group 2), outside in a group pen with a thermoregulated (TRS) and a unthermoregulated (NTRS) shelter connected with a yard (group 3). Each group consisted of six calves, which were 8-16 days of age on average at the beginning of the test. The lying behavior of the calves was observed for 48 hours, 4 times during the test. The calves in group 3 spend more time in TRS than in NTRS, except in two of the observation periods when also the highest minimum observation temperatures were measured. The total lying time decreased with age in all the groups. The calves in group 2 laid down more and ruminated less as procentages of lying observations (OBS) than the calves in the other groups. Lying in the side position (SP) with 3-4 legs straightened out was most common in group 1.

Key words: dairy calf, behavior, lying, non-thermoregulated rearing system, temperature

Introduction

The calf rearing methods in Finland in single boxes and with bucket feeding, do not fulfil their physiological and behavioral needs. There is an increasing interest of housing dairy cows in simple, unheated buildings. Calves can cope in relatively low temperatures (Woivalin 1990, Ylipekkala 1990, Bøe 1993). The ability to lie down in different positions improves resting and thermoregulation of young calves (de Wilt 1984).

The aim of these experiments was to develop and test methods for a project that compares the performance of young dairy calves in thermoregulated (TRS) and unthermoregulated (NTRS)

group housing systems in a Nordic climate and to develop practical ways for rearing calves in these conditions with respect to their physiological and behavioral needs.

Material and methods

The experiments were conducted in autumn 1995 and in spring 1996. The six dairy calves were brought to each test environment at the age of 8-16 days on average. The experiments lasted for 7 weeks. The calves were reared inside a barn in a 10.7 m^2 group box (group 1) and 1.2 m^2 individual boxes (group 2), both with a wooden slatted floor. In the outside pen (group 3) the calves could choose either 12 m^2 straw bedded TRS or NTRS connected with a 40 m^2 yard covered with bark. All the calves were fed 3 times a day with 2 liters of whole milk using individual teat-buckets. Hay, an oats-barley-mixture with minerals (5%) and water were given *ad libitum*.

The behavior of the calves was observed every second week for 48 hours. The observation interval was 2 minutes. The studied parameters were 1) the average % of lying down from the total observations (OBS) 2) the average % of lying in a side position (SP), with 3-4 legs straightened out, from the total lying OBS 3) the average % spent ruminating from the total lying OBS and 4) the average % of shelter usage from the total location OBS in the outside groups. Environmental conditions, temperature and humidity, were registered automatically.

This was a pilot test and due to a small sample size (n=2) the behavioral data can not be analyzed statistically. The gained information is used as a basis for further studies.

Results and discussion

During the two experiments temperature varied inside the barn between 15..20 ^oC and 12..22 ^oC, in the heated shelter between 9..21 ^oC and 13..25 ^oC, in the unheated shelter between -9..25 ^oC and -2..25 ^oC and outside in the yard between -8..25 ^oC and -1..29 ^oC, respectively.

The calves preferred the TRS over the NTRS in both of the pilots. However, there was one observation period during both of the experiments when the calves preferred the NTRS, 3rd in experiment 1 and 4th in experiment 2. The highest minimum temperatures during observation periodes were measured then (**Table 1.**).

Lying OBS decreased with age in all the groups and the rumination increased. This is also shown by other authors (Sato, 1993). The smallest averages of lying OBS were for group 3 and the largest for group 2. (**Table 2.**) The calves in group 2 ruminated less as procentages from lying

OBS than calves in the other groups. The rumination behavior is described more detailed by Hepola et al. in these proceedings.

The calves in group 1 were observed more often in a SP, than calves in the other test groups. The SP observations decreased with age, also the relative portion of SP from lying OBS declined. Similar results are reported in the other studies (de Wilt 1984, Albright 1991). The maximum number of OBS in SP was in the groups 1 and 2 during the 1st OBS periode but only in group 2 during the 2nd OBS periode. However, the standard deviation inside the groups was wide.

Conclusions

Sato (1992) suggested that frustration from restricted space and a lack of social contact and decreased exploration behavior is increasing lying and rumination. In our experience the calves in single boxes (group 2) were observed to lay down more. They spent, however, less time ruminating as a procentage from lying OBS. Calves in the more enriched environment in the outside pen (group 3) were observed to lay down less than calves in the other groups.

Calves in the inside group (group 1) were observed to lay down more in SP than calves in the other groups. The reason might be a lack of space (0.99 x 1.20 m^2 per calf) in group 2 or avoidance of draft in groups 2 and 3.

The temperature seemed to have an effect on the preference of young calves for NTRS or the TRS, but it needs further studies. A strong effect of the behavior of the leader calf on the group preference can not be excluded.

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Table 1. The average % of shelter usage/ calf (S.D.) from the total observation and temperatures outside during observations

	Experiment 1					Experiment 2			
	obs1	obs2	obs3	obs4		obs1	obs2	obs3	obs4
Ntrs	1.16	25.52	68.85	6.26	**Ntrs**	3.84	4.83	4.64	42.17
S.D.	2.2	2.23	6.26	0.13	S.D.	5.09	4.72	2.81	2.3
Trs	37.62	53.32	2.83	70.51	**Trs**	60.07	74.13	59.82	0.34
S.D.	31.97	2.72	2.59	5.77	*S.D.*	14.51	3.99	3.38	0.32
Tmax	-	17	12	7	Tmax	15	6	21	23
Tmin	-	2	6	-2	Tmin	-1	3	5	11
Mean	-	10.6	9.8	1.0	Mean	7.3	10.6	10.60	14.3
S.D.t.	-	5.0	1.8	3.0	S.D.t.	4.3	0.8	2.9	3.4

S.D. = standard deviation for the variation of behavior of calves in 2 d NTR = non-thermoregulated shelter, TR= thermoregulated shelter, mean = average temperature outside during OBS, S.D.t.= standard deviation for the variation of temperatures during OBS

Table 2. The lying behavior of calves, % of observations

Experiment 1					Experiment 2				
Grp	obs1	obs2	obs3	obs4	Grp	obs1	obs2	obs3	obs4
1	73.00	68.01	67.21	66.77	1	69.84	69.62	60.45	65.60
S.D.	5.87	6.00	4.76	4.79	S.D.	6.05	4.33	6.13	4.49
2	76.11	69.33	71.09	72.28	**2**	72.04	67.01	72.35	72.40
S.D.	6.06	3.49	3.79	3.81	S.D.	7.38	7.11	4.87	5.58
3	*65.14*	*66.73*	*60.04*	*62.34*	***3***	*69.33*	*66.28*	*57.86*	*54.38*
S.D.	*3.59*	*3.45*	*4.02*	*3.53*	*S.D.*	*3.48*	*3.07*	*5.73*	*8.45*

Adaptation of calves to a cold micro-climate

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Summary

Modern dairy husbandry has tended to emphasize the low cost of production.The purpose of this study was to investigate the influence of a cold micro-climate on the welfare of calves. Three groups of newborn Ayrshire bull calves were housed under different climatic conditions for 9 weeks in winter. One group was in warm (+10 - +16 ^{o}C), the second group was in cool (0 - +5 ^{o}C) and the third group in cold (with temperature varying according to the ambient conditions) experimental rooms. The first part of this investigation shows that the calves grow as well in the cool and cold micro-climates as in warm. The calves housed in cold had lower skin surface temperature which indicate a lower heat loss.

Key words: calf, cold micro-climate, adaptation, growth

Introduction

In Finland, there is an increasing interest in housing cows in simple, low cost buildings. This is due to higher building costs here than in most European countries. In the future it will be necessary to find new and more economical ways of building cow houses. However, a low cost building implies lower and also more variable indoor temperatures than those presently recommended i.e. +10 - +16 ^{o}C (Rajala 1990). According to Webster (1974) the lowest temperature which a newborn calf can survive is +9 ^{o}C and even at one month it should not be in sub-zero conditions. On the other hand, it was reported that the growth and welfare of calves were not adversely effected even though the ambient temperature fluctuated from -10 ^{o}C to +30 ^{o}C (Hansen 1984). Rawson et al. (1988) also reported that the newborn calves did not experience any physiological problems in extreme cold conditions.

It was decided to study the effect of a cold micro-climate on health, welfare and growth rate of calves to identify housing conditions that do not overtax their physiological and behavioral capacity to cope. The aim of this study was to study adaptation of young calves to a cold and variable micro-climate.

Materials and Methods

In the first part of this study 21 Ayrshire calves (males) were subdivided into three test groups living in different climatic conditions during winter. The experiment will be repeated next year. Each group consisted of 7 calves and they entered the 9 weeks test at the age of 7 - 12 days.

They were evenly divided into three test groups (K, T_1, T_2) according to weight and the level of gammaglutamyltransferase enzyme. The groups were placed in experimental rooms measuring 500x450x250 cm, (LxWxH). The group pen with a concrete floor covered with a 40 cm straw litter bed was situated in the middle of the room. Control group K was in the warm room (+10 - +16 ^{o}C). Test group T_1 was in the cool room (0 - +5 ^{o}C) and test group T_2 was in the room where the temperature was changing (+6 - -22 ^{o}C) according to the climate outside. The air velocities were <0.1 m/sec in all three group pens. The calves were fed from buckets three times a day and they also had free access to hay, water and a feed concentrate.

The groups were video recorded for 24 hours per day for the five first weeks. Blood samples were taken weekly and the calves were weighed every third week. Health was registered daily. Hair samples were taken at the end of the test. At the end of the experimental periods all animals were first allowed to adapt to a constant temperature for 2 h after which their skin temperatures were recorded using an infrared video camera.

Results

The first part of this experiment started on October 30th 1996 and finished on February 2nd 1997. There were no significant differences in mean daily growth rate between the test groups ($p>0.05$, Friedman one-way ANOVA) during this first experimental period (**Fig. 1**). The infrared photographs showed that the skin temperatures were lower in those calves housed in the cold ambient temperatures.

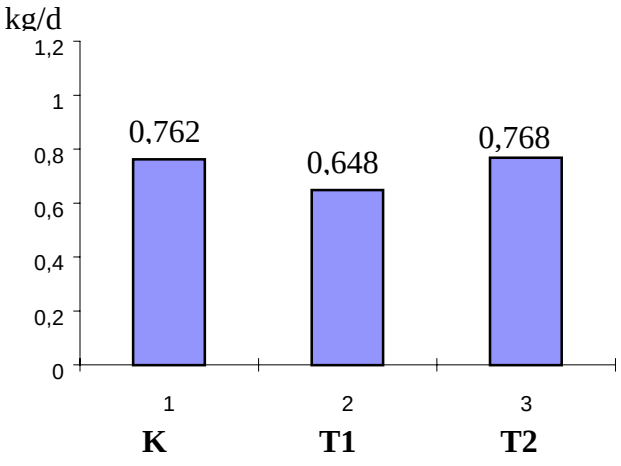

Fig. 1. Mean daily growth during 1 - 9 weeks in calves housed in warm (K, +10 - + 16 ^{o}C), cool (T1, 0 - +5 ^{o}C) or ambient cold (T2, +6 - -22 ^{o}C).

There were no statistical differences between serum glucose values ($p>0.05$, Friedman one-way ANOVA) during the first period (**Table 1**.). The results of the other blood parameters and hair analyses will be reported later.

Table 1. Mean weekly values for serum glucose of calves (mmol/l) housed 9 weeks in warm (K, +10 - +16 ^{o}C), cool (T1, 0 - +5 ^{o}C) or ambient cold (T2, +6 - -22 ^{o}C).

	Week								
Group	1	2	3	4	5	6	7	8	9
K	3.5	3.7	3.6	3.6	3.7	3.6	3.7	3.6	3.9
TI	3.5	3.8	3.7	3.5	3.6	3.3	3.7	3.9	4.0
T2	3.6	3.2	3.7	3.9	3.9	4.2	4.1	4.1	4.1

Conclusions

The results of this study indicate that calves could be housed in simple, low cost shelters although the temperature is even sub-zero. Exposure to cool and cold temperatures did not reduce the calves´ daily growth during 9 experimental weeks. In this study the calves were kept in groups of 7 animals enabling them to reduce the impact of cold through huddling behaviour. A dry, 40 cm deep litter bed and a lower air velocity than that often considered the limit for winter (0.25 m/sec) further reduced the severity of the cold. On the other hand, the lower skin surface temperatures in calves housed in the cold indicate that they had undergone adaptive changes in response to the low temperature history, either through circulatory adjustments or through changes in hair coat insulation.

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The Suitia Research Unit for Animal Production Environments

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Summary

The Faculty of Agriculture and Forestry at Helsinki University has set up a research unit for full scale testing of various animal production environments on one of it's experimental farms. The first research setup will be focusing on different aspects of unisolated loose housing systems for dairy cows. The building project of the research unit was completed in june 1997.

Key words: production buildings, animal production environment, unisolated loose housing systems

Introduction

Research activities in Finland concerning various aspects of animal production environment have missed facilities that are flexible and effective in terms of research objectives. Certain trials, especially comparative studies, cannot easily be realized on practical farms. When dealing with new production methods, it must be possible to discontinue the trial, and the animals must have other shelters during renovation of the trial set up. These facts among others have lead to the new facilities that were built next to the 80-head isolated loose housing dairy barn on Suitia research farm in Siuntio.

The research facilities

The research facilities consist of a research hall (650 m^2) and adjacent laboratories for individual animal monitoring / operations and specimen preparation (230 m^2).

The hall has been designed in such a way, that practically all constructions (except the truss and foundations) can be changed when needed. This kind of flexibility allows different research setups without high reconstruction costs. In compliance with the first research setup, the building has been given the form of an unisolated loose dairy cow house with three partially slatted walls and open ridge.

The laboratory facilities have been renovated inside an old animal building. They consist of an animal monitoring / operations room with places for up to three cows, an adjacent observation room and rooms equipped with basic specimen preparation instrumentation.

Research activities

The main aim of current research dealing with animal production environment is to find out, how the production costs per animal due to the building can be lowered to an economically sustainable level without compromising welfare of the animals and animal keepers, environmental aspects or product quality. The following strategic research topics relating to different approaches have been specified:

- Organization / management of different tasks and parts of production.
- Interaction between production methods and production environment.
- Effect of production methods on animal behavior and health
- Performance and economy of technical solutions
- Effect of production methods on field operations
- Effect of production methods on the environment

The first research projects at the research unit for animal production environments will cover six subjects that focus on unisolated loose housing systems:

- Cubicle alternatives for dairy cows
- Health and behavior of calves
- Health of dairy cows
- Safety risks of the animal keeper due to dairy cow behavior
- Feeding technique and eating behavior of cows
- Development of environment and welfare measurements

Conclusions

The research unit provides facilities that have previously not been available and that are needed in various animal production research approaches. It will be used by several research institutes within and outside Helsinki University.

Friesian x finncattle (fr x sk) cows rearing two foster calves

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Summary

The objective of the experiment was to study friesian x finncattle crossbred cows as suckling cows each fostering one extra calf with her own calf (5.5 mo) and one additional calf (4.5 - 5 mo) after the weaning of the first two. Data was obtained from the same 10 - 14 cows over three consecutive years starting from the first lactation. The cows' own calves were a cross between fr x sk and either aberdeen angus (1st calving) or limousine (2nd - 3rd calving). All foster calves were ayrshire bulls. The nature of calving, cows attitude towards foster calves, milk production and growth of calves in the suckling period were studied. Milk production was measured in the third lactation by the calf weighing method. The trial was conducted in a cold loose housing system.

The cows had more calving difficulties when they were primiparous than multiparous. The older cows accepted the foster calves easier than young cows, but variation between individuals and years was large. There was no difference in the manner that the cows treated the extra or additional calves. Milk production was the highest at 120 days after calving (18.1 kg d^{-1}), and in the suckling period (165 d) the cows produced on average 17.4 kg milk daily. In the suckling period the cows' own calves grew better than the foster calves, but after weaning (the data not shown here) there was no difference.

Key words: crossbred dairy cows, multiple suckling, extra calves, additional calves

Introduction

In Finland the number of suckling beef cows is rather small (30 000), the main reason being the low profitability of suckler herds. One possibility to increase profitability and make the production more intensive is to have cows of dairy breeds suckling more than one calf per lactation. The milk production of a dairy or crossbred cow is high enough to suckle several calves, but also mothering ability and good nature are important. In a cold housing system a suckling cow has to be healthy and resistant to environmental changes.

Cows of beef breeds have been shown to be slightly better dams than dairy cows (Selman et al. 1970), but the differences between breeds in accepting foster calves have been variable (Nilsson 1973, Perez et al. 1983). In general, the strength of a cow-calf bond depends on individuals. With age a cow often becomes more maternal and has a stronger bond with her own

calf, which can make the acceptance of alien calves more difficult (Burger 1980). On the other hand, a cows experience in multiple-suckling can facilitate fostering. Several tactics have been used to get a cow to accept foster calves: the calf has been given to the cow immediately or 5 days after calving (Le Neindre et al. 1978), the cows own calf has been removed temporarily (O'Neill 1979) or alien calves have been treated with foetal fluids (Rosecrans and Hohenboken 1982).

The objective of this experiment was to study the ability of friesian x finncattle cows to be suckling cows in terms of nature of calving, acceptance of foster calves, milk production, feed intake and growth of the calves in cold housing conditions.

Materials and methods

Fourteen cows started in the experiment as heifers and they were studied over three years. Four cows had to be removed during the trial. The cows' own calves (24) were aberdeen angus (aa) (1st year) and limousin (li) (2nd - 3rd year) crossbreds, whereas all extra (29) and additional (18) calves were ayrshire (ay) bulls. The experiment was carried out in practical conditions, and the production was based on home grown feedstuffs as well as low building and machinery investments. The building was a cold uninsulated loose house with three walls. A calving and weighing room was located between the cow-calf area and the compartment for growing cattle. Feeding was based on wilted silage, grass and rolled barley treated with propionic acid. The cows had silage *ad libitum* and 2 kg of barley per sucking calf daily. In the suckling period the calves were not given any other feed than milk, but they did have access to silage and barley on the cows' feeding table as well as grass at pasture.

The desired calving season was March-April. All calvings were ranked into four categories: easy (no assistance), fairly easy (light assistance), difficult (strong assistance) and dead calf. The extra calves were given to cows within a week from calving and the additional calves one day after weaning of the first two at 5.5 months. The age of the foster calves was 5-20 days. To get used to sucking, the foster calves were first allowed to suck a calm cow twice a day when the cows were tied to the feeding table. When the foster calves had learned to suck and were accustomed to the environment they were allowed to stay together with the cows and other calves. The acceptance of foster calves was estimated by the cows willingness to let the calf suck: easy (from the beginning), fairly easy (within 7 days), difficult (within 4 weeks) or not at all.

Both milk production and feed consumption were determined during the third year at 60, 120 and 165 days after calving. Each cow and her calves were in a single pen during four days. Feeds

and orts were weighed during the first three days and milk production was measured at the fourth day by calf weighing method.

Results

The cows had more difficult calvings when they were primiparous (28.6%) than multiparous (8.7%) in spite of the aa bull and lighter calves in the first year. Calving difficulties increased with the birth weight of the calf within each year. However, the differencies in calving difficulties were not statistically significant.

The daily milk production of the cows in their third lactation was highest at 120 d after calving (18.1. kg) being 0.9 kg higher than at 60 d and 1.6 kg higher than at 165 d. The average daily milk yield was 17.4 kg in the 165 d long suckling period.

The acceptance of foster calves by the cows was highly variable. The behaviour of an individual cow could change between the years. However, if a cow was known to be a good dam, she always accepted foster calves easily. The older and more experienced the cows became the easier they seemed to accept foster calves than in the first year. There was no difference in the behaviour towards the extra and additional calves.

In general, the cows did not like to suckle alien calves especially when they were tied up during feeding. On the other hand, when loose even a difficult cow would let the extra calf suck at the same time as her own calf. The additional calves were able to suck only during the cows' feeding time, because they did not have the support of the own calves. However, the foster calves quickly learned how to get milk and did not give up easily even when being kicked. The cows did not show much affection or care for the foster calves and therefore human help and observation during the cows' feeding times (twice a day) was needed for about two months to ensure sufficient sucking.

The amount of feed consumed by the cow-calf pair increased towards the end of the suckling period (**table 1**). Milk was enough to cover the energy (FFU, fattening feed unit) and protein (DCP, digestible crude protein) demand of the growing calves but their capacity to consume dry matter (DM) was greater.

Table 1. The average daily feed consumption of the cows and their two calves, and the calculated proportion of forage intake by the calves (%).

	kg DM			FFU			g DGP		
d	forage	barley	forage/ calves	forage	barley	forage/ calves	forage	barley	forage/ calves
60	14.2	3.3	14.9	11.0	3.9	7.3	1948	305	6.6
120	18.5	3.3	21.8	14.0	3.9	16.8	2479	296	11.1
165	22.2	3.3	43.5	16.8	3.7	28.8	3170	305	16.0

In the suckling period the daily growth rate of the li x frsk bulls (1300 g) was higher than in any other group ($p<0.01$). The difference in the daily growth rate between the aberdeen angus crossbred bulls (1000 g) and extra (873 g) or additional calves (996 g) was not significant, but the additional calves grew faster than the extra calves ($p<0.05$).

Conclusions

The results of this experiment show that the friesian x finncattle cows produce enough milk to suckle their own calf and two foster calves succesfully. The cows learn to suckle foster calves with experience but as the individual differences are big it is important to select the most suitable cows for this type of production. The dairy type cows also managed well in the cold housing system and did not develop mastitis or other illnesses. During the most cold period in the winter the cows were dry.

The use of limousine bulls does not increase calving difficulties in older cows, but for heifers it is advisable to use smaller breeds. In addition, li enhances the growth of calves especially before weaning. The performance of foster calves is poor in the suckling period, but after weaning they show compensatory growth and produce good carcasses.

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Cold housing and open housing – effects on swine health, management and production

Cold housing and open housing - effects on swine health, management and production

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Summary

As intensive indoor pig production has evoked strong reactions in society due to morbidity and behavioural problems and as building and energy costs have increased, the interest is now focussing on out door or cold housing of pigs. Systems have been developed that function well in terms of production results and disease prevalence. However, it is crucial to gain more knowledge about maternal characteristics in sows as these to a large extent influence the reproductive success. It is also crucial that the farmer has the proper interest and skills to manage such housing systems.

Key words: Housing, climate, health, behaviour, management, production

Introduction

Since 9000 years back, the domesticated swine has been kept outdoors under different conditions as well as in various forms of cold housing. It is really only during the very last portion of this time period that man has intensified pig production and used insulated and climatized buildings. This was mainly to control the production better, maybe to some extent to prevent exposure to predators but also to make use of feed more efficient. The increase in production led to a development where litter size born, feed efficiency and growth rates governed decisions on where to go for the future. However, as a result of this husbandry systems were developed which were not accepted by the public anymore. High incidences of MMA, piglet diarrhoea, injuries, respiratory disease and leg problems as well as behavioural disorders such as bar biting, tail biting, aggression and apathy were all signs of environmentally evoked illness and suffering. On top of this, building costs as well as costs for energy have increased substantially over the last decades why there has been both consumer and producer interests to find other housing systems that better allows for healthier pigs and lower investments.

Out door piglet production

Rearing sows and piglets in huts is nowadays a common practice in the UK as well as in many other countries in the northern part of Europe . Many types of huts have been tested and as a general rule it can be said that the farrowing hut should be big enough to allow the sow to move around in her nest, but not so big that two sows choose to use the same hut. Arch shaped huts allow for some space between the sow and the wall which prevents crushing of pigs and the length/width ratio has been proven important to get the sows to lay down diagonally in the hut. This, in turn, reduces piglet crushing (Ebner, 1993). The placement of the huts, as well as the character of the soil will determine whether the dirt will turn into mud during rainy periods. Enough straw should be provided so that the temperature in the nest keeps at reasonable levels (Algers and Jensen, 1990; Ebner, 1993). When the litters are a couple of weeks old the pigs will start lying together and it is important to take the huts away and provide the pigs with a communal lying hut.

When it comes to the animals and their functioning in these out door systems we need to rely on the natural behaviour of the sow and her ability to care for her young (Jensen, 1988). It is experienced by many farmers that some sows show better maternal care and some sows do not care enough for their piglets and losses might increase. At present there is very little knowledge available about what causes such differences in maternal behaviour. Environmental as well as genetical factors have been suggested to play important roles (Algers, 1992) and research has only just started within this area (e.g. Visser, 1995). It is of great need to gain more knowledge as the production results to a large extent depends on the maternal abilities of the sows. Production results are, however, generally reported to bee good, 20-22 piglets weaned per sow and year (e.g. Visser, 1995; Ingold and Kunz, 1997).

Out door finishing pigs

To keep finishing pigs out doors demands well thought through solutions in terms of allocation of paddocks, grazing rotation scemes etc. If the ground is to heavily exposed to the pigs there is not only a build up of parasite infestation pressure, but also a deteriorated state of claw health. Access to drinking water as well as a mud hole is crucial. Management routines need to include a daily supervision to ensure that diseased animals get proper assistance and care. A separate pen for treatment is needed but is often not built on the farms (Lindsjö, 1996).

In a large swedish survey the disease prevalence in out door finishing pig production was lower than the national average disease prevalence regarding abscesses, pneumonia and pleuritis, similar regarding ”white spots” and higher regarding joint infections (Lindsjö, 1996). Management procedures, such as vaccinations, grazing rotation scemes and rearing conditions of the piglets will affect these results substantially.

Hooped structures

Hooped structures constitute simple shelters from rain, wind and extreme sunshine. In recent years, the interest in using such structures for finishing pigs have increased especially in the US (Weber, 1996). With a better technology for using heated water systems, such buildings could prove cost effective as well as having the prerequisits for allowing the pigs to express their natural behaviours. As the air quality corresponds to out door air, infection pressure will be low and the prevalence of respiratory disease may prove to diminish. At present, however, few large scale studies on disease prevalence have been carried out studying such environments.

Management skills

It is evident, not least from the experience of many farmers, that the management skills needed when growing pigs in out door or cold open housing systems are different from those of many farmers using conventional indoor systems (Thornton, 1988). First of all, the farmer need to have an interest in his animals. many of the first signs of something going wrong are just subtle changes in the animals normal behaviour. A piglet, that keeps lying in the straw when the rest of the piglets run away as the farmer walks around inside the paddock is a sign that someting is not right. A gilt, that goes into other sows huts as she is about to farrow might be annother sign.

Further, when using deep bedding, as in hooped structures, the farmer need skills to keep the bed in the right condition so that it can be used for the pigs to regulate their comfort in cold weather.

In summary, the farmer must conceptualize his pig farming so that all parts of it, including his use of land, functions well together. It is only when this is achieved that the pig husbandry can be pursued with low morbidity, allowing for the pigs to behave naturally and with appreciation from the consumer and society.

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BEHAVIOURAL AND PHYSIOLOGICAL THERMOREGULATION IN DRY SOWS HOUSED IN AN UNINSULATED BUILDING.

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Abstract:

Behavioural and physiological responses of pregnant sows to cold exposure were investigated. Ten groups, each of six pregnant sows, were housed in an uninsulated building during winter. There were two pens in the house, each containing kennels with straw bedding and individual feeding stalls. General activity (standing, sitting, movement, manipulating straw), lying posture, degree of huddling and the use of kennels were continously recorded by four video cameras. Blood samples were taken from group 9 and 10.

The results show that with decreasing temperature outside, the sows spent more time lying on the belly in contact with another individual than with higher temperatures. The sows spent more than 80% of their time inside the kennels. There was a negative correlation between outdoor temperature and time spent inside the kennels and manipulation of straw in the kennels. The sows spent more than 50.8% of their active time manipulating straw. At outdoor temperatures below -10 ^{o}C, the sows had a higher level of free fatty acids and free thyroxine than at higher temperatures.

Introduction

Lower critical temperature is influenced by many factors, such as body weight and condition, provision of a micro climate and type of floor. A study on thermal micro climate in winter-farrowing nests of free-ranging domestic pigs, showed that nest temperatures were virtually unaffected by outer climatic conditions and by the number of piglets in the nest (Algers and Jensen, 1990). This shows that it is possible to construct an optimal micro climate just by using straw. Verstegen and van der Hel. (1974) found that the effective critical temperature of animals weighing 40.0 kg was 11.5-13.0 ^{o}C on straw bedding, 14.0-15.0 ^{o}C on asphalt and 19.0-20.0 ^{o}C on concrete slats.

Compared with sows housed individually, social groups have a better energy budget. According to Close et al., (1971), Geuyen et al. (1984) and Verstegen (1971), groups of pigs show a considerable lower extra heat production below the effective critical temperature in

comparison with solitary individuals. The lower critical temperature for sows on concrete floor, are estimated to 20 ^{o}C when housed individually, and 14 ^{o}C for group housed sows (Geuyen et al., 1984).

According to Bøe (1990), an animal in confinement have at least four alternative strategies to reduce their heatloss; altering lying time, changing lying posture, changing time of the day for lying (lie more in the middle of the day when temperature is higher) and social thermoregulation. A stratgy to increase heat production is to increase the activity level.

The aim of this study was to investigate behavioural and physiological responses of dry sows exposed to low temperatures, and the effect of competitive ability of the sows on the use of kennels with straw bedding.

Material and methods

Ten groups, each of six dry crossbred sows (Landrace x Yorkshire) of different parities were used in the experiment. They were kept in an uninsulated building with two experimental pens (one group of six sows in each) with individual feeding stalls and an uninsulated kennel with straw bedding, for six to eight weeks during wintertime. The sows were fed 2,5 kg of a standard concentrate mixture every morning. Blood samples were taken from the ears of 10 sows in group 9 and 10 at four different days with temperatures ranging from -13.9 °C to +0.4 °C. The animals were videorecorded for 48 hours when outdoor temperature (diurnal average) was below -10 °C, around -5 °C and around 0 °C. When analysing the tapes, different thermoregulatory behaviour categories were scored using instantaneous sampling at 15 minutes intervals.

Results

When cold, the sows spent more time lying on their belly in contact with another sow than if the temperature was medium or warm (**figure 1**). Whether the sows were lying in contact with another sow or not, was not affected by the temperature. The sows spent more than 50 % of their active time, manipulating straw inside the kennels. When the temperature was cold, the sows spent more time manipulating straw inside the kennels than when it was medium or warm. The sows spent more than 80 % of their time inside the kennels. The use of kennels, however, seemed to be independent of temperature. The activity level among the sows were slightly higher in the cold periods, although this was not significant some of the groups.

The use of kennels was dependent of competitive ability (ranked from 1-6) among the sows. Sows given a low rankorder (5 and 6) according to competitive ability, used the kennels less frequently, and spent less time manipulating straw than did sows with a high competitive ability (scored 1 and 2). There was a tendency for sows with low competitive ability to lie more on their own without body contact than sows with a high competitive ability.

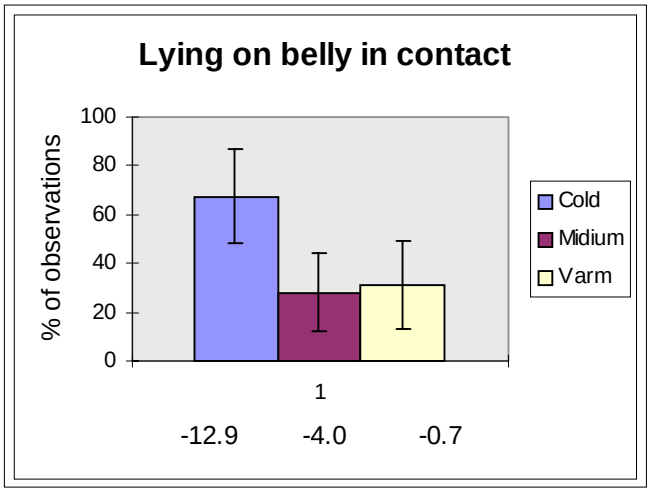

Figure 1. Social thermoregulation in group 9 and 10 (N=12)

At the lowest temperature (period 1), the sows had a higher level of free fatty acids (figure 2) and free thyroxine (figure 3) than at higher temperatures (period 2, 3 and 4). However, there was no difference between temperature period 2, 3 and 4 in any of the blood parameters measured. The plasma level of glucose and total thyroxine did not differ between temperature periods.

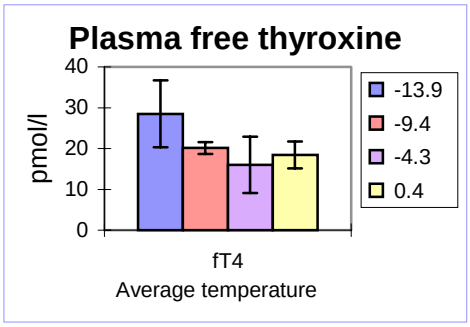

Figure 2. Plasma level of free thyroxine for group 9 and 10 (N=10)

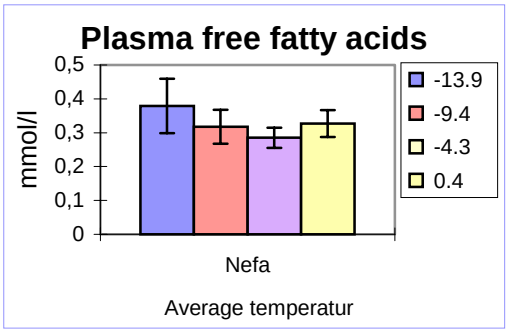

Figure 3. Plasma level of free fatty acids for group 9 and 10 (N=10).

In summary, the most predominant thermoregulatory strategy used by the sows, were social thermoregulation. Activity level were not significantly affected by outdoor temperature. The use of kennels seemed to be independent of outdoor temperature. However, there was a clear effect of competitive ability among the sows in the use of kennels - the low-ranked individuals visited the optimal micro climate less frequently.

The increase of plasma free fatty acids and free thyroxine at an outdoor temperature below -10 ^{o}C, may indicate an increased metabolism. It is therfore possible that this temperature was below lower critical temperature for the sows.

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AN INFLUENCE OF MICROCLIMATE TO THE SANITARY SITUATION AND GROWTH IN FATTENING PIGS

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During the time of four years the author scientifically analysed a series of parameters, significant for maintenance, production and health promotion in various piggeries with fattening accommodation in cooperation, in the vicinity of Varaždin.

Comparative analysis of the axterior average microclimatic values of piggeries belonging to group I (houses with partially slatted floors and slurry), and of piggeries belonging to group II (houses with full floors in the adapted pens previously intended for other purposes), revealed that the exterior air temperatures followed the temperatures in the above mentioned piggeries. These temperature rates were caused by building and technical characteristic of piggeries and by the inadequate thermoinsulation. The average air temperatures were the lowest in piggeries belonging to group II during the winter period, and were relatively high in the summer period. The lowest average minimal and the highest maximal temperatures were marked in the summer period in piggeries belonging to group III.

The average value sequence of relative humidity in the environment and in the piggeries showed a considerable deviation. The highest values which were established in the fall-winter period, showed a certain ventilation insensitivity on account of warmth which wae being kept in piggeries.

In the summer period, the average values of air velocity were at their highest in the environment as well as in the piggeries belonging to groups I and II, causing the possible combination of the natural and mechanical ventilation effect. In piggeries belonging to group III the situation was found to be quite opposite, the lowest air velocity was established in the summer period due to the inadequate ventilation caused by small openings and the density of premises within the structure of other farm and housing buildings.

The central radiation temperature in considered to be significant as far thermoinsulation and heat factors are concerned. The central radiation temperature follows the air temperature and a few oscillation found can be attributed to the influence of air velocity speed difference.

The findings of the average values of the central radiation temperature confirmed the poor thermoinsulation in all buildings, and supported the congruence of speed with the average air temperature.

The gas structure of the in the premises described, war particularly distinctive with regard to CO_2 and NH_3 concentrations. The increased amounth of CO_2 and NH_3 and dust found in the water period, and CO_2 in the summer period, were due to the inadequate fresh air supply of the premises. The increased amounts of gas in the winter period obviously pointed to the temperature preservation on account of inadequate ventilation.

Problems concerning the concentration of ammonia in the piggeries are tightly connected with the manipulation of waste fecal materials. Piggeries belonging to group I with partially slatted floor and slurry, compared to those belonging to group II and III, with full floor, where occasional cleaning of the premises was done, strongly supported the facual situation.

The parameter of ammonia concentration could, similarly as the parameter of CO_2 concentration in human hygiene, be a chief indicator of waste fecal materials as well as the abundance of ventilation.

With regard to the amount of hydrogen sulfide in the examined premises the author would like to point out that he was unable to establish its presence by applying the usual technique. This fact confirmed the findings of other authors who all agreed that no special attention should be paid to its presence in evaluating the hygienic conditions of piggeries with fattening accommodation.

The findings confirming the presence of oxygen in the examined premises were extremely inportant with reagard to the overallhygienic point of view.

The lowest average oxygen values were established in the buildings belonging to group III. The reason explaining that fact was found to be the lack of mechanical and natural ventilation in this group of buildings. The average high concentration of oxygen was found in the buildings belonging to group I, in the spring period, whed other microclimatic parameters were at their nearest to the optimal requirements, and whed the food intake per 1 kg of weight gain was at its lowest.

Up to now the concentratioof oxygen as hygienic parameter has only been studied in special microclimatic environments. The author´s scientific work confirmed the necessity of establishing this parameter doubting about the radiation deficit and animal's metabolism; he suggested it be included in regular analyses along with analyses of ammonia and carbon dioxide concentrations.

Concentration of dust and a number of microorganisms in the air of an animal house (with regard to the existing technology, housing and food intake) is being explained by the unfavourable dilution conditions and building locators, or by the short supply of air ventilation. It was established that dust concentration war at its highest in the buildings no. 2 belonging to groups I, II and III. It was also established that the average number of mesophilic bacteria was at its highest in the buildings belonging to groups III/1, and II/2 in the winter period, and in the buildings belonging to groups I/1 and I/3 in the summer period.

The established conditional pathology is an original reflection of given circumstances for placing, keeping and use of genic potentiality.

In brief, experience has shown that in the given geografico-climatic conditions of the Varaždin region, it is necessary to build houses with extremely good insulation and ventilation facilities, i. E, with full or slatted floors if optimal rates of temperature-humidity conditions are being fulfilled, and if the air pollution rate is at its lowest. Otherwise, the worst results can be achieved with regard to fattening and promotion of general health; ring the extreme temperature oscillations, primarily in the winter and then, in the summer period.

Economical evaluation of animal health and welfare

Economic evaluation of animal health and welfare

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Summary

Animal health and welfare economics is a relatively new discipline, which is progressively developing a solid framework of concepts, procedures, and data to support the decision-making process in this area. Research in this field deals with various interrelated aspects, such as: (1) quantifying the economic losses of animal health and welfare problems, (2) developing methods for optimising decisions when individual animals, herds or populations are affected, and (3) determining the costs and benefits of measures that are aimed at improving animal health and welfare. In the paper four examples were chosen and presented to illustrate the potential of some of the modelling techniques in this area, and the type of output they provide (i.e., the type of questions they can help to answer). The importance of a close link between economics and the more technical disciplines is stressed, as well as the need and possibilities for an international exchange of models and procedures.

Key words: economics, animal health, animal welfare, simulation modelling

Introduction

Controlling the costs of production is becoming critically important in modern livestock farming. Improving animal health and fertility can play a major role in achieving efficient and economically rewarding production. In the Netherlands, for instance, losses of common health and fertility problems in dairy cattle were estimated to average Dfl. 400 to 500 per cow per year, which corresponds to about 7% of gross return and 35% of farmers' net return to labour and management. The best 20% of farms manage to realise only half of the calculated losses on the average farm, and so it should be possible to achieve considerable economic improvement on many farms. In swine, calculations on the losses of disease are not widely available yet, but there are reasons to expect an even bigger economic impact than in dairy (Dijkhuizen and Morris, 1997).

In most countries animal welfare has received increased attention as being an important consumer concern. Although improved animal health will add to the welfare of animals, it is more commonly related to factors such as space, type of housing and way of handling the animals (Fraser and Broom, 1990). Improving animal welfare by changing these factors will increase the cost price of the product. As most consumers are not prepared to pay for these costs, there is considerable economic pressure to balance animal welfare preferences and the additional costs (i.e., the decrease in farmers' income).

Animal health and animal welfare have in common that it is rarely an all-or-nothing affair to achieve improvements. Usually several measures or programs are available, each of them offering a different degree of improvement and requiring a different level of investment. Determining the optimal input level, therefore, is to a large extent a matter of economic decision making. This is not only the case for the individual livestock owner, but also for a national government that must determine the optimal policy against specific infectious diseases, or for the farmers and other participants in an integrated livestock production chain aimed at producing in a more animal welfare-friendly way.

In the paper first some basic issues with respect to economic analyses in livestock will be presented and discussed. Subsequently, some examples to quantify and illustrate the

economics of animal health and welfare are presented. The paper is concluded with suggestions for further research in this area.

Modelling the economics of animal health and welfare

Models are essential tools for understanding the economics of animal health and welfare. Mathematical models are especially useful in this context and generally defined as a set of equations to describe or simulate an interrelated part (system) of the real world (Hillier and Lieberman, 1990). Three broad functions can be distinguished: (1) to provide an objective basis for assessing and assimilating available information about the system, (2) to detect where essential knowledge of the system is lacking or inadequate, indicating needs for further research, and (3) to assist in the management control of the system.

Basically, there are two different modelling approaches to be considered: a positive approach and a normative approach. The positive approach can best be indicated as a description of relevant processes and characteristics by statistical/epidemiological data analysis (the so-called empirical modelling). Traditionally, research in livestock production has mainly been conducted in this way. In animal health and welfare economics more attention is paid to the normative approach, which includes computer simulation techniques (the so-called mechanistic modelling). Computer simulation is a method for analysing a problem by creating a simplified mathematical model of the system under consideration which can then be manipulated by modification of inputs. This method is especially attractive where real-life experimentation would be impossible, costly or disruptive (as is the case with highly contagious diseases), and for exploring options that have not been widely applied yet (such as newly developed animal-friendly housing systems). Special attention has to be paid to the correspondence between model and reality to obtain meaningful results for real-world situations.

During computer simulation, usually six interrelated steps are being considered (Dent and Blackie, 1979): (1) define the system and objectives for modelling, (2) gather the data and specify relations relevant to the model, (3) construct the model, (4) validate the model, (5) carry out a sensitivity analysis, and (6) use the model to provide the results. Validation is considered a very important but difficult step in the entire modelling procedure. The key issue here is to judge whether or not the model mimics reality sufficiently well to fulfil the purposes for which it has been developed. This may include the sensitivity analysis (fifth step), in which the values of relevant input parameters are systematically varied over some range of interest to determine their impact on the results. Good knowledge of sensitive parameters should be available and entered into the model. If this is not available, which is still often the case with animal health and welfare related issues, sensitivity analysis can help to set priorities for further empirical research. In this way a valuable interaction between computer simulation and field data analysis is possible. Computer simulation may be used to quantify the significant gaps in (veterinary) knowledge, while knowledge obtained from field data research increases the realness of economic models. This interaction is considered fundamental to the study of animal health and welfare economics.

Example 1: Partial budgeting to analyse the economics of vaccination against APP in fattening pigs

In a field research experiment of one of the pharmaceutical companies, vaccination against APP showed to improve the major performance parameters in fattening pigs, such as daily weight gain, feed conversion rate and mortality. Improvements in performances after vaccination were available from compartments with both acute and chronicle outbreaks of APP, and compartments with chronicle outbreaks only. With acute and chronicle outbreaks together feed conversion efficiency (defined as kg of feed per kg of weight gain) improved by 0.12, daily weight gain by 30 grams and mortality by 1.6%, whereas costs for medicines (antibiotics) reduced by Dfl. 3.90 per pig. In the chronically infected compartments (without mortality or

clinical APP outbreaks) feed conversion efficiency improved by 0.08 and daily weight gain by 25 grams, whereas costs for medicines reduced by Dfl. 3.69 per pig (Dijkhuizen and Valks, 1997).

The question then was whether these (small) improvements in technical performances were sufficient to pay for the cost of vaccination, which are about Dfl. 4 to 6 per pig. To carry out a cost/benefit analysis, first a computer spreadsheet model was developed to derive economic weights for each of the parameters, using the partial budgeting technique. Partial budgeting is simply a quantification of the economic consequences of a specific change in farm procedure, such as vaccination against APP (Dijkhuizen and Morris, 1997). The economic weights were then combined with the improvements in technical performances, added, and compared to the cost of vaccination. The economic calculations were carried out for a typical commercial Dutch farm, but can easily be modified to other farm and price conditions.

A typical commercial pig fattening farm in the Netherlands has a herd size of about 1800 fattening places. The weight of the piglets at the start of the fattening period is on average 25 kg. Fattening pigs are slaughtered at a live weight of 112 kg, implying a slaughter weight of 87 kg. The duration of the fattening period is 119 days, which means that the average daily weight gain is 730 grams and that there are 2.8 deliveries (fattening rounds) per year. The mortality rate is 2.8%, and hence a total of 5000 fattening pigs are sold per farm per year. Feed conversion efficiency averages 2.8. Gross return per kg of slaughter weight varies considerably through time, but is typically about Dfl. 3.25, which leads to a gross return per fattening pig of Dfl. 283. Feed price is Dfl. 0.42 per kg, which adds to total feed costs of Dfl. 103 per fattening pig. The purchase price for piglets (at 25 kg) is on average around Dfl. 100 per head. Net return to labour and management per fattening pig averages Dfl. 13, which is 4.5% of the gross return.

The economic weights for the three performance variables under consideration (i.e., feed conversion efficiency, daily weight gain and mortality rate) are summarised in Table 1. They are also calculated and presented for a (much) lower price level of piglets and fattening pigs, which was the case in most recent years.

A difference in *feed conversion efficiency* of 0.1 affects feed consumption by 0.1 x (112 - 25 kg) = 8.7 kg per fattening pig sold and income by 8.7 kg x Dfl. 0.42 = Dfl. 3.65 per head. This equals (Dfl. 3.65 / Dfl. 13) x 100% = 28% of typical net return to labour and management. In quantifying the economic impact of differences in *daily weight gain*, it is necessary to determine which single cost item is related to the length of the fattening period, and which is not. Purchase price of the piglet and cost of transportation of the fattened pig are examples of costs which are not related in this way. Moreover, no relationship should be included for total feed costs, because differences in this parameter manifest themselves already in the feed conversion efficiency and thus should not be counted twice. The other cost items are more likely to be related to the length of the fattening period. So, the income margin per day of fattening period in the starting situation equals: gross return - (purchase price piglet + feed costs + cost of transportation) = Dfl. 283 - (Dfl. 96 + 103 + 5) = Dfl. 79 in 119 days or Dfl. 0.66 per day. A 10-gram increase in daily weight gain decreases the initial fattening period by 1.6 days, implying an economic value of 1.65 x Dfl. 0.66 = Dfl. 1.10. With a 10-gram decrease in daily weight gain these values are about the same. So, the economic value per gram of daily weight gain equals about Dfl. 0.11. The economic weight for *mortality* depends, among others, upon the age of the animal at death. Assuming that, on average, mortality occurs halfway the fattening period, the losses include costs before death (purchase price piglet and feed costs) and return to labour and housing foregone after death, which in total average about Dfl. 220 per dead fattening pig, or Dfl. 2.20 per percentage of fattening pig mortality.

Table 1. Economic weights of performance variables in pig fattening.

Selling price fattening pig (Dfl. per kg slaughter weight)	3.25	2.60
Purchase price piglet (Dfl. per head)	96.00	78.00
Feed conversion efficiency (Dfl. per 0.1)	3.65	3.65
Daily weight gain (Dfl. per 10 grams)	1.10	0.55
Mortality rate (Dfl. per %)	2.20	1.65
Typical net return to labour and management (Dfl. per head)	13.00	-25.00

As shown in Table 1, economic weights of daily weight gain and mortality rate decrease considerably with lower prices for piglets and fattening pigs, as does the typical net return to labour and management on fattening farms. Weights for feed efficiency remain unchanged, and hence become relatively more important with lower prices for the animals.

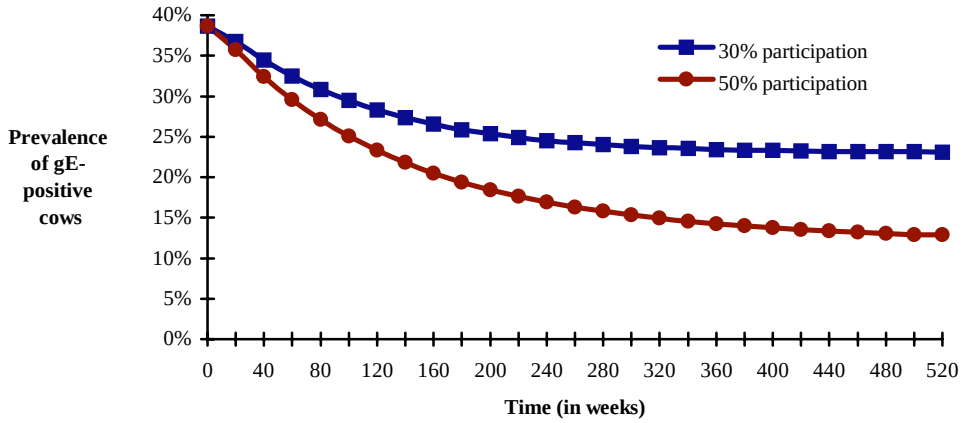
When multiplying the effects of vaccination on technical performances, mentioned before, with the respective economic weights presented in Table 1, it turns out that vaccination against APP is economically very attractive. Compartments in which acute and chronicle infections are present show economic benefits of Dfl. 15.10 per pig at normal price levels and Dfl. 12.57 at lower price levels. These benefits are Dfl. 9.36 and Dfl. 7.99 respectively in compartments with chronicle infections only. So, the benefits are much higher than the costs of vaccination (i.e., Dfl. 4 to 6 per pig) and hence could be considerably lower before the break-even is reached. Built into a spreadsheet model, the approach is simple to use for on-farm advice about vaccination against APP.

Example 2: State-Transition modelling to explore the economics of a nation-wide vaccination campaign against IBR in dairy cattle

The Bovine herpesvirus type I (BHV1), causing Infectious Bovine Rhinotracheitis (IBR), was introduced into the Netherlands in 1971. In 1993 about 42 percent of the dairy cows had antibodies against BHV1. In the future more restrict demands are to be expected considering the health status of exported breeding cows, semen and embryos. Therefore, it is considered in the Netherlands to start a nation-wide and compulsory vaccination campaign to eradicate the disease. To help make better decisions on what type of program should be preferred, a simulation model was developed in which the epidemiological and economic consequences of various vaccination strategies can be explored (Vonk Noordegraaf et al., 1997).

In simulating the spread and control of the infections over time, the so-called State-Transition approach is used. Central to this approach are the states animals or herds can be in, and the transitions between states (Dijkhuizen and Morris, 1997). In the IBR-study the modelling unit is a dairy herd, because the focus is on the spread of BHV1 between herds, and transitions are possible on a weekly base. In the model herds can be in different states, based on (1) the reproduction ratio R, which is the number of secondary cases caused by one infectious animal/herd, (2) the prevalence of gE-positive animals within each value of R, and (3) the expected number of infectious animals in an infectious herd within each prevalence range. The dynamic transition probability of a herd going from one state to another depends on direct contacts between animals, and other contacts, such as transmission through fomites, indirect transmission through other species, and airborne transmission. By multiplication of the current state vector with the probabilities in the transition matrix, the development of the infection over time can be simulated. An example of such a dynamic outcome is given in **Figure 1.**

Figure 1. Development of the prevalence of gE-positive cows with 30% and 50% of the herds participating in a vaccination program, using live vaccine.



As shown in Figure 1, participation of 30% of the farms leads to an equilibrium situation with a prevalence of about 25%. In the vaccinated herds the average prevalence in equilibrium is about 10%, towards 30% in the non-vaccinated herds. When 50% of the herds participate in the program, a prevalence of about 15% will be reached. So, IBR is not likely to be eradicated with a voluntary vaccination program.

Therefore, besides this voluntary program (from here onwards indicated as strategy I) also compulsory vaccination strategies were included in the analyses. Strategy II is based on a compulsory program for all herds. Strategy III stimulates the farmers to cull their last gE-positive cows before the campaign starts, because herds that are IBR-free can be certified, and exempted from a compulsory vaccination. It is required that these certified herds purchase cows from other certified herds only, in this way reducing the probability of introduction of the virus in a certified herd. Strategy IV is subdivided into IVa and IVb. These two strategies differ in the exemptions that are given to closed herds (i.e., herds that do not purchase any animals), the first exempting all young stock on closed herds to be vaccinated and the second exempting all gE-negative animals on closed herds with a prevalence less than 50% to be vaccinated. Strategy V is a combination of two years application of Strategy I, with 30% participation, followed by Strategy III. Results are summarised in **Table 2.**

Table 2. Most important outcomes for the different vaccination strategies.

	Time till 5% (weeks)	Costs till 5% (Million Dfl)	Diagnosis for culling cows (Million Dfl)	Culling (Million Dfl)	Pay-back period (weeks)
	1	2	3	4	5
Strategy I	Does not lead to eradication of IBR				
Strategy II	288	320	25.9	56	598
Strategy III	241	225	6.0	55	405
Strategy IVa	241	219	6.0	55	397
Strategy IVb	242	217	5.9	56	394
Strategy V	312	197	5.5	51	400

The first column in Table 2 displays the number of weeks before the prevalence of gE-positive cows reaches the pre-defined threshold value of 5%. The total costs per vaccination program, made in the period displayed in the first column, are shown in the second column. The

costs of detection of the last 5% gE-positive cows and the costs for culling of these cows are presented in the third and fourth column respectively. The most important economic parameter of a vaccination strategy is the 'pay-back period', in this study defined as the number of weeks after the start of the strategy that the cumulative benefits (i.e., reduced losses of IBR) are equal to the cumulative costs of the program. The benefits included in this study are a reduction in (1) lower milk production for gE-positive cows, (2) clinical and subclinical losses for infectious cows, (3) outbreaks on AI-stations and (4) potential losses due to export bans on live animals.

Based on Table 2, preference should be given to a strategy that exempts certified herds and youngstock in closed herds from a compulsory vaccination program with live vaccine (strategies IVa and IVb). According to the simulation model, these strategies yield an estimated pay-back period of between 394 and 397 weeks (i.e., almost 8 years) and take 241 to 242 weeks to reach a prevalence of less than 5% gE-positive cows. It is the intention to start a campaign along these lines (i.e., strategy IVa, which is easier to control than IVb) in the Netherlands at January 1, 1998.

Example 3: Spatial and stochastic modelling to evaluate foot-and-mouth disease control strategies

Outbreaks of contagious animal diseases, such as foot-and-mouth disease (FMD) and classical swine fever (CSF) can be very costly, especially for major exporting countries such as the Netherlands. In case of an outbreak rapid and adequate elimination of all virus sources has the highest priority. Policy makers are faced with many uncertainties regarding the development of the outbreak, expected efficiency of control strategies and possibility of export bans set by other countries. Despite these uncertainties they have to make decisions on how to control the outbreak and what measure to take. Experiments with different control strategies are not feasible, and therefore computer simulation is the adequate tool to evaluate the consequences of different options.

A set of models is being developed that simulates the epidemiological and economic consequences of different control strategies for outbreaks of FMD taking into account these uncertainties. Policy makers can use this information to prepare and make their final decisions about what control strategies to apply. In terms of managing an FMD epidemic using stamping-out, strategies include adjusting the size of protection and surveillance zones around diagnosed infected herds, instigating pre-emptive slaughter of serious contact herds and implementing a ring vaccination buffer. The modelling approach is part of EpiMAN, a decision support system for the control of FMD. EpiMAN has been developed in New Zealand and its applicability for the European situation has been investigated within a European Union funded project (EpiMAN-EU; Jalvingh et al., 1995). Currently, the system is further modified to suit Dutch conditions and will be implemented for use by Dutch disease control authorities (EpiMAN-NL).

The modelling approach for the economic evaluation of FMD control strategies requires simulation of (a) disease spread between farms, (b) direct costs of eradication and (c) indirect costs due to export bans. In that, the simulation of disease spread serves as starting point for the economic calculations. Input parameters on disease spread are especially difficult to obtain, since (data about) epidemics of FMD are scarce. Therefore, quantification of the uncertainties in the model output that result from uncertainties in the input is essential.

As part of EpiMAN, the spatial and stochastic simulation model InterSpread simulates within a region from day to day the spread of FMD between farms. Starting point of the simulation is infection seeded to an index farm. Via different spread mechanisms, i.e., (a) movements (animals, people, vehicles, material), (b) local/neighbourhood spread and (c) airborne spread, the virus can spread to other farms, and if infected the further spread is simulated for these farms as well. Once diagnosis of the first infected farm has been made, several control mechanisms can be put into place (e.g., slaughter, tracing, surveillance, movement control, pre-

emptive slaughter, emergency vaccination). Starting point is data on the location of individual farms and corresponding animal numbers. The geographic location is used in simulating disease spread and its control (e.g., which farms are in the surveillance zone and which in the protection zone around a certain farm).

All processes (disease spread and its control) are modelled stochastically using Monte Carlo simulation (Dijkhuizen and Morris, 1997). Random numbers are drawn from appropriate probability distributions and the outcome determines what happens (i.e., whether a movement results in infection depends on the probability of infection and the random number drawn). In this way, the model mimics what happens in real life; one can be very lucky in controlling the disease or one can be very unlucky. In order to get reliable results replicate calculations with one and the same input set are necessary, each of them representing a possible pattern of the outbreak. The main outputs of the model are the probability distributions of the number of affected herds, the number of days the outbreak lasts and the number of farms that are faced with movement restrictions. These results are used in calculating the economic losses.

A prototype version of the model was used to carry out preliminary calculations (Jalvingh et al., 1997). Calculations, with 50 replications, were carried out for an area of 50*50 km, with the average Dutch farm density (2 per km2). So in total almost 5000 farms (dairy, pig and mixed farms) were situated in the area. The first infected farm was located in the centre of the area. The control strategy applied represented the basic EU strategy. Since the results are rather skewed, next to the mean number of infected herds, parameters reflecting the variation in number of infected herds are presented in **Table 3.**

Table 3. Number of infected herds in various situations (basic and sensitivity analysis)

Situation	Average	SD	Minimum	Median	95% Perc.	Maximum
Basic	39.4	31.9	2	32	108	140
Animal movements +10%	46.0	37.6	1	35	131	147
Local spread +10%	40.6	32.5	2	34	111	139
Animal movements -50%	12.7	9.5	1	11	27	51
Distance movements +50%	54.1	39.0	6	47	118	206

In the basic situation the number of infected herds varies from 2 to 140. On average 39 herds were infected, but the standard deviation was large (31.9). In 50% of the cases the number of infected herds was 32 or less and in 6% of the cases above 100. The time period with controls in place lasts on average 50 days (ranging from 37 to 76 days).

Several input parameters in InterSpread are unknown or difficult to estimate. Therefore, sensitivity analysis is important to find out which parameters have a large impact on the outcome. An extensive sensitivity analysis of the model is currently undertaken. For illustration purposes a few results are also shown in Table 3. An increase in the number of animal movements with 10% (from on average 2 per week to 2.2) results in a 17% increase in the average number of infected herds. When the daily probability of local spread (radius of 1 km) is increased by 10% (0.011 instead of 0.01), the number of infected herds is only slightly increased. When animal movements are reduced by 50%, the size of the epidemic is strongly reduced from on average 39 to 13 infected herds. In the basic situation the majority of movements takes place within 5 km of the infected herd. By shifting part of these movements to distances between 5 and 30 km, the average distance of movements is increased from 7.1 to 12.7 km. As a result the number of infected herds increases, since less herds that have been infected through movements will be in the zones with movement control (max. 10 km radius) around the herd that infected them.

The model is currently being extended to include four types of losses, i.e., (1) losses due to slaughtering of infected herds, (2) additional losses on (a) diseased farms, and (b) in the area with movement controls, (3) trade losses and 4) organisational costs of disease control. Moreover, an application to CSF is under development.

Example 4: Linear programming to optimise animal welfare at minimum costs in the pork production marketing chain

Consumers show increasing interests in the quality of the product and the production process, including animal welfare, environmental pollution and food safety issues. To evaluate the economic impact of pig welfare, an economic pork chain simulation model was developed (Den Ouden, 1996). The model includes a farrowing stage for producing feeder pigs, a fattening stage for producing fattened pigs, and a slaughtering stage. Also, transportation between the stages was considered. As the various stages of the pork chain are linked vertically, their behaviour may influence both their own technical and economic performances, and those of the successive stages. These so-called interstage relations were also included in the model.

As indicated in Figure 2, both the animal welfare evaluations and the costs of the corresponding attributes served as input values in an optimisation model (i.e., linear programming). The optimisation model is used to define chain concepts in which the additional costs for realising increased levels of pig welfare are minimised.

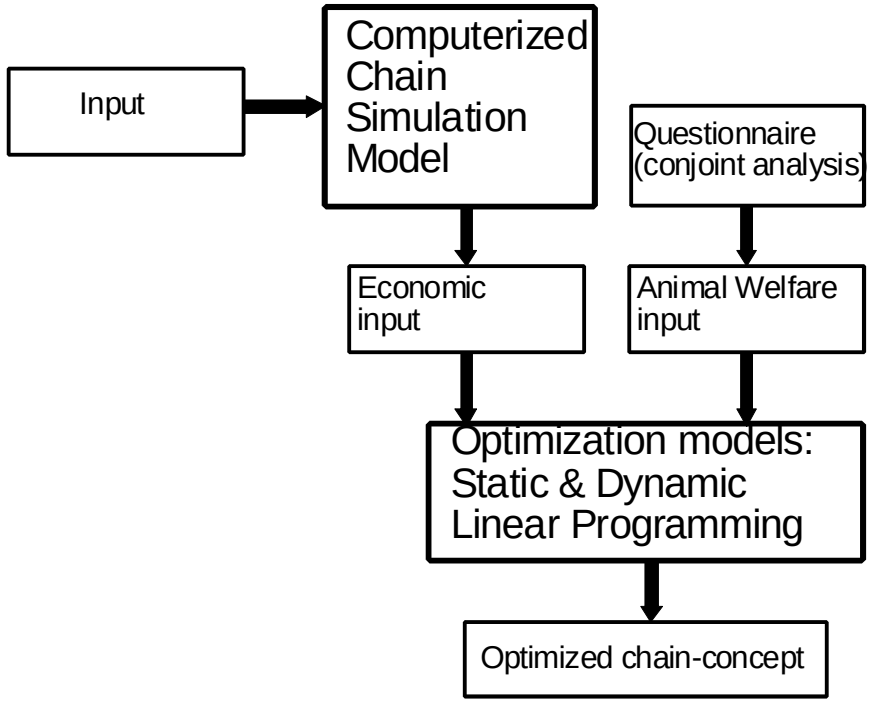
To weigh animal welfare measures against their costs, some sort of quantitative scale for animal welfare is required. Such weights are not commonly available (yet) in literature,. Therefore a pig welfare questionnaire was designed to derive these of both pig welfare experts and consumer-related respondents, such as groceries and animal welfare pressure groups, dedicated to influence the public opinion. A total of between 40 and 50 pig welfare attributes were included in the questionnaire, such as space, type of floor, illumination, mixing of socially unfamiliar pigs, weaning age of the pigs, and way of handling during transport and at the slaughterhouse (Den Ouden, 1996). These attributes were combined into different pig welfare profiles, of which each one had to be judged by the respondents on an interval scale from 0 to 100. This technique is called conjoint analysis, which is widely used in consumer research (Green and Srinivasan, 1978). Conjoint analysis offers the advantages of allowing for quantitative evaluation of subjective and differently-scaled attributes using only a limited number of alternative profiles, consideration of interactions and testing for consistency in the answers of the respondents (Hair et al., 1990). Compared to compositional methods such as direct questioning, conjoint analysis provides the advantage of higher realism because attributes are evaluated in combination with one another, as in the ‘real world’, instead of separately (Huber et al., 1993). Other advantages may refer to the absence of groups effects, reducing the likelihood of socially desired answers and probably being less time-consuming than repeated rounds of group- or individual elicitation procedures. Based on their predictive performances, conjoint techniques were often found to give better results than the compositional methods (Huber et al., 1993). Ordinary least-squares (OLS) regression analysis was used to break down the respondent’s overall judgements on the set of concept alternatives into the contribution of each attribute level.

The economic effects of the pig welfare-related attributes were calculated using an economic pork chain simulation model, as also indicated in Figure 2. Costs calculated inlcude labour, interest, depreciation, and other costs such as feed, drugs, water and electricity (Den Ouden, 1996).

As an example, results of the linear programming approach are shown in Table 4 for one of consumer-related respondents. The model was asked to define the chain concept that meets the required welfare level at 10, 20, 30 and 50 welfare points, respectively (within a scale of 0 to 100), at minimum costs.

To satisfy the required pig welfare level for the first step (10 points), five attributes were incorporated into this concept, as indicated in Table 4, ranging from 'not keeping pigs at the slaughterhouse overnight', 'reducing the stock density in the slaughterhouse lairage rooms from 300 to 235 kilograms of live weight per m^2 to 'raising the illumination standards to 20 lux per m^2 per 12 hours period'. These measures increased the costs by Dfl. 0.23 per head (whereas the total costs in the default situation were Dfl. 357 per pig from farrowing to the slaughter stage). While increasing the pig welfare constraint, values of some attributes which were already included were enhanced and new attributes were included, as also shown in Table 4. Besides raising the illumination standards in the farrowing and fattening stage, in particular transportation and slaughtering attributes were incorporated into the optimal concept.

Figure 2. Schematic overview of the pork chain model.



The differences in additional costs incurred between the consumer-related respondent and the expert are summarised in Figure 3. At a maximum welfare level of 100 points the additional costs from the farrowing to the slaughtering stage equalled Dfl. 77 and Dfl. 114 per pig respectively, as shown in Figure 3. The latter higher costs resulted from the attributes 'outdoor space', 'straw' and 'roughage supply', as quantified by the expert. In total, the maximum extra costs accounted for approximately 22% and 32%, respectively, of the total chain production costs in the default situation (which was Dfl. 357, as indicated before). In Figure 3 it can also be seen that the additional costs incurred increase progressively at higher desirable levels of additional pig welfare.

Table 4. Optimal chain concept at different levels of welfare requirements.

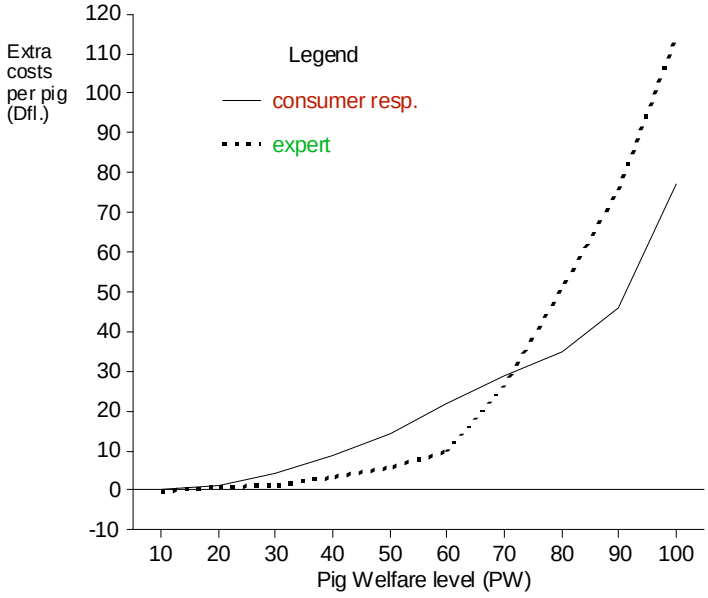
Figure 3. Additional chain production costs per pig at increasing levels of extra pig welfare, using the coefficients of an expert and a consumer-related respondent.

Table 4. Optimal chain concept at different levels of welfare requirements.

Welfare points			10	20	30	50
Costs (Dfl. per head)			0.23	1.20	4.01	14.52
Pig welfare attributes:	Default	Stage				
Stay overnight	Yes	S	No	No	No	No
Stock density (kg/m^2/pig)	300	S	235	235	235	235
Handling	Rough	T	Quiet	Quiet	Quiet	Quiet
Concrete floor nursery (m^2)	0	Far	0.07	0.07	2.31	2.31
Illumination (lux/12 hrs/day)	No	Far	20	20	20	20
Water spraying during resting	No	S	-	Yes	Yes	Yes
Automated lifting platforms	No	S	-	Yes	Yes	Yes
Automated ventilation	No	T	-	Yes	Yes	Yes
Illumination (lux/12 hrs/day)	No	Fat	-	20	20	20
Stock density (kg/m^2/pig)	300	T	-	275	235	235
Handling	Rough	S	-	-	Quiet	Quiet
Total floor non-lactating (m^2)	1.1	Far	-	-	1.4	1.4
Mixing during transportation	Yes	T	-	-	No	No
Mixing during resting	Yes	S	-	-	No	No
Concr. floor space (m^2/place)	0	Fat	-	-	0.03	0.20
Outdoor space (m^2/sow)	0	Far	-	-	-	5
Housing non-lactating sows	Teth.	Far	-	-	-	Cubi.
Straw supplied (kg/pig/week)	0	Fat	-	-	-	0.64

Far = farrowing, Fat = fattening, T = transportation and S = Slaughtering stage

Figure 3. Additional chain production costs per pig at increasing levels of extra pig welfare, using the coefficients of an expert and a consumer-related respondent.



Final remarks

The management of animal health and welfare is becoming increasingly important in modern livestock farming. A critical aspect of good management is making the right decisions. The process of decision making is commonly described in five steps (Boehlje and Eidman, 1984): (1) define the problem or opportunity, (2) identify alternative courses of action, (3) gather

information and analyse each of the alternative actions, (4) make the decision and take the action, and (5) evaluate the outcome. Current animal, herd and national information systems are restricted mainly to data recording and analysis, which especially support step 1 and partly step 2 of the decision-making process (Jalvingh, 1993). Research in animal health and welfare economics focuses on the development of models that allow for the evaluation of alternative decisions and strategies, as illustrated in this paper, supporting primarily steps 3 and 5. Economic models can in the first place be helpful in an early stage, when almost no data are available yet. Calculations have then to rely on estimates of input values, to be obtained from experts and/or practical experiences, but these can systematically be varied to determine their impact on the results (sensitivity analysis). Such preliminary outcomes can be very helpful in setting priorities for further empirical research. Once available more reliable input values can be entered into the model to increase the realness of the outcome. This is an on-going process until no further significant improvement is made. A major challenge in future research is to integrate these models (where appropriate) into existing information systems, making them more accessible for actual use in (veterinary) decision making at the animal, herd and national level. These systems in general and the economic models in particular should be flexible in their structure, and suitable to be tailored to individual farm and price conditions. That also opens the possibility for a sound international exchange and application. Once available, they can provide a solid and uniform basis for a mutual comparison of the economics of animal diseases and their control, and may also be a starting point for further (and joint) international research. Experienced gained so far in this respect with Dutch models are promising.

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COMPUTER SIMULATION APPROACH TO QUANTIFY THE RISK AND ECONOMIC CONSEQUENCES OF EXOTIC ANIMAL DISEASE OUTBREAKS

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Summary

Outbreaks of diseases such as Classical Swine Fever (CSF) and Foot and Mouth Disease (FMD) are a major problem for countries with a high concentration of farms and an export oriented production, such as the Netherlands. To support policy-makers involved in disease prevention and control, simulation models were developed and combined, describing the various aspects of outbreaks, risks and economic consequences. The integrated approach provides a useful tool for policy-makers to maximize the efficacy of existing disease prevention programs and to evaluate possible alternatives.

Key words: contagious animal diseases, simulation modelling, economics

Introduction

EU regulations provide a strict prescription about the eradication of contagious animal diseases such as Foot and Mouth Disease (FMD) and Classical Swine Fever (CSF), including measures such as the establishment of surveillance zones, stamping-out of infected herds, etc. It is obvious that outbreaks will have serious economic impacts, even if exports bans are not enforced. Hence adequate disease prevention programs are of major interest, especially for countries with areas with a dense population of farms, such as the Netherlands. In the Netherlands, extensive research was started to develop an integrated approach, which combines the various aspects of outbreaks and quantifies the risk and economic consequences. Research of Jalvingh et al. (1996) and Saatkamp (1996) already provided models describing the spread of outbreaks of respectively FMD and CSF within the Netherlands. Therefore, aim of this study was two-fold: 1) the development of a simulation model describing the introduction of virus into the Netherlands and 2) the integration of the results and insights obtained by the various models. The paper gives a short description of the developed model which was named VIRiS (Virus Introduction Risk Simulation) and illustrates the overall approach for FMD and CSF in the Netherlands.

Material & Methods

Introduction of virus into the Netherlands

The model VIRiS is a Monte Carlo simulation model. Using the Monte Carlo technique it is possible to incorporate variation and uncertainty realistically into the model. The model is based on available historical, statistical and research information relevant to this study. However, as outbreaks occur irregularly in time and place and circumstances change over time (i.e., more open trade, better disease prevention, etc.) it is difficult to derive general properties and estimates which describe the current and future situation.

Therefore, additional information was sought and found by experts, people involved in disease eradication, prevention and research. For the elicitation of expert opinions an experiment was performed, in which special techniques were used in order to obtain the information objectively and controllable. The experiment and results are described by Horst et al (1997). VIRiS provides information concerning the expected number of primary outbreaks and their location. The latter is important for the calculation of the losses, i.e., losses are directly related to the farm density of the affected area. VIRiS also provides insight into the source of the outbreak, i.e., countries and

risk factors. VIRiS is currently available describing the introduction of FMD and CSF into the Netherlands but the structure/approach is general and can be applied for other countries and diseases.

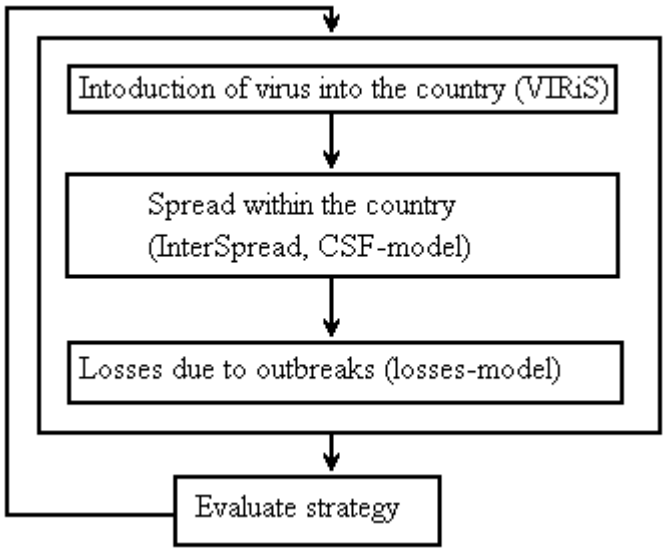

Figure 1 *gives a schematic impression of the various parts of the integrated research approach and the way in which they are combined and can be used to support policy-makers.*

Spread within the Netherlands

The Monte Carlo simulation model InterSpread was developed to describe the spread of FMD within a country. The conceptual model has been developed in New Zealand. Jalvingh et al (1996) extended the model and adapted it to Dutch circumstances. InterSpread simulates the day to day spread of the virus between farms. This spread can be due to: 1) contacts (animals, people, material, transport vehicles), 2) local contacts (vermin, cats, dogs), and 3) air. Several eradication strategies can be implemented into the InterSpread model, the base situation uses the strategy prescribed by the European Union. InterSpread provides insight into the number of infected and affected farms (affected by the movement restrictions), and the duration of the outbreak. Saatkamp (1996) developed a state-transition model describing the spread of CSF within Belgium. This model was adapted to the Dutch situation and provided insight into the number of infected and affected farms an the duration of outbreaks, under various eradication strategies (base situation uses the standard EU-strategy).

Losses due to outbreaks

In close corporation with representatives from the livestock production sector, losses due to outbreaks of FMD and CSF were calculated, for all parts of the production chain. Losses include slaughtering of infected herds, production standstill on affected farms, movement restrictions, market support, and organizational costs.

Strategy evaluation

Combining and integrating the three parts within the box presented in figure 1, provides insight into the economic consequences of the prevention strategy used. Evaluation of VIRiS-results showed that important aspects determining the risk of primary outbreaks of CSF and FMD in the Netherlands were the risk factors import of livestock and returning trucks and the duration of the so-called High Risk Period (HRP). The HRP defines the period in which the virus is not yet detected and/or measures are not yet effective, i.e., the period in which the virus may easily be transferred to other countries.

Results

The base scenario describes the current situation in the Netherlands, based on the assumption that 2.5 CSF and 1 FMD-outbreak are expected to occur in the next five years. The calculations presented in table 1 were based on the average duration and magnitude of outbreaks. The results show that complete elimination of the risk associated with returning trucks would result in a reduction of losses of more than 10 million Dutch guilders annually, which is an indication of the financial space available for measures aimed at this factor.

Table 1. Number of outbreaks per year and losses per year (in million Dutch guilders)

Scenario	FMD Outbreaks/year	Losses/year	CSF Outbreaks/year	Losses/year
Base	0.2	25	0.5	77
HRP*2	+ 0.17	+ 80%	+ 0.13	+ 26%
No risk of:				
- import livestock	- 0.098	- 66%	- 0.28	- 61%
- returning trucks	- 0.066	- 30%	- 0.08	- 6%

Concluding remarks

Simulation modelling is a powerful aproach to gain insight into complex problem situations. The study shows that this technique, together with the integration of epidemiological and economic information provides useful information for the evaluation of animal disease prevention programs.

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Economic assessments of relationships between productivity and health in Swedish dairy herds

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Summary

In order to understand the relative contribution of different biological traits for the economic efficiency in the dairy production, economic weights were estimated for each of the traits: milk yield, inseminations per service period, calving interval, age at first calving, culling frequency, % veterinary treated mastitis and other diseases, cell count in bulk milk, and in some calculations wasted milk, i.e. milk not delivered to the dairy industry. A ”bioeconomic” index for each herd was thereafter calculated by combining the recorded trait with its economic weight. The repeatability between years of the traits and the costs in the bioeconomic index were reasonably high, indicating that a significant number of herds has a considerable economic potential hidden in high health costs. A higher frequency of cows with sub-clinical mastitis was associated with a higher proportion of milk not delivered to the dairy industry, and undelivered milk contributed considerably to the final financial outcome.

Key words: dairy herds, economic efficiency, mastitis, udder health, milk yield.

Introduction

For a long time, the predominant strategy to increase profitability among Swedish dairy farmers has been to increase the milk yield and thereby the total income. However, along with the introduction of a milk quota system and over-production, the possibilities to increase the income are limited, and controlling the costs are thus becomes increasingly important. We have earlier presented data showing that high-yielding herds with high frequencies of veterinary treated mastitis and sub-clinical mastitis are less efficient compared to herds with the same yield but with low frequencies of udder health disturbances (Hallén Sandgren & Emanuelson 1995). In this study we present data on repeatability of the different traits and costs included in the bioeconomic index and also show that adding the trait undelivered milk to the model contributes considerably to the final financial outcome.

Materials and Methods

All herds with more than ten cows and enrolled in the official Swedish milk recording scheme during 1993/94 to 1995/96 were eligible for inclusion in the study. The material thus consisted of 9,669 herds. Data on production, health, fertility and culling were available on each herd for the period September 1 to August 31, in three consecutive years beginning in 1993. Average kg milk per cow and year was the measurement of production used. Fertility parameters were: average calving interval, average number of inseminations per service period, and average age at first calving. Health parameters were: incidence of cases of clinical mastitis (recorded as cases per 100 cow-years), incidence of any other disease (cases per 100 cow-years), and monthly bulk milk somatic cell counts (BMSCC). Culling was recorded as the number of cows leaving the herd, for other reasons than ”sold for dairy purposes” or due to disease eradication programmes (BLV or BVDV), and expressed as number of cows per 100 cow-years.

A bioeconomic index was calculated for each herd and year, combining the different measures of production, fertility, and culling, according to economic weights. The weights used are presented in Table 1. The index represents an estimate of the average economic return per cow and year. Additionally, an average over the three years was calculated for each trait. From these values a mean bioeconomic index and also a mean total costs and disease cost were obtained.

Herds were classified into quartiles according to: average kg milk per cow and year, incidence of clinical mastitis, and frequency of sub-clinical mastitis. The latter was calculated as the average proportion of cows having a low udder health score (0-2). The score is based on three consecutive individual somatic cell counts, where 0-2 indicates 0-30 % risk of having a sub-clinical infectious mastitis (Brolund 1990). As a result of the classification, herds were divided into 64 different groups (4*4*4). Herds that were low with respect to both types of mastitis were designated as LOLO, while LOHI herds had a low incidence of clinical mastitis but a high frequency of sub-clinical mastitis. HILO and HIHI were formed accordingly.

Finally, the impact of the frequency of cows with sub-clinical mastitis on the amount of milk not delivered to the dairy was estimated by an analysis of variance. For this part, data were collected during 1996 from 2,462 randomly selected herds in the milk recording system. Undelivered milk was estimated by subtracting milk delivered to the dairy from the total amount of produced milk and expressed per cow. Geographic region, breed, herd size, milk yield and frequency of cows with sub-clinical mastitis (udder health score 6-9, indicates 60-100 % risk of having an infectious sub-clinical mastitis) were the variables included in the model.

Table 1. Economic weights, in SEK, used to calculate an index combining different parameters of production, health, fertility and culling.

Parameter and unit of measurement	Economic weight
Milk production[a], kg	1.60
Calving interval, month (over 12 months)	- 300.-
Inseminations per service period, insemination	- 150.-
Age at 1st calving, month (over 24 months)	- 300.-
Mastitis incidence[b], %-unit	-34.-
Incidence of other diseases[c], %-unit	-20.-
Months with BMSCC>400', month	100.-
Culling rate[d], %-unit	-60.-
Wasted milk[e], kg	3.00

[a] Calculated as milk price minus feed cost.
[b] The cost of one case of mastitis was estimated to 3,400.- SEK.
[c] The cost of one case of "other diseases" was estimated to 2,000.- SEK.
[d] The culling rate used to calculate the economic index was first adjusted for the direct effects of mastitis and other diseases.
[e] The cost of discarded milk equals milk price.

Results

The medians of the bioeconomic index within herd classes for herds in the highest milk yield quartile are given in Table 2.

Table 2. Median values of the economic index (year 1995/96) for high-producing herds classified according to incidence of clinical mastitis and frequency of sub-clinical mastitis (undelivered milk not included).

	LOLO	LOHI	HILO	HIHI
Economic index	10891	10506	9989	9753
Diff. from LOLO	0	-385	-902	-1138
Relative to LOLO	100	96	92	90

The threshold values for the upper and lower quartiles for one single year (95/96) and for the mean values for the three-year period are given in **Table 3.** The thresholds are rather similar, indicating that economic results are stable over the period.

The result from the analysis of the effect of sub-clinical mastitis on amount of milk not delivered to the dairy showed that each cow with a high udder health score (6-9) contributed to approximately 500 kg undelivered milk per year. Adding this information to the index presented in Table 2 yields an index for LOHI-herds of 10095, i.e. a loss of 796 SEK compared to LOLO-

herds. An account of produced but not delivered milk is apparently necessary to fully appreciate the cost of sub-clinical mastitis.

Table 3. Threshold values for the upper and lower quartile for some economic indexes based on one single year (95/96) and based on the mean over a 3-year period.

		Lower 25 %	Upper 25 %
Economic index	95/96	7116	9677
	3-year mean	7306	9501
Total costs	95/96	3124	4538
	3-year mean	3419	4492
Disease costs	95/96	819	1771
	3-year mean	1063	1847

Conclusions

Health and fertility costs drain the dairy profit. In herds with high health costs, the problems are permanent over a long period of time and deleterious for the economic efficiency. The costs for having cows with sub-clinical mastitis are severely underestimated and are mainly due to an increased amount of undelivered milk.

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Optimal decision making in animal disease epidemics management : a note

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Summary

An economic model for the transmission dynamics of livestock communicable disease is proposed where farmers must decide to engage in transmissive or (imperfect) protective behavior. It follows that the hazard function may be a decreasing function of prevalence level. When the disease is endemic, prevalence elasticity lowers the total effect of Pigouvian price subsidies and increases the total effect of an improvement of protection efficiency.

Key words: animal epidemics, endemic disease, aggregate demand for protection, disease prevalence level.

Introduction

We propose a note on the decision-making process of animal health management when herds are affected by a contagious disease. The farmer has to choose between two behaviors : a transmissive behavior or a protective behavior. The protective activity, such as vaccination, is costly. We analyze his optimal strategy under uncertainty and we define the aggregate demand for protection. We derive the economic model for the transmission dynamics of animal disease epidemics from the standard epidemiological model. When the disease is endemic the sensitivity of stationary prevalence level to protection price or its risk of failure is also dependent on the prevalence elasticity of demand.

The economic model

The model of behavior during an epidemic is described as follows. The basic unit is the herd. At period *t*, the health status of the herd is susceptible ($h_t = s$), infected ($h_t = i$) or removed ($h_t = r$). Each farmer adopts a decision v_t about his herd : exposure to risk ($v_t = 0$) or protection against risk ($v_t = 1$). This protective activity is not completely effective. The farmer with characteristics θ is assumed risk neutral. His expected utility per period in health state $h \in H \equiv \{s,i,r\}$ is denoted $u(h;\theta)$, where we assume $u(s;\theta) > u(i;\theta) > u(r;\theta) = 0$. The protective activity is assumed to be costly. We denote $C(p;\theta)$ the cost of protection for farmer. It's an increasing function of the price of one unit of protection p. Farmers discount future utility

at a discount rate $\delta \in [0,1]$. They are assumed to maximize the time-present value of utility given the transmission probabilities among health states. The farmer's problem can be written as

$$J(I_t, h_t; \theta) = \max_{v \in \{0,1\}} \left\{ u(h_t; \theta) - vC(p; \theta) + \delta E_t J(I_{t+1}, h_{t+1}; \theta) \right\} \text{ subject to}$$

$$\begin{cases} \Pr[h_{t+1} = i / h_t = s, v_t = 0] = g(I_t) \\ \Pr[h_{t+1} = i / h_t = s, v_t = 1] = \pi g(I_t) \\ \Pr[h_{t+1} = r / h_t = s] = \lambda \\ \Pr[h_{t+1} = r / h_t = i] = \lambda \\ \Pr[h_{t+1} = r / h_t = r] = 1 \end{cases}$$

Since the disease is communicable, the force of infection $g(I_t)$ for a susceptible and exposed herd depends on the prevalence level at period *t*, I_t. We assume that this probability of contamination is proportional to prevalence, thus we have $g(I_t) = \beta I_t$ where β is the dissemination rate. It represents the average fraction of susceptible herds to which disease agent is delivered by each infected herd. It depends upon a number of factors : environment, type of farming, animal movements. We denote π the probability that the protection will not be effective. The mortality rate due to the disease is assumed equal to zero, so the probability of mortality unrelated to the disease is denoted λ.

The recursive nature of the problem allows us to derive the following objective functions. For the sake of simplicity, the discount factor is normalized at unity. Because the value of death is normalized to zero, $J(I_t, r; \theta) = 0$. This implies that the value function when the herd is infected is $J(I_t, i; \theta) = \dfrac{u(i; \theta)}{\lambda}$ since no selfishly rational farmer engages his infected herd in protection. This value function is the average duration of the infection times the expected utility per period when the herd is infected. The value function of a susceptible herd is

$$J(I_t, s; \theta) = \max\left\{ j(I_t, s, 1; \theta), j(I_t, s, 0; \theta) \right\} \text{ where}$$

$$j(I_t, s, 1; \theta) = u(s; \theta) - C(p; \theta) + \pi\beta I_t J(I_{t+1}, i) + (1 - \pi\beta I_t - \lambda) J(I_{t+1}, s)$$

$$j(I_t, s, 0; \theta) = u(s; \theta) + \beta I_t J(I_{t+1}, i) + (1 - \beta I_t - \lambda) J(I_{t+1}, s)$$

Consequently, under the population distribution type at period *t* $F_t(\theta)$, we define the aggregate demand for protection $D(Z_t, p, \pi) = \dfrac{1}{1 - I_t} \int_{\Theta_t} dF_t(\theta)$ where $Z_t = \{I_t, I_{t+1}, I_{t+2}, \ldots\}$ is the

future disease prevalence path and the set of susceptible herds which are protected at period *t* is

$$\Theta_t = \left\{ \theta : \frac{1}{\beta(1-\pi)} \frac{C(p;\theta)}{J(I_{t+1}, s;\theta) - J(I_{t+1}, i;\theta)} \le I_t \right\}.$$

If the number of herds is large enough, by the law of large numbers, the stochastic epidemic model can be approximated by the corresponding deterministic model

$$\begin{cases} S_{t+1} - S_t = \lambda_0 - \beta I_t S_t \left[1 - (1-\pi) D(Z_t, p, \pi) \right] - \lambda S_t \\ I_{t+1} - I_t = \beta I_t S_t \left[1 - (1-\pi) D(Z_t, p, \pi) \right] - \lambda I_t \end{cases}$$

By equating the birthrate with the deathrate, $\lambda_0 = \lambda$, one can focus on the case of a constant population size which, without loss of generality, is normalized to one. The fraction of susceptible herds is S_t and the fraction of infected herds, the rate of prevalence, is I_t. Note that, contrary to the standard epidemiological model, the economic model for the transmission dynamics of infection allows to distinguish susceptible and exposed herds from susceptible and protected herds. This implies different relationships between the hazard rate into infection from susceptibility and the prevalence rate. In a standard epidemiological model, the demand for protection is prevalence inelastic,($D \equiv 0$), then the hazard function is increasing since it satisfies $\kappa(I_t) = \beta I_t$. In this economic model, it satisfies $\kappa(I_t) = \beta I_t \left[1 - (1-\pi) D(Z_t, c, \pi) \right]$. This function may be decreasing in prevalence rate.

An endemic disease

In order to investigate the effect of public health measures on animal disease, consider the steady state denoted (S^*, I^*) and defined by the conditions $S_{t+1} = S_t$ and $I_{t+1} = I_t$ for all *t.* Direct algebraic manipulation yields that, given the stationary prevalence path $Z\{I, I, ...\}$ the aggregate demand for protection is

$$D(I, p, \pi) = \frac{1}{1-I} \int_{\Theta} dF(\theta) \text{ with } \Theta = \left\{ \theta : \frac{1}{\beta(1-\pi)} \frac{C(p;\theta)}{u(s;\theta) - u(i;\theta)} \le \frac{\beta I^*}{\beta I^* + \lambda} \right\}$$

It follows that the aggregate demand for protection is a decreasing function of the protection price ($D_p \le 0$), a decreasing function of the probability of failure ($D_\pi \le 0$) and an increasing function of prevalence ($D_I \ge 0$). Given $R_0 \equiv \frac{\beta}{\lambda} > 1$ and $D(0, p, \pi) = 0$, we prove that there exists a unique prevalence level $I^* \equiv I^*(p, \pi) > 0$. The reproductive ratio R_0 represents the expected total fraction of susceptible herds infected by a single infective. An epidemic will only occur if $R_0 > 1$. Furthermore, algebraic manipulations show a positive relationship between protection

price and disease prevalence, $\frac{\partial I^*}{\partial p} \geq 0$, and between probability of failure and disease prevalence, $\frac{\partial I^*}{\partial \pi} \geq 0$. In this stationary equilibrium, the total effect of a price decrease or an improvement of protection on the aggregate demand for protection is respectively

$$\frac{dD(I^*, p, \pi)}{dp} = \left[1 - \frac{D_I}{D_I + \Gamma}\right] D_p \text{ with } \Gamma > 0$$

$$\frac{dD(I^*, p, \pi)}{d\pi} = D_\pi \left[1 + \frac{D_I}{(1-\pi)(D_I + \Gamma)} \pi \left(\varepsilon_\pi^{-1} + \frac{(1-\pi)}{\pi}\right)\right]$$

where $\varepsilon_\pi = \frac{D_\pi \pi}{D} \leq 0$ is the inefficiency elasticity of demand. The total effect of protection price on aggregate demand is the partial effect on demand times a factor smaller than one. The larger the prevalence elasticity of demand, the smaller the price elasticity of demand in absolute value. Therefore the prevalence elasticity lowers the role of Pigouvian price subsidies and other demand-stimulating public health measures. If the inefficiency elasticity of demand is large enough, the total effect of an improvement of protection, characterized by a decrease of probability π, is the partial effect on demand times a factor greater than one. The larger the prevalence elasticity, the larger the ineffectiveness elasticity of demand in absolute value.

Conclusion

In this article, we develop an economic model for the transmission dynamics of animal contagious diseases. The aggregate demand for protection is specified when the disease is endemic. The prevalence elasticity of demand turns out to be a key factor to analyze the total effect of a price subsidy on the aggregate demand for protection.

Production, animal health and economic results of commercial layer flocks in aviary systems.

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Summary

The Dutch government introduced in 1992 the demonstration project animal friendly housing systems . In this project about 20 layer farms invested in aviary systems designed to substantially improve bird welfare. Data on production, economics and animal health were collected from these farms. The zootechnical results of 29 flocks in aviaries were compared to 50 flocks kept in battery cages. The aviary flocks had lower egg weights, lower mortailty and poorer feed conversion. The lower food intake in cages was not significant. As the distribution of the different strains of hens in both housing systems was not equal, a comparison was made between 17 and 33 Lohmann White flocks housed in aviaries and battery cages respectively. The aviary flocks had significant lower egg weights, higher feed intake and poorer feed conversion.

Production costs per kg eggs produced in aviaries were 8.2% greater than in cages. This increase was mainly caused by higher costs for pullets (rearing on deep litter), housing (lower bird density) and labour (collecting floor eggs).

Mortality rates on the aviary farms were low and there were not many serious health problems. The treatments reported were mostly related to parasites: 75% and 50% of the flocks were treated for worms and red mites respectively. The treatments for worm infections probably accounts for the slightly higher costs for animal health in aviary flocks.

Key words: layers, aviary, economics, production results, animal health, welfare

Introduction

In 1992 the Ministry of Agriculture in the Netherlands started a project to introduce the aviary system on commercial farms in order to improve the welfare of the layers. At the moment more than 20 farmers keep layers in aviary systems. The Agricultural Economics Research Institute (LEI-DLO) is collecting the zoötechnical and economic results from these farms. As the LEI-DLO is also collecting data on farms with layers in cages the results of both housing systems can be compared.

Materials and Methods

The results of 29 white layer flocks kept in different aviary systems were registered. The average flock size was 17,000 hens, ranging from 4,000 to 29,000 hens. The number of hens kept per m^2 groundsurface was 20. The aviary flocks are compared to 50 flocks in cages with an average size of 30,000 hens. Production data of aviary and cage flocks were registered through the standard method used by LEI-DLO.

The animal health status of the aviary flocks was monitored by the Animal Health Service (GD). This control consisted of dissection of birds, blood and manure research and registration of vaccination and treatments. LEI-DLO registered the costs for animal health: expenses for treatments, veterinarian and costs for disinfection and vermin control.

The production data were subjected to ANOVA to test the effect of the housing system. Significant differences were identified at $P<0.05$.

Results

production

Table 1 gives the zoötechnical results of all white layer flocks kept in both systems. The results show that there is no difference in the number of eggs produced. The aviary flocks had lower egg weights, lower mortality rates and poorer feed conversion. The difference in feed intake was not significant. The average percentage of floor eggs in aviaries was 5.2, ranging from 0.9 to 13.9.

The distribution of the different strains of hens in the group with aviaries and cages was not equal. In the aviary group the percentage of Lohmann white layer (LSL) flocks was lower and the farmers more often used the Bovans bird. LSL hens have a significant higher feed intake combined with a higher egg weight compared to Bovans (Lelystad, 1995). In the second part of table 1 the zoötechnical results of the LSL birds in both systems are given. The results show that for LSL flocks in aviaries egg weight was lower, feed intake per hen per day higher and feedconversion poorer.

Table 1. Zoötechnical results of aviary and caged field flocks with white laying hens.

	all flocks		LSL flocks	
	cages	aviary	cages	aviary
Number of flocks	50	29	33	17
Laying period (days)	410	415	409	408
Eggs/hen housed	326	329	325	329
Egg weight (g)	62.3[a]	61.0[b]	63.0[a]	62.2[b]
Total egg weight (kg)	20.36	20.10	20.50	20.45
Feed intake (g/ hen d)	112	114	113[a]	116[b]
Feedconversion (kg/kg)	2.19[a]	2.29[b]	2.20[a]	2.26[b]
Mortality (%)	8.5[a]	6.8[b]	8.2	6.5

animal health

The registered costs for animal health in cages and aviaries were 11 and 14 Dfl. cents per hen respectively. The treatments reported on aviary farms were mostly related to parasites: 75% and 50% of the flocks treated for worms and red mites respectively. No data were available on frequency of health problems in cages. Worm infection can be related to housing systems with litter (Bosch et al., 1995). On the average the aviary flocks were treated twice for worms with costs of 2.5 to 3 Dfl. cents per hen. The treatment for worm infections probably accounts for the slightly higher costs for animal health in aviary flocks.

economics

The recently collected data on aviary flocks don't change the results of earlier published economic calculations (van Horne, 1996). Production costs per kg eggs produced in aviaries were 8.2% greater than in cages. This increase was mainly caused by higher costs for pullets (rearing on deep litter), housing (lower bird density) and labour (e.g. collecting floor eggs). The field data show there is a wide variation in zoötechnical results and production costs for both aviaries and cages. Based on Swiss data Meierhans et al. (1992) reported a 9.5% increase in production costs of aviary eggs compared to eggs produced in cages.

Discussion

The farmers with aviaries in this project invested in new equipment and most of them built a new poultry house. The farms with cages were a randomly chosen group with both new and old buildings and equipment. On the other hand, the farmers with aviary systems didn't have any experience with keeping hens in aviaries.

In this paper only white layers are compared. More recently the number of brown layers placed in aviaries has increased rapidly. The brown eggs are sold under a free range label. Combined with the low number of flocks this is the reason that no comparison was made for brown layers.

It should be mentioned that the beaks of the layers kept in cages and aviaries were trimmed.

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Implementation and costs of mastitis control actions in French dairy herds

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Summary

Mastitis control actions were described in 265 farms. Classical control measures (*i.e.* udder preparation, postmilking teat disinfection and drying-off treatment) were widely in use. Economic losses were estimated from frequency of clinical mastitis and somatic cell counts. Costs of milking hygiene, costs of treatments and losses averaged respectively 64, 135 and 348 FRF per cow-year. Relationships between losses and control costs showed widely different farm-patterns. The results suggested needs for better effectiveness in implementation and for better ex-ante assessment of economic worth of actions *1 FRF = .15 ECU = .17 USD*

Key-words: dairy cow, mastitis, control, economics

Introduction

Mastitis is today recognized as the most worrying disease for dairymen. Recommendations about preoccurrence and postoccurrence control actions are nowadays often cast over doubt by farmers facing a lack of results or obvious economic worth of these control actions. Little reliable information is available on current implementation and economic aspects of mastitis control actions in French dairy herds. The present study aimed to provide descriptive information about: *(1)* implementation and costs of milking hygiene, *(2)* implementation and costs of mastitis treatments, and *(3)* relationships between these control costs and estimated losses caused by mastitis.

Material and methods

To be included in the survey, farms had to be located in the Pays de la Loire area (West of France), to be enrolled in the Milk Recording Scheme and to be willing to cooperate in a herd-health survey for 2 years. 265 farms were involved. Herd size and cow yield averaged respectively, 44 cow-year and 7 450 kg milk per cow-year.

Data collection consisted in recording of the main health disorders by farmers with a monthly check for consistency by a vet or a trained technician. Data describing practices in milking hygiene and treatments were collected through a questionnaire and an observation of a milking session by a member of the research team. Costs of the implemented measures were recorded from the book-keeping system of the farm.

Clinical mastitis incidence rate (CMIR) was defined as the average number of cases per cow-year. CMIR and bulk milk cell count (BMCC) were used to estimate economic losses caused by mastitis (Appendix 1). Twenty-three farms with a CMIR below 4% or for which mastitis control costs could not be separated from other costs were excluded.

Results

Implemented actions are displayed in tables 1 and 2. Milking hygiene protocols included mostly the classical measures (*i.e.* wet udder preparation and postmilking teat disinfection). Antibiotic therapy at drying-off was systematic in quite all the surveyed herds. Half of farmers did not treat themselves cows with systemic illness signs. Average costs of milking hygiene, treatments and total control actions were respectively 63.9, 134.6 and 198.5 FRF per cow-year. Drying-off treatment costs accounted for 34% of the treatments costs for mastitis. Average BMCC was 199,000 cells/ml.

Average CMIR was 51 % resulting from 43% cases without and 8% cases with systemic illness signs. Estimated economic losses averaged 347.7 FRF per cow-year and 0.048 FRF per kg of milk. Total economic impact of mastitis averaged 547 FRF per cow-year and 0.075 FRF per kg of milk. Relationships between estimated losses and control costs did not show a clear systematic trend. Farm patterns differed widely. Losses were higher than costs in 74% and lower in 26% of the farms (Fig. 1). The relative importance of losses *vs.* control costs showed a trend to increase when total economic impact of mastitis per kg of produced milk became higher (Fig.2).

Table 1: Implementation of milking hygiene

Control action	Percent
Premilking udder hygiene	
- classical wet udder preparation	86.2
- premilking teat dipping	4.9
- dry preparation or no systematic protocol	8.9
Check of first streams before milking	
- systematic implementation	28.3
- only in suspect cows	37.3
- not implemented	34.4
Procedure for mastitic cows milking	
- milking order or specific cluster unit	6.2
- cleaning and/or disinfection in between	77.6
- no systematic procedure implemented	16.2
Postmilking teat disinfection	
- systematic teat dipping or spray	82.1
- seasonal teat dipping or spray	6.5
- not implemented	11.4

Table 2: Implementation of treatments

Control action	Percent
Drying-off treatment	
- systematic and unique protocol (same protocol for all cows)	50.5
- systematic but different protocols (based on SCC lactation-pattern)	48.3
- implemented only in high SCC cows	0.8
- not implemented	0.4
1st treatment of clinical cases without systemic signs	
- intramammary antibiotics	83.8
- intramammary and systemic antibiotics	12.8
- no antibiotic treatment (homeopathy)	3.4
1st treatment of clinical cases with systemic signs	
- no case with systemic signs	5.7
- intramammary and systemic antibiotics	90.2
- only intrammary antibiotics	4.1

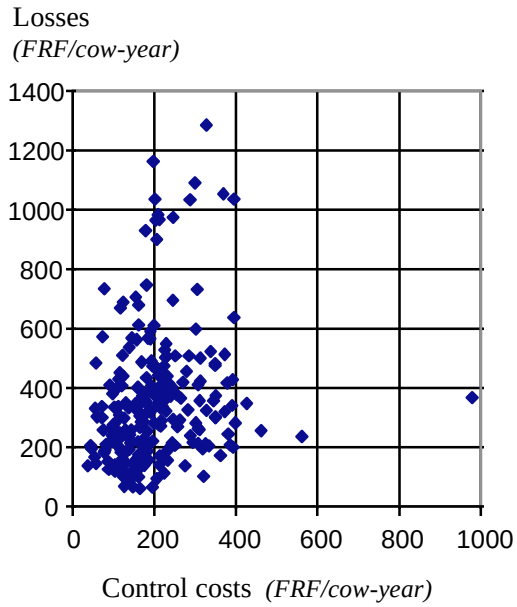

Figure 1: Relationships between estimated losses and control costs.

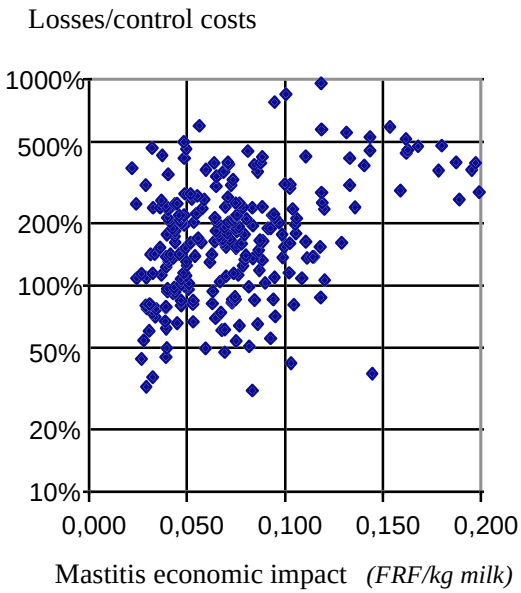

Figure 2: Relationships between the ratio losses/control costs and the economic impact (control costs and losses).

Discussion and conclusion

Quality of implementation and effectiveness of actions to limit the risk of incidence or the extent of consequences of mastitis were not reported in this paper. However, it is noteworthy that this effectiveness could be improved in our sample. Udder preparation was frequently a critical procedure as also reported by Yalcin et al. (1997).

Mastitis control-costs definition did not include all actual related costs. However, average level was found high. This was first to relate to the treatment costs reported here which were higher than in previous French data, but study populations were different. Average CMIR was found considerably higher than in previous French reports although average BMSCC was lower. Recent studies in other countries showed also high herd incidences when this parameter is expressed per cow-year (Agger et al. 1997).

Total economic impact in our study was slightly higher than the result provided by Schakenraad and Dijkhuizen (1990) for the average Dutch dairy (136 DFL = 405 FRF per cow-year). However direct comparisons are not valid when study populations and methods differ (Schepers and Dijkhuizen 1991). Our calculation took into account the quota constraint and at contrary, did not include opportunity costs for extra-labour.

Our study aimed a descriptive purpose and results should be considered only at the global level. Among the estimated losses, some are avoidable and some non avoidable. In farms with high control costs, their economic relevance might be questionable. However, to investigate in a given farm, *(1)* a more reliable calculation of losses based on intra-farm-specific values for prices

and costs and *(2)* relevant estimates of effectiveness of control actions on incidence and consequences of mastitis are needed.

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Appendix 1: Simplified estimation of economic losses due to mastitis

Basic assumptions

1. Actual 12-month milk production is assumed to be equal to the quota of the farm. No cow milk is routinely given to calves after 1 week, but 75% of the discarded milk is given to calves.
2. In case of absence of mastitis, milk production capacity of the farm will exceed the quota. Adaptations adopted by the farmer are assumed to be: (1) to give more cow milk to female calves : a maximum of 200 kg can be used per cow-year; (2) to reduce the herdsize to produce the same total amount of milk with less cows and using less hectares. Released hectares are assumed to be devoted to another production.

Losses calculations

1. Estimation of excess in milk production capacity (EXMPC): addition of discarded milk and not produced milk due to clinical mastitis (average loss of 285 kg per case) and not produced milk due to high BMSCC (loss of 2 % per step of 2-fold increase of BMCC above 100,000 cells / ml).
2. Estimation of herdsize reduction: division of EXMPC by average rolling herd average. For each entire unit, 1 cow and 0.3 heifer are supposed to be in excess on the farm. The modulo gives the amount milk available to feed calves.
3. Final calculation of estimates of economic losses:
   (1) penalties for high bulk milk SCC (thresholds of 250,000, 300,000 and 400,000cells/ml);
   (2) in comparison with the theoretical reference of complete absence of mastitis:
   - loss of 3 850 FRF per step in possible herdsize reduction resulting from reduced expenditures (for cow: maintenance-feeding, health control, milk recording and breeding, and for heifer: total operational costs), reduced receipts (1.2 calf not sold per year) and increased gross margin (.70 hectare released);
   - loss of .5 FRF per possible kg of cow milk fed to calves (difference between milk-replacer equivalence value and feeding costs for 1 kg of milk);
   - loss of 20 FRF per percent point of CMIR and cow-year to account for forced replacement and mortality.

Economical values for health and fertility traits in Finnish dairy cattle population

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Summary

Economical values for independent traits are needed when defining the breeding goal. The aim of this study was to estimate economical values for fertility, udder health and production traits for a change of one genetic standard deviation unit (gen. sd.) in each trait independently. Milk recording data in Finland in 1995 were used to estimate the average feeding cost of maintenance and production. All product prices and production levels were from the fall 1996 and genetic standard deviations from the national genetic evaluation of dairy cattle in December 1996.

Following components were taken into account in the calculation of the genetic merit for fertility: 1) cost of an extra day open, 2) costs of re-inseminations, 3) costs of treatments for fertility problems and 4) replacement costs due to culling of cows because of fertility problems.

Following components were included in the calculation of the genetic merit for udder health: 1) cost of mastitis including discarded milk and treatment costs, 2) reduction in milk price due to higher somatic cell count, 3) replacement costs and lower production level of the herd due to involuntary culling of cows because of udder problems.

The value of improving udder health by one genetic standard deviation unit was about three times the value of improving fertility. The protein production had about twice the value of fertility and somewhat lower than udderhealth. According to these results the total merit index currently used in Finland has higher weight on fertility and lower weight in udderhealth than these calculations suggest.

Key words: Economical weights, breeding goal, selection index, functional traits, health, fertility, dairy cow

Introduction

The aim of the national breeding program is to improve the economical efficiency of milk production by genetic progres in economically important traits. Finnish dairy cattle breeding

program has been succesfull in improving the production traits and simultaneously achieving a positive or at least not negative genetic change in many functional traits, e.g.udder health, milkability and conformation (Korhonen and Juga, 1996). The only trait clearly declaining beeing daughter fertility.

When designing and running a breeding program one has to take care that all sectors of it are optimized, namely the breeding goal, prediction of breeding values (BV) and selection of animals. The aim of this study was to estimate the economical value of production, fertility and udderhealth to be used in designing the total merit index in the selection of dairy bulls and cows.

Methods

Groen et.al. (1996) gave a detailed review of the methods used in defining the breeding goal, calculating the economic weights and constructing the total merit index. Breeding goal can be written as

$$\mathbf{H}_{kl} = \mathbf{a}_{kl}'\mathbf{g}, \quad \text{where } \mathbf{a}_{kl} = \mathbf{c}_l\mathbf{v}_k$$

and $\mathbf{H}_{kl}$ is the agregate genotype of an animal, k is the time for comparison, l is the selection path, $\mathbf{a}_{kl}$ is a m*1 vector of discounted economic values of m genotype traits, **g** is a m*1 vector of genetic superiorities of m gentype traits, $\mathbf{c}_l$ is a m*m diagonal matrix with cumulative discounted expressions of m genotype traits and $\mathbf{v}_k$ is a m*1 vector with economic values of m genotype traits.

The aggregate genotype can not be observed, since the genotypic values of the traits in aggregate BV are not measurable. The practical tool is to predict it with a selection index method which was presented already in 1943 by Hazel. The information does not necessarily need to be on the same traits included in aggregate genotype (total merit), but also some correlated traits can be used. Generally a selection index can be presented as (Groen, et.al., 1996)

$$\mathbf{I}_{kl} = \mathbf{b}_{kl}'\mathbf{x}, \quad \text{where } \mathbf{b}_{kl} = \mathbf{P}^{-1}\mathbf{G}\mathbf{a}_{kl}$$

and $\mathbf{b}_{kl}$ is a n*1 vector with regression coefficients of n index traits, **x** is a n*1 vector with observations, **P** is a n*n matrx with covariances between index traits and **G** is a m*n matrix with covariances between m genotype traits and n index traits.

No universally best method exists for deriving economic values, but what is best will depend on traits and production circumstances considered and what is possible in practice (Groen et.al., 1996). Groen (1989) lists five criteria to be considered when deriving economical weights:

1. Efficiency: biological versus economic definition
2. Perspective: to maximize profit (=revenues-costs), to minimize costs or to maximize revenues/costs
3. Planning term: strategic versus tactical
4. System level: animal, farm, sector or international
5. Method: positive approach (data evaluation) versus normative approach (data simulation)

All five aspects provide alternative strategies, which can be justified. This means that no universally acceptable economic values exists, but these values need to be derived in each country.

Results

The economical valus for production, health and fertility traits were calculated on animal level utilizing the information on Finnish milk recording and progeny testing of dairy bulls. Finland has a farm and national quota, why derivation of economical values on herd level would be logical, but Finland has not exceeded the country quota since joining the EU, which means that farm quotas have not been in effect either. This make it also practical to use animal level. Improvement of genetic merit of animals increases the efficiency of production, which will decrease the market prices of the product in the long run (Groen et.al., 1996).

Following components were included in the calculation of the genetic merit for udder health: 1) cost of mastitis including discarded milk and treatment costs, 2) reduction in milk price due to higher somatic cell count, 3) replacement costs and lower production level of the herd due to involuntary culling of cows because of udder problems. The economical values for different traits per genetic standard deviation and currently used index weights in Finnish total merit index are prsented in **table 1.**

Conclusions

Minimizing the production costs on aimal level leads to more efficient production and reduction in the consumer prices in the long run. The Finnish breeding goal in recent years has been to increase the efficiency of production without worsening the functional traits. Estimated economical values for individual traits give evidence that the strategy is correct. Although the economiacl value of udder health is higehr than the currently given weight in total merit index,

more weight to udder health is given by weighting the udder conformation, which is positively correlated to udder health.

Table 1. The economical value of a change of genetic standard deviation unit in different traits in Finnish Ayrshire.

Trait	Economical value of a genetic s.d.	Relative to prot. yield	Weight in total merit index
Milk yield	238 Fmk	83 %	-
Prot-%	280 Fmk	98 %	0.3
Fat-%	147 Fmk	51 %	-
Fertility	82 Fmk	29 %	0.5
Prot. kg	106 Fmk	100 %	1.0
Fat. kg	106 Fmk	37 %	-
Udder health	412 Fmk	144 %	0.3
Growth rate	104 Fmk	36 %	-

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Economic aspects of health control in organised sheep farms in India

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Summary

The information on economic aspects of maintenance of health of sheep is worked out utilizing, the data collected for a period of six years (1990 to 1995) from organised sheep farm. The expenditure incurred on medicines was on antiboitics and sulphanarmides, anthelmintics, antidiarrhoeals, antiseptic creams and dressing materials, dipping agents and general tonics. During the six years an amount of INR 25933/- was spent on medicines including anthelmintics with an annual average of INR4322/-. Maximum amount of INR 9137/-(34.65%) was spent on anthelmintics followed by antiboitics and sulphanamides INR 6322/- (24.23%) general tonics INR 2817/-(II .55%), deticking agents INR 2050/(8.09%) and anti diarroheals INR 1494/(5.73%). The average cost of treatment per sick sheep was worked out to be *INR 20/-* and the over all expenditure was *INR 19/-* per head in the flock. An annual average expenditure of *INR* 7/- per sheep was incurred on anthelmintics. The over all expenditure per sheep increased gradually due to increase in the cost of medicines particularly antiboitics and anthelmintics.

Key words: Economics, health of sheep, organised sheep farm, Medicines, anthelmintics, dipping agents.

Introduction

Sheep industry in India is developing at a faster rate. The major source of income from sheep is through sale of lambs, wool, manure and skins. The major components of expenditure are feeding and coverage of health. Economics of organised farms is usually based on the above components. Health coverage is one of the most important factors in sheep production. The information on economic aspects of maintenance of health of sheep is scarce. Hence as attempt was made to work out the expenditure incurred on sheep health maintenance at organised sheep farms.

Materials and methods

The data on expenditure towards maintenance of health of sheep of Livestock Experimental Station, Rajendranagar. Hyderat; id, (AP) - India was utilized. The data were collected for the period of 1990-91 to 1995-96 and analysed. The expenditure on medicines was on antiboitics and sulphanarnides, anthelmintics, anti-diarrhoeals, antiseptic and dressing materials, deticking agents etc. The annual number of sick sheep treated, average flock strength and climatic data were recorded.

The cost of treatment per animal was derived by the expenditure on medicines (excluding anthelmintics) by dividing the number of sick sheep. Similarly the expenditure on anthelmintics per sheep was obtained by dividing the total number of sheep available and the average expenditure incurred per head was worked out.

The indigenous breeds that are maintained include Nellore and Deccani sheep which were on semi-intensive systems for meat production. The animals were generally allowed for grazing for 6 hrs and were supplemented with mixed grass in the night shelters. They were also fed with concentrate mixture (@ 200 gms/head per day during lean period.

Results and discussion

The information on average flock strength, number of cases treated, cost of treatment per sick animal, expenditure incurred per sheep available for each year and average for six years is given in Table.

Expenditure on medicines:

During the six years an amount of INR 25,933/- was spent on medicines including anthelmintics with annual average of INR 4322/-. Maximum amount of INR 9137/- (34.65%) was spent on anthelmintics followed by antiboitics and sulphanamides INR 6322/- (24.23%), general tonics INR 2817 - (11.55%) deticking agents INR 2050/- (8.09%), Intra-Venous therapy of dextrose, calcium and magenesium INR 1943/- (7.61%), antidiarrhoeals INR 1494/- (5.73%) and miscellaneous items INR 1106/- (4.27%). The expenditure incurred was increased progressively every year and there was about 38.63% increase towards the cost of medicines in the year 1995-96 as compared to 1990-91. Balakrishna *et a/ (1985*) reported 1.71% of the total investment towards cost of medicines in sheep rearing in small size (21 sheep) sheep flocks maintained by farmers.

Expenditure on anthelmintics:

An thelmintics used by rotation were Fenbendazole, Albendazole, Nilverm and Thio bendazole. A total amount of INR 9137/- was spent on anthelmintics with an average of INR 1523/- per year. The percent expenditure on anthelmintics varied from 28.98% (1990-91) to 40.29% (1995-96) with an overall average of 34.65% of total expenditure. An annual average expenditure of INR 7/- per sheep was incurred on anthelmintics. The expenditure on anthelmintics was increased gradually due to increase in the cost of anthelmintics, type of anthelmintics used, agro-climatic conditions and number of drenches given and sheep population of various age groups available. The sizable expenditure incurred on anthelmintics is justifiable as parasitic gastro-enteritis caused by nematodes is one of the major cause of enormous economic loss to sheep farms. A modern worm burden can bring down growth rate and wool production. Rotation of pastures has been suggested for the control of parasites which may not be a practical consideration under Indian conditions.

One US $ is equivalent to INR 35/-

Cost of treatment per sick sheep:

An amount of INR 14,746/- was spent on medicines for the treatment of 757 cases of different ailments during six years with an annual average of INR 2458/- and 126 cases. The average cost of treatment per sick sheep varied from INR 17/- to INR 23/- during different years. An average expenditure of INR 20/- was spent on sick sheep.

Expenditure per available sheep:

An average annual expenditure of INR 19/- was incurred on every sheep available. It was found to be maximum (INR 25/-) during 1995-96 and minimum (INR 15/-) during 1990 - 9l . Price rise of medicines, disease conditions encountered, climatic conditions and flock strength

are the major factors affecting the average expenditure from available sheep. The over all expenditure per sheep increased gradually due to increase of the cost of medicines.

Conclusions

* Maximum amount (34.65%) was incurred on anthelmintics followed by antiboitics and
* sulphanamides (24.23%) to maintain health of sheep.
* An average amount of INR 20/- per sheep was spent on sick sheep.
* An average amount of INR 7/- was spent per sheep on anthelmintics.
* The overall expenditure per sheep increased gradually due to increase in the cost of
* anuboitics and anthelmintics.

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TABLE: Health expenditure of sheep flock

TABLE

Health expenditure of sheep flock

Year	Average flock strength	No. of cases treated	Expenditure on			Total Expen-diture	Treatment per sick sheep	Anthelmintics per sheep	Overall expenditure per sheep
			Medicines	Anthelmintics	Deticking				
			INR	INR	INR	INR	INR	INR	INR
1990-91	244	128	2199.00 (61.27)	1040.00 (28.98)	350.00 (9.75)	3589.00	17.18	4.26	14.71
1991-92	239	132	2261.00 (59.80)	1200.00 (31.74)	320.00 (8.46)	3781.00	17.13	5.02	15.82
1992-93	231	117	2167.00 (56.89)	1350.00 (35.44)	292.00 (7.67)	3809.00	18.52	5.84	16.49
1993-94	219	120	2398.00 (57.01)	1417.00 (33.69)	391.00 (9.30)	4206.00	19.98	6.47	19.21
1994-95	223	125	2586.00 (55.02)	1774.00 (37.74)	340.00 (7.23)	4700.00	20.69	7.96	21.08
1995-96	238	135	3135.00 (53.61)	2356.00 (40.29)	357.00 (6.10)	5848.00	23.22	9.90	24.57
Average	232.33	126.17	2457.67	1522.83	341.67	4322.17	19.45	6.58	18.65

N.B.: Figures in parenthesis indicate percentages

Economical evaluation of sheep health and reproduction control by bio-technical methods

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Summary

The credibility of agricultural economic analysis, especially production economics stem from its links with identifiable technical processes and confronted decisions about resource use. Despite its importance as a serious imperfection in production the health and reproduction problems of animals has not been widely explored. The paper provides presentation of the micro-economic principles, couched in an attempt to see that the self-organizing principles of the markets are in conflict with the self-organizing principles of ecosystems. In a classical, so-called Carpathian sheep breeding two years program only 71.0 and 53.1 % of ewes were mated. The change in the breeding system by bio technical methods of reproduction control enable all treated clinically healthy ewes to be mated within 36 to 42 days. The change brought about a significant increase in lamb rearing, a reduction of barren ewes *(Fig. 1)* and significant decrease in lamb and ewe morbidity and mortality. Reproduction control offers better conditions for control of parturition, thus reducing neonatal mortality.

Key words: Oestrus synchronization, reproduction control, breeding, production economics, demand and supply.

Introduction

In conditions of our country the classical, so-called Carpathian system of breeding when sheep are seasonally bred in the autumn and lambs are delivered in winter so that they would come at the time of weaning to fresh, young spring pasture, requires good foodstuff supply for winter season. On agricultural farms with higher concentration of animals where the main attention in animal production is paid to cattle breeding and sheep breeding is a complementary branch, modest foodstuff supply for sheep winter season is not infrequent.

Materials and methods

To prepare the use of biotechnical method of the reproductive cycle induction, an objective analysis of fertility for preceding farming year and for the last two to three reproduction cycles is needed (4). The breeder has to evaluate the feedstuff situation, quality of pastures, and production orientation of the breeding (wool-meat or meat-wool production). It is not advisable to implement this method in breeding with dairy production and orientation for production of lumpy cheese. This output, however, must be taken into account when round-the-year sheep milking is done with herd changing in combination with a very early weaning of lambs and round-the-year production of specialties based on sheep cheese. To use the method of output, the nursing staff must be properly trained as well as acquainted with the emerging tasks. Cadres have to be stabilized for the time of the whole reproduction cycle. It is necessary to ensure properly the activities and cooperation with biological services (both veterinary and breeding) *(3).*

Results and discussion:

Using the above-described method, it is possible to mate 95 to 100 % of animals selected for breeding use in the course of induced oestrus well as during two successive reproductive cycles and lambing 72 to 92 % of sheep intended for mating. The losses will be decreased so that with

classical system, at mean 86 lambs for 100 ewes were raised and when the utility herd is halved to a changed system and to classical system of breeding, at mean 156 lambs for 100 ewes were raised. In 1984, of animals selected for breeding use 71.0 % were mated, in 1985 - 53.1 % and with classical system of breeding organization, 71.1 % of sheep in 1986. In 1986 and 1987, however, in a herd with a changed breeding system all the included animals - i.e. 100 % were mated. A more serious problem for a breeder appears to be the number of lambed animals of those selected for mating and of those mated. In 1984, 64.7 % of sheep included for mating lambed and 91.1 % of mated, in 1985, 49.8 and/or 93.8 % and in 1986 with classical breeding system, 63.0 % of included and 88.7% of mated sheep. With changed system of sheep breeding in 1986, 72.4 % of both included and mated sheep lambed like in 1987, in a herd with classical breeding 90.3 % lambed and in a herd with a changed breeding system 92.2% of animals lambed *(Tab.1).* The elimination of the difference in the number of lambed for a number of included for mating and for a number of mated consists in the fact that, when culling of breeding sheep, those sheep were culled that were not mated successively within the season and off-season period.

To obtain real positive results approaching to those obtained when verifying the described method, conditional state, standard of breeding, and good pasture conditions are the limiting factors. It must be emphasized that method described in a realization output cannot replace the inadequate nutrition, poor breeding care, and insufficient organization of breeding as well as management shortcomings.

Prior to the selection of animals and beginning with realization, it is necessary to carry out the dehelmintization and immunoprophylactic treatments required in a respective locality; the body weight of animals should reach the breeding standard. When condition of animals is poor or reproduction indices obtained in the preceding reproduction cycle are unsatisfactory, it is necessary to evaluate the state and level of foodstuff tease from both the qualitative and quantitative viewpoints, to determine the metabolic profile, and to ensure reasonable improvement of a ration according to the results of examinations.

Due to a number of reasons, primary agricultural suppliers are in an unsatisfactory economic position. Decrease in demand, increase in retail prices, increase in input prices, real decrease of agricultural commodity prices are just a few of these. Such effects have led to the situation where the effectiveness of the agriculture industry has decreased or has no existence at all *(1).* The question needs to be asked, what has happened to the demand curve for agricultural commodities, and on which section of the supply curve is it increasing or decreasing (marginal and average cost curve)? If the demand is in the decreasing section of the cost curve, this is possible due to the increased supply of agricultural commodities after decreased prices of commodities during the transformation, then the average cost of production is higher then marginal cost. But the solution depends on the reasons in inefficiencies. If the reason is the management practices, then the cost by economic theory, should be borne by the producers. On the other hand, if there are objective reasons (transformation), cost is communal and the community should bear the cost, mostly due to the fact that the deepening inefficiency and time increase the cost of the solution *(2).*

The use of bio technical methods of reproduction today, during the transformation are more relevant than during the stable agricultural development. The reason is that agricultural firms are in a bad economic situation, mostly in mountain regions. But also economic significance of sheep farming in spite of changes in crop production - change in crop structure, increase of pasture crop area- has decreased.

Total number of sheep has decreased since 1988-1989 by 20-25 % (1988: 650000. Sheep and 370000 ewes, 1995: 420000 sheep and 290000 ewes). Even though there were changes in the specialization in production from wool to milk and meat, as well as rapid aging of flocks. It is due to these reasons that we need to address the sheep breeding with respect to total agricultural industry. Things we need to address:

1. Economical situation ofthe individual firms and the industry itself.
2. Economical efficiency of sheep breeding.
3. Reproduction of the flock.

It is possible to address these problems by detection and synchronization of sheep breeding and increase the number of lambing to 1.5-2.0 per year. In such a way it is possible to guarantee fattening of weaning lambs (Christmas and Easter) for which there is a demand in EU for better prices.

There will also be other benefits like: reduction of the devoted to oestrus detection; facilitation of the artificial insemination; insemination on a predetermined term in conjunction with ovulation time controlling procedure; shortening the overall period of parturitations in a herd or flock as a sequel of synchronized breeding; supervision at birth in order to reduces neonatal mortality; successful control of breeding and lambing give possibility weaning, fattening and better marketing; induction of stricter measures for control of disease, especially with regard to building usage; facilitation the use of the embryo transfer technique; rationalization of the labour use, buildings and other resources in a general management.

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Table 1. Observed Parameters of Reproduction

Fig. 1 Applyed of oestus synchronization for decrease of barren ewes

Table 1. Observed Parameters of Reproduction

		Number of					
Year	Heard	ewes for breeding	mating ewes	lambed ewes	total lambed born	ewes lambing twins	weaning lambs
1984	A	280	260	247	280	33	226
	B	473	336	306	355	49	272
1985	A	342	340	318	345	28	303
	B	456	242	227	254	27	132
1986	A	346	346	329	382	53	345
	B	211	150	133	151	18	118
	$B_{synch.}$	250	250	181	230	49	208
1987	A	333	327	309	343	34	303
	B	236	236	293	230	17	183
	$B_{synch.}$	257	257	237	280	43	261

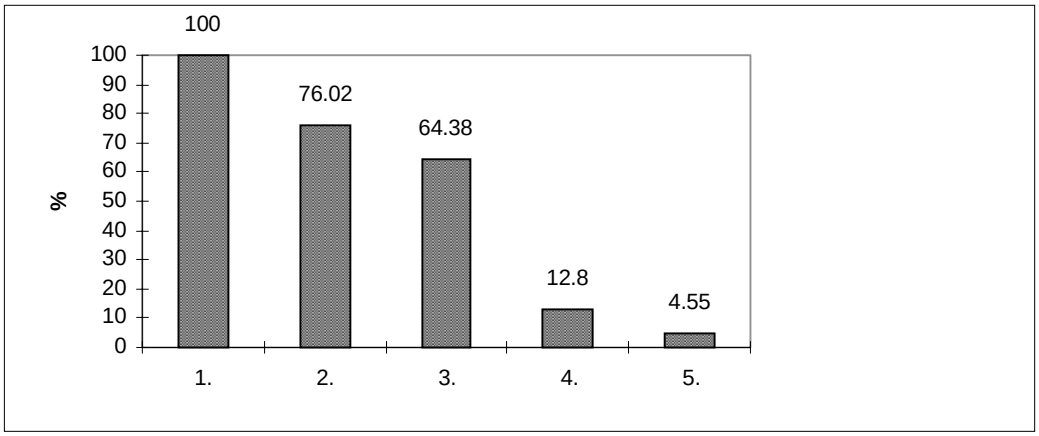

Fig. 1 Applyed of oestus synchronization for decrease of barren ewes
1 - synch., 2 - mated, 3 - lambed ewes, 4 - barren ewes in heard,
5 - barren ewes aver oestrus synchronization.

Losses due to porcine reproductive and respiratory syndrome in a large swine farm

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Summary

In the summer of 1994 an acute onset of maternal reproductive failure occured in a 2,330 sow farrow-to-finish farm. Clinical signs observed in the affected sows were typical for porcine reproductive and respiratory syndrome (PRRS). During the first 6 weeks of the epizootic 1,117 sows farrowed; 216 (19.33%) farrowed before the 110th day of gestation. The majority of piglets born before term died within a few days of birth and the mortality rate for term piglets increased to a maximum of 75.56% during the 5th week of the epizootic when 1,562 out of 2,067 piglets were either born dead or died prior to weaning. Preweaning mortality rates gradually returned to normal values within 16 weeks. The incidence of respiratory disease in the weaned and fattening pigs increased during this time. Although specific prophylactics against respiratory diseases were administered, the death rate doubled for the weaned and fattening pigs.

Key words: PRRS infection, swine, economical losses

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a new disease that initially spread as a pandemic (2) but which has become endemic in many countries throughout the world. It appeared in the USA in the 1980s where it was known as a mystery swine disease (1). Since then it has been reported in Canada in 1987, Germany and The Netherlands in 1990, Belgium, Britain and Spain in 1991 and subsequently many other European countries as well as the Far East. The true extent of the spread of the disease certainly is far greater than has been documented.

In 1992 first clinical cases of porcine reproductive and respiratory syndrome were detected in a small pig farm situated in the western part of Poland. Serological examinations performed at the Lelystad laboratory, of blood samples collected from pigs showing typical signs of the disease, revealed the presence of antibodies specific to PRRS virus.

The aim of the studies was to demonstrate the losses and economical consequences of PRRS outbreak in a large pig farm „X" in Poland.

Material and methods

Farm X is a farrow-to-finish operation consisting of 2,330 sows and the annual production of piglets was about 46,000. Every 10 days approximately 200 (120-260 depending of the season of the year) sows and young females farrow. Prior to the occurence of PRRS the pig mortality index, from birth to weaning, did not exceed 6% and losses among weaned piglets and fattening pigs were lower than 2%.

Serological examinations were performed by the immunoperoxidase monolayer assay (IPMA) (4).

Virological examinations were carried out using lung tissue collected from 5 piglets which died some hours after birth and blood samples collected from 6 sick sows. Virus isolation was carried out on a culture of lung macrophages obtained from 8-week-old piglets. The presence of PRRS virus was detected by immunofluorescence (IF) (3).

Results

From July 11, 1994 the first cases of premature farrowings were detected in this farm. After a few days the incidence of the disease was considerably increased. Among 181 females which farrowed from July 21 to July 30, 13 (7.2%) farrowed before the 110th day of pregnancy, including 4 sows (2.2%) that farrowed before the 105th day of pregnancy. A similar situation was found in August. Altogether from July 11 to the end of August, 1,117 females farrowed of which 216 (19.3%) farrowed before the 110th day of pregnancy. In September, October and until November 10, the number of premature farrowings gradually decreased.

In the first period of the enzootic in some sows the following typical signs of PRRS were observed: hyperthermia (up to 40.8^0C), inappetence, cyanosis of the ears and mammary gland, stillborn piglets and mummified foetuses. An acute course of the disease caused deaths in sows. Twenty-five (2.2%) out of 1,117 farrowed females died. In some piglets congenital splayleg and paralysis of hind quarters were observed. Some piglets were lying on one side and performed paddle-like movements. Some litters had both dead mummified and normal foetuses. Neonatal piglets occasionally had oedema of the eyelids. Agalactia and disorders in the behaviour of about 30% of females showing reluctance to feed their newborn piglets caused deaths in many of them. In some litters only single suckling remained alive a few days after birth.

Piglets of low weight and vitality had significant difficulties in suckling and went into hypoglycaemic coma. Some suckling piglets had difficulties breathing. In litters of sows which farrowed in due time an increased number of cases with diarrhoeal symptoms were obserwed. The mortality rate between farrowing and weaning was about 6.0% before the occurence of

PRRS. From July 21-30 it reached 36.7% and in the succesive analysed periods it increased rapidly. The highest losses of piglets were stillborn or died in the period between farrowing and weaning. From September the mortality rate in sucklings gradually and slowly decreased. It should be underlined that in the last analysed period the mortality rate was still very high at 29.9%. Altogether in all the analysed period (from July 1st to November 10th) 9,915 out of 19,574 (50.7%) piglets were stillborn or died. A peak of losses was recorded in the fourth and fifth 10-day period of the outbreak. In that period total losses of piglets was 80%. It should be stressed that the main component of these losses were dead and stillborn piglets. The frequency of piglets was 28.8 to 46.5% and that of stillborn piglets ranged from 8.3% in the first period of the disease to 40.9% in the peak of the enzootic. The percentage of mummified foetuses ranged from 0.2 to 35.0%. Acute losses due to PRRS in this large herd lasted 14 weeks.

Effects of PRRS in farm „X" were also observed in weaned and fattening pigs. An increase in the number of disease outbreaks with respiratory symptoms was also found in those animals about 2 weeks after detecting the first sick animal. Although immediate therapeutic measures were introduced the mortality rose significantly. The obtained results show that prior to the occurence of the disease some 60-70 fattening and weaned pigs died within a 10 day period. In the peak period, these losses were 4 times greater and, for example, in the third 10-day period of August 299 young pigs and 290 fattening pigs died. After 5 months from onset, the production and health of pigs returned to normal levels.

Bacteriological examinations revealed the presence of the following organisms in the lung tissue of dead pigs: Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Pasteurella multocida and Bordetella bronchiseptica.

No pathogenic changes were found at autopsy in sows. In some cases necrotic foci were detected in the placenta. In piglets an enlargement of the heart and some changes in the blood vessels were visible. An increased amount of exudate was found in the thorax. In several sucklings inflammatory lesions were visible in the apices of the lungs, histologically the lungs showed interstitial pneumonia characterised by increased cellularity of alveolar septa.

The results of serological and virological laboratory examinations for brucellosis, leptospirosis, swine fever and Aujeszky ' s disease were negative. Based on the course of the disease and results of clinical and laboratory examinations it has been unambiguously found that the disease and heavy losses in farm „X" were caused by PRRS.

Conclusions

Field observations indicate that quality of management and size of the farm may influence the course of PRRS. According to the observations the most serious losses are observed in large herds and in farms in which sanitary status is very high.

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Economical consequences of fasciola hepatica infection in sheep

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Summary

The studies has been done on 54 lambs at the average weight of 21.5 kg, by using the coproscopical methods. The animals were divided in 3 groups with 18 lambs in each. In the first group were non-infected animals; the second group was infected with 200 metacercaria and was treated with Fasinex (10 mg/kg body weight) 6 weeks after infection. The third group was infected with 200 metecercaria, but has not been trea-ted with Fasinex. The best results were obtained in the first group. In this group the weight gain was about 4 kg (14.7 per cent) higher comparing with the group third (in-fected, non-treated) group. The weight gain in group two (infected and treated) was 2.42 kg (11.0 per cent) higher as in third group (infected and non-treated).

Key words: experimental fasciolosis, treatment, Fasinex, weight gain

Introduction

The economical consequences of *Fasciola hepatica* infection of sheep in Poand were mainly established on the basis of losses after veterinary-sanitary examination in slauther-houses (Kuczyñski 1970, Walkowiak 1970, Lis 1989). It is worthy of notice that the subclinical infections are underestimated through the veterinarians and breeders. The subclinical infections caused heavy losses, which are demonstrated through worse weight gain (Düwel et al. 1972, Düwel 1982, Ducos de Lahitt 1988), worse wool production (Düwel 1982, Ducos de Lahitt 1988) and worse feed intake (Tschubarjan 1964, Sykes et al. 1980).

The aim of these studies was to etablish the influence of experimental *F. hepatica* infection on the economical consquenseces in sheep.

Material and methods

The studies has been done on 54 lambs at the average weight of 21,5 kg, in a farm in South Poland.

The lambs were divided in 3 groups with 18 animals in each. In the first group were non-infected animals; second group was infected with 200 metacercaria and treated with Fasinex (10mg/kg of body weight) six weeks after infection. The third group was infected with 200 metacercaria but has not been treated with *F. hepatica*.

The extensity of infection was established on the basis of coprological examination 10,13 and 16 weeks after infection with the sedimentation method (Stefañski and Zarnowski 1971). From each group 3 lambs were autopsied 16 weeks after infection and the number of *F. hepatica* in liver were established.

The results were analysed statistically with the t-Student test for small groups.

Results

The results are presented in the **tables 1** and **2** Fasinex prove to be a highly effective drug against Fasciola hepatice. After the drug was used against immuture flukes 6 weeks postinfection only in faeces of one lamb single eggs at the end of the studies (16 weeks postinfection) has been found.Muture *F. hepatica* has been prove in one case (3 flukes) on the basis of autopsy 3 experemental infected lambs (group 2) 16 weeks postinfection. The highest weight gain (3.96 kg per lamb) was obtained in the first group (no infected). This results was about 14.7 per cent higher comparing with the group III (infected and no treated). The weight gain in group II was 2.42 kg (11.0 per ceny) higher as in group third (infected no treated).

Statistically were high sigmificant differences between groups I (no infected) and II (infected and treated) and group III (infected and no treated) on the level $P \leq 0,01$.

Conclusions

1. Fasciolosis is on important factor, which has an influence on the production results of lambs. The weight gain of experimentally infected with *F. hepatica* animals was about 4 kg (14.7 per cent) lower as in no-infected lambs.
2. Fasinex is a high effective drug against immutare *F. hepatica* infection. Lambs treated 6 weeks postinfection were practically free of *F. hepatica* infections after 16 weeks.

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Tab. 1. The effect of treatment after Fasinex administration (10 mg/kg of body weight) on experimental with *Fasciola hepatica* infected lambs.

Experimental group, number of sheep	Number of infected animals- 200 metacercaria	Number of treated animals- 6 weeks post infection	:Evaluation of treatment - number of infected lambs			
			coprological examination			autopsy
			10 weeks after infection	13 weeks after in fection	16 weeks after infection	16 weeks after infection
I-18	0	0	0	0	0	-----
II-18	18	18	0	1	1	$\frac{1}{3}, \frac{2}{0}, \frac{3}{0}$
III-18	18	0	1	18	18	$\frac{1}{45}, \frac{2}{64}, \frac{3}{73}$

Explanation: Number of animals
Number of *Fasciola hepatica* found in the liver

Tab. 2 The mean weight gains and the standard variation of the control (III) and experimental groups (I and II).

Tab. 2 The mean weight gains and the standard variation of the control (III) and experimental groups (I and II).

Groups	Weight		Mean weight gain X_2-X_1	Standart variation
	at the beginning of studies X_1 (kg)	at the end of studies X_2 (kg)		
group I -non infected	22,20	36,10	13,98[xx]	1,523225
group II -infected (200 metacercaria) and medicated	22,18	34,62	12,44[xx]	2,07189
group III -infected and non medicated	20,80	30,82	10,02	1,50840

[xx] Statistically high significant differances on the level $P \leq 0,01$

Management and economic results associated with the BHV1 status of dairy farms

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Summary

A more closed farming system may prevent introduction of infectious diseases on dairy farms and can be a good starting point for eradication of these diseases. In phase 1 of the project data were gathered to make up an inventory of the existing more or less closed farming systems in the Netherlands. The objective of the study was to analyze the risk factors for introduction of Bovine Herpes Virus type 1 (BHV1) on a dairy farm. The BHV1 status of 214 farms was known by means of blood and/or bulk milk samples. Case farms had animals with antigens against BHV1 and control farms were free from BHV1. Logistic regression was used to analyze the multivariate relationships. The risk factors associated with BHV1 status were (professional) visitors, purchase of cattle, participation in cattle shows, and distance to other cattle farms.

Furthermore, a 2-year follow-up study will be carried out to quantify the determined associations between farming systems, disease status and technical and economic farm results and prove possible causal relationships.

Key words: BHV1, risk factors, closed system, dairy farms

Introduction

A more closed farming system may prevent introduction of infectious diseases on dairy farms and can be a good starting point for eradication of these diseases. The study explores the possibilities of adaptations in management (i.e., a more closed system) on dairy farms to prevent introduction of diseases and improve the health status on the farm. Diseases can be introduced on a farm in several ways and every infectious agent has its own routes (Koole, 1995).

In an exploratory study the technical and economic results of more or less closed farms were investigated (Van Schaik et al., 1997). Data from an accounting system showed that ‘closed’ farms realized better economic results than ‘open’ farms. ‘Closed’ farms had a positive influence on net profit per 100 kg milk of almost Dfl 1. A survey was carried out to make up an inventory of the existing more or less closed farming systems in the Netherlands. In this survey introduction of BHV1 was chosen because good records existed of the BHV1 status of dairy

farms. BHV1 causes the disease called IBR. Most studies only mentioned a few possible risk factors for introduction of BHV1 but did not quantify these risk factors. The objective of this study was to quantify the risk factors for introduction of BHV1 on Dutch dairy farms using logistic regression.

Material and methods

A case-control design was used (Martin et al., 1987). The farms were non-randomly selected dairy herds with a known serological status for BHV1. The farms were selected on the basis of geographical position, namely the northern provinces of the Netherlands. The cases were farms on which 1) all of nine monthly bulk milk samples were antibody titre positive (n=131), or 2) half yearly blood samples (n=83) showed that antigens positive cows were present in the herd. Milk and blood samples were tested by undiluted gB-blocking. The controls were farms on which all bulk milk samples or all blood samples were negative for antibodies against BHV1.

Data about husbandry practices, disease status, personal characteristics and possible risk factors for introduction of infectious diseases on the farms were collected by means of a questionnaire. The questions were asked about 1995 and four preceding years. Technical and economic farm results were derived from secondary databases. For the analysis a subgroup of 107 farms which said to have never vaccinated against BHV1 was used. The BHV1 status of the farms in the subgroup could basically only be caused by introduction of BHV1.

Hosmer and Lemeshow (1989) recommended a subset approach for situations in which the total number of possible predictor variables greatly exceeds the degrees of freedom allowed in the model, as was the case in this study. First a univariate analysis was carried out on BHV1 status of the farm. The univariate analysis gave an idea which variables would be useful in further analyses. Furthermore, all risk factors based on the major routes of introduction according to the extension services (Koole, 1995) were also included in the multivariate analysis. In the second part of the analyses logistic regression was used to analyze the multivariate relationships between risk factors and BHV1 status (Hosmer and Lemeshow, 1989). The statistical analysis was carried out using the LOGISTIC REGRESSION procedure in SPSS 6.1 (SPSS Advanced Statistics 6.1, 1994). The odds ratios were derived by exponentiation of the betas.

Results

Sixty-two farms were free of BHV1 and 45 farms were BHV1 positive. In Table 1 the model of associations between risk factors an BHV1 status is shown. The model correctly classified 76% of the farms for BHV1.

Table 1. Multivariate logistic regression model of risk factors associated with positive BHV1 antibody status in unvaccinated dairy farms (n=107, $\chi^2 = 0.54$)

Variable	β	SE(β)	*P*	OR	95% CI	AP or PF
# of visits of AI technicians (10 visits)	0.04	0.05	0.43	1.04	0.94-1.15	0.04[1]
presence of farm clothing and/or boots	1.91	1.13	0.09	6.73	0.74-61.9	0.17
distance to nearest cattle farm (100 m)	-0.36	0.12	0.00	0.70	0.55-0.88	0.74[2]
participation in cattle shows	1.26	0.65	0.05	3.54	0.99-12.6	0.22
temporary worker	1.18	0.68	0.08	3.27	0.86-12.3	0.19
number of cattle purchased in 1995	0.28	0.07	0.00	1.32	1.15-1.52	0.31[3]
at least every week occasional visitors such as neighbors, family, friends etc. in the barn	1.40	0.59	0.02	4.06	1.28-12.9	0.35
interaction AI technician*farm clothing	-0.03	0.01	0.02	0.97	0.95-0.99	0.03[4]

[1] The AP was calculated by comparing 10 visits per year or less with on average 101 visits of AI technicians per year.

[2] The PF was calculated by comparing a distance of less than 100 m with on average 400 m.

[3] The AP was calculated by comparing no cattle purchased with on average 6.6 cattle purchased per year.

[4] The PF was calculated by comparing the lowest exposure (0) with the mean of the exposure of the rest (108).

Farms which never vaccinated against BHV1, and had a positive BHV1 status were more often visited by AI technicians, more often had farm clothing and/or boots, were closer to other cattle farms, more often participated in cattle shows, more often had a temporary worker, purchased more cattle, and had more occasional visitors in the barn. An odds ratio of 1.32 for 'number of cattle purchased' means that a farm which purchased one cow was 1.3 times more likely to be a BHV1 positive farm. 'Farm clothing' is more often present on farms with a positive BHV1 status. An interaction term of 'AI technician' and 'presence of farm clothing' was found significant at $P \leq 0.05$. The interaction term means that when AI technicians (or other visitors) used farm clothing and/or boots a farm was less likely to be BHV1 positive. Farm clothing was a so called effect modifier. The AP of 'number of visits of AI technicians' and the interaction term of this variable with 'presence of farm clothing' of 0.04 and 0.03 respectively were very low. The

AP or PF of the other variables in the model were of a considerable size, ranging from 0.17 till 0.74.

Concluding remarks

This was an exploratory study. It provided a way to examine the influence of factors that might be associated with a positive BHV1 status of a farm. The purpose of the study was to evaluate the advice of the extension services and identify factors that could be further examined in other ways or that might be amenable to manipulation for the purpose of prevention of introduction of infectious diseases on a farm. Our study resulted in several factors that deserve more attention of farmers. Final decisions about implementations of the management measures found to reduce the chance of having BHV1 on the farm must eventually be made on the basis of economic comparisons of the expected benefits of a more closed farming system with the costs of implementing those measures.

In a prospective cohort study farms which differ in risk factors for introduction of diseases will be investigated for a two year period. Introduction of several infectious diseases (BHV1, BVD, Leptospirosis hardjo, Salmonella dublin and Strept. Agalactiae (mastitis)) on the farms will be measured as thoroughly as possible. The farmers will be followed and interviewed about their management according to (introduction of) diseases. Records will be kept of technical and economic farm results, mastitis, and low fertility to measure a possible relationship with (introduction of) infectious diseases. With the data of the two-year period we hope to quantify the more causal relations between management, infectious diseases, factorial diseases and economic farm results. The results of the project will be used to create a computer model which can be used to support farmers in their decisions to derive a more or less closed farming system to prevent introduction of diseases.

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Animal wastes as a risk for animal and human health

Animal wastes as a risk for animal and human health

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Summary

The causative agents of many infectious diseases are excreted by the fecal route and also with other excretions of the body. Some pathogens are also excreted from clinically healthy animals, from those with latent infections and in cases of transmissible multifactorial diseases. In all types of livestock housing, the pathogens finally reach the floor with the installations for collecting manure as a solid (farmyard manure, FYM) or liquid (slurry). Livestock owners very often do not realize that manure may contain pathogens and therefore do not take precautions against possible spread of diseases by utilization of manure. The pathogens do not survive very long in properly stored FYM because of the temperature rise and biological and biochemical activities prevailing in the middens. In slurry the conditions are different because the temperature does not rise and biochemical activity is low so that the pathogens survive for rather long periods in slurry. Despite this fact, there are only very few documented records of disease transmission to animals or man by animal wastes. Although a certain risk always exists, it can be reduced to an acceptable level if care is taken to treat FYM and slurry according to appropriate guidelines.

Further, epidemiological considerations, dangers emerging from herds infected with notifiable diseases and from the agro-industry with slaughterhouse offal and animal rendering are discussed. Finally some gaps in scientific knowledge are identified.

Key words: manure, slurry, pathogenic bacteria, viruses, parasites, notifiable diseases, slaughterhouse offal, animal rendering, gaps in research

Introduction

The causative agents of infectious diseases are excreted by the animals via feces and urine and also with other excretions or secretions of the body. In all types of livestock housing, the pathogens finally reach the floor with the installations for collecting manure or slurry. Farmyard manure (FYM) refers to a mixture of excreta together with substantial quantities of bedding materials (e.g. straw, wood shavings, sawdust, peat) dense enough to be handled as a solid. Slurry is a mixture of feces and urine which may be mixed with washing water, rain water, small quantities of bedding material and spoiled feed particles. Therefore these causative agents can be found in both FYM and slurries.

The so-called „transmissible factor diseases“ can lead to multifactorially caused diseases characterized by normally harmless infections leading to clinically recognizable symptoms of disease released by non-microbial factors. If they exist in a population for a longer time, a gradual accumulation of infectious agents results, and if further factors are added, in the outbreak of a dangerous multifactorial disease. Today, this type of disease is a particular threat to larger and specialized livestock enterprises with a lack of population heterogeneity such as fattening of only pigs, only calves or only broilers. In many countries salmonellosis has been steadily spreading among man and animals for more than two decades. In some areas, therefore, more than one-third of pigs and more than one-half of broilers produced for slaughter are infected with salmonellas. These are the reasons for regarding manure from larger animal production units to be generally infected and for requiring certain safety measures to be observed in disposal.

The spread of many classical human diseases such as cholera, typhoid fever or bacillary dysentery has been controlled by insulating man from human excreta by improvements in personal hygiene and the use of sewers, and sewage and water treatment processes. In areas of the world where such improvements have not been made, or where available systems are inefficient, these diseases remain endemic. The treatment of animal wastes with methods used for the purification of municipal sewage is, with the exception of a few gigantic intensive units in various parts of the world, generally considered as too expensive and not suitable for farm use. Therefore, one has to look for simple methods to minimize the environmental hazards emerging from "normal" manure which is only possibly infected and for more sophisticated methods to render manures, which are evidently infected, innocuous for further utilization as fertilizers or for other purposes.

Until the intensification of agriculture and the increase in the size of herds, a simple separation of farm animals from their excreta was achieved by collecting their waste in straw bedding and removing it to a midden to compost. The resultant compost is free of most pathogenic organisms and can be spread on land. On the majority of farms some compost is still produced but this system has largely been superseded by the disposal of animal excreta as a liquid slurry.

Potential health hazards

Long lists of organisms which may be spread by slurry have been compiled, but of most importance are those responsible for salmonellosis, brucellosis, leptospirosis, tuberculosis, yersiniosis, campylobacteriosis, erysipelas, listeriosis and swine dysentery. However, these diseases may spread through a herd under conventional husbandry conditions. Therefore, if

diseases are to be spread by slurry, it is necessary for the waste to become infected with the causative organisms and for these to survive storage or treatment, to remain capable of causing disease and to survive on pasture until it is grazed.

Bacteria: Theoretically in all cases of bacterial infections, the respective bacteria can occur and may be found in the excreta and manures of infected animals. Under practical conditions the number will be limited by many factors and the variety of pathogens actually isolated is comparatively small. These factors include: location of the farm, species of animals, temperature, physical, chemical composition and dry matter content of the manure, age of the slurry, and ease with which small numbers of pathogens may be isolated.

Viruses: Considering that viruses are completely dependent on their cellular hosts for their "existence", it is reasonable to state that they can survive in nature only if they are able to pass from one host to another, within the same animal species but, depending on their biological properties, also to hosts of different species.

The information about the occurrence and tenacity of viruses in animal wastes are less than for bacterial infections. Enteroviruses are relatively tenacious in slurry.

Based or knowledge about the occurrence and survival of viruses of human origin in waste water, sludge, soil and on plants which were fertilized with sewage sludge (more than 100 different strains of enteroviruses were isolated), it can be assumed that also the agents of many virus diseases of domestic animals are excreted in the faeces and urine of infected animals and, therefore, must frequently occur in manures (Strauch, 1991).

Parasites: All the workers meeting in Dublin in 1977 at an EC-conference (Kelly, 1978), agreed on the parasites which should receive priority for research purposes. The top two priorities for cattle and sheep were strongly nematodes. They also agreed the following procedures to reduce the potential hazard to animals when slurry is applied to land: (a) use slurry on tillage land where possible, (b) delay the application of slurry to grassland until July at the earliest, (c) do not allow calves or lambs to graze treated pasture, (d) where possible slurry should be injected into the soil, (e) cattle should be treated with anthelminitic three weeks after being housed and (f) slurry may be treated by aeration or chemicals in order to destroy pathogens.

In cattle slurry, trichostrongyles are likely to be the most important parasitic hazard because of their widespread prevalence, and because, in their free-living stage, they are resistant to external factors and cattle slurry is widely used on grassland. Fascioliasis has been proposed as a potential problem for cattle where slurry is used on pastures in the Netherlands. However direct

evidence of such a risk to animal health from slurry application to pasture is lacking. ln pig slurry ascaris eggs can be present in large numbers and can remain infectious for 267 days in the soil of East Slovakia Lowlands. *Ascaris suum* may contaminate lamb and calf liver. Broiler and hen litter will normally be contaminated by oocysts of different *Eimeria* species.

Cryptosporidiasis, which is caused by a protozoan parasite, is now almost as common in patients with presumed acute infectious diarrhoea as salmonella infection. It is higher in rural populations and could possibly be linked to the excretion pattern in cattle and sheep. Within the past few years, this parasite has become recognised as an important and widespread cause of diarrhoeal illness in several animals including man.

A further report has been published on the importance of animal manures as vector for parasites. The authors drew the conclusion that cattle manure is a vector for *Eimeria* species, *Cryptosporidium parvum, Sarcocystis* species, *Taenia saginata* and *Fasciola hepatica*. Pig manure is classified as a reservoir of infections with *Toxoplasma* gondii, *Sarcocystis* species and *Ascaris suum* and it represents a favourable culturing medium for stable flies(Hiepe and Buchwalder, 1991).

The priority of any contaminant as defined above according to its prevalence in the effluent, and its innate properties of resistance and survival potential, may not only be modified by the conditions in which it is kept or stored after having been shed by the host, but also by the destination of further use. The hygienic hazard, for instance, of pig slurry is very different depending on whether it is going to be used as fertilizer on arable land, as fertilizer on sow or cattle pastures, or as recycled feed for cattle. The variety of parasites will also be influenced by its composition and age. The parasites discussed above have to be modified depending on the geographical area where a relevant problem arises.

Fungi: Animal wastes are not usually considered as a source of pathogenic fungi although solid manure may provide a good growth substrate for many species.

Survival of pathogens during storage of slurry

Bacteria: it is generally accepted that most pathogens are reduced by storage. The type of slurry, storage temperature and serotype of salmonellas may all affect the survival time which will also depend to a considerable extent on the number of organisms contained in the slurry at the beginning of storage. A considerable amount of literature is available on the survival time of pathogenic bacteria in excreta and manure(Strauch, 1991; Strauch and Ballarini, 1994).

Viruses: several factors are responsible for the survival of viruses in nature: subclinical infections, persistent infections, population size.

Only insufficient information is available on the survival time of viruses in farm effluents. Many more investigations have concentrated on survival of viruses in human sewage or soil and herbage treated with sewage and sewage sludge. The causative agents of many virus diseases are excreted in the feces and urine and a number of virus diseases, particularly virus enteritis, swine vesicular disease or foot and mouth disease, could be spread by slurry although such cases have yet to be reported. The latter may be due to the fact that these diseases are partly notifiable, and therefore subject to specific and very stringent regulations which emphasize the control of movement and utilization of animal wastes from infected farms and usually are based on biological (dung packing) or chemical disinfection of solid and liquid manures (Haas et al. 1995)

Parasites: Many parasitic infestations of farm animals are transmitted by ingestion of infective stages in materials, including pasture, contaminated with feces. The viability of parasite eggs and larvae can vary greatly. In particular, ascaris eggs and certain larval stages of trichostrongylids have a rather long survival time and may give rise to further infestations of animals after spreading the manure on crops or pasture land. Fortunately, parasitic infections are seldom fatal, except in young animals, and the effect of spreading infected slurry is probably merely to increase the parasite burden of animals which are already infected.

Epidemiological considerations

Conventional livestock units where bedding is used do not cause a special epidemiological problem because, assuming proper management procedures are carried out and enough straw is used, dungsteads develop temperatures high enough to destroy pathogens that may be present. The relative safety of this procedure is demonstrated by its being stipulated in many countries in the official provisions of dung disinfection for the control and eradication of notifiable infectious diseases, in terms of the socalled dungpacking. After five weeks the dung is considered to be disinfected and can be used on untilled arable land. After 10 weeks it can be used on pasture and forage land (German regulation).

Stored slurry is usually anaerobic and therefore no spontaneous generation of heat that could destroy pathogens will occur either in summer or winter. Therefore, slurries containing pathogens always pose a health hazard to the animals of the particular farm and its neighbourhood. A certain degree of "self-disinfection" during storage of slurry together with the adverse influences of the environment after spreading on pastures and arable land add to the fact that reports about

severe outbreaks of infectious diseases after utilization of slurry, especially in grazing animals, are rather rare.

As referred to above, there is a rare possibility that certain pathogens (e.g. salmonellas) can also be detected once or twice in the slurry of farms with clinically healthy animals but then vanish again („passing pathogens“).To minimise even such a low risk, the already referred to expert group of CEC has elaborated Interim Minimum Guidelines for the utilization of "normal" slurries.

Since the risks of infection associated with the spreading of slurry on farmland have not yet been clearly established, the safest procedure will be to aim at the best possible decontamination of infected slurry during the storage phase, e.g. before spreading the slurry. Although our present knowledge of the initial concentrations of potentially pathogenic bacteria in fresh slurry under practical conditions is generally sparse, several reports are available on the concentration levels of certain bacteria. So far, no decision has apparently been reached with respect to an "acceptable final level" of various pathogenic bacteria or viruses in relation to decontamination of slurry.

In the field of parasitic diseases, the epidemiological situation might be somewhat different from that of bacterial and viral diseases. In contrast to the epidemiological facts of infectious diseases caused by bacteria and viruses, the parasites may need more than one host for their final development and they may have several larval stages with a different sensitivity to environmental conditions. Furthermore, it is possible by certain measures to interrupt chains of infection for parasites without disinfection of the inanimate vector, e.g. utilization of pig slurry on cattle pastures and *vice versa*.

In cases of notifiable diseases all countries have special legal regulations procedures for disinfection and handling of the excreta and manures. Solid manures are usually incinerated or composted, slurry chemically disinfected.

Larger livestock enterprises sometimes use **anaerobic digestion** of slurry to produce biogas. Most of these biogas plants are operated at mesophilic temperatures in the range of 30-35°C and some operators believe that pathogens are destroyed by the anaerobic digestion process but this is incorrect. Digester effluent will be disinfected under certain conditions only by temperatures in the thermophilic range of 55°C, provided an unfailing plug-flow system is used. Therefore, the effluent of biogas plants with mesophilic digestion has the same hygienic status as any slurry which was stored in a normal slurry tank.

Dangers emerging from herds infected with notifiable diseases

The declaration of an animal disease as a „notifiable disease“ places an economic and/or zoonotic importance on that condition which warrants direct action by the State in order to protect its own wider interests. The epizootic diseases such as foot and mouth disease, swine fever and rinderpest readily fit into this category and the concern expressed worldwide regarding such diseases is exemplified by the measures taken by governments to guard borders against their entry, for the above reason. The Office International des Epizooties (OIE), acting in concert with the national agencies and such other bodies as the Food and Agriculture Organisation of the United Nations (FAO) and, in this region, the Commission of the European Communities (CEC), serves as an alerting agency in the event of outbreaks. Within the European Union (EU), each Member State is obliged to notify the Commission and the other Member States of the existence of certain epizootic diseases on their territory (Directive 64/432/EEC, as amended). Other notifiable diseases, such as brucellosis in cattle and pigs and tuberculosis in cattle, which have a more direct zoonotic importance are dealt with at national level. Meanwhile, the known existence of rabies in an EU Member State calls for notification and direct action, for its own protection and that of its neighbouring States and trading partners.

The regulatory measures which are required to be taken at farm level initially to contain and later control and eradicate notifiable animal diseases, should reflect the seriousness of each situation and the need to prevent or curtail the direct or indirect spread of the infective agent from the infected holding to adjacent and more distant parts.

Precautions which guard against the dissemination of such agents through the agency of infected solid and/or liquid manures from restricted farms, as well as from meat plants and market-places, should be included in such measures. The extent to which manures constitute a hazard in each case for animal and/or human health may vary from disease to disease, and may or may not be accurately known. Likewise, the extent of the risk involved in the handling, storage, landspreading and wider utilization of animal manures from controlled holdings, will vary according to, *inter alia* the agent involved, the numbers present, and the manner in which the population at risk may become exposed. Also for consideration are the susceptibility of the target host and the animal related factors which further determine whether or not the animals or persons thus exposed may become infected and, later, diseased.

The hazards such diseases may pose for animal and human health in the context of animal manure management and utilization on controlled farms and other restricted holding, under current production conditions are briefly described. It includes:

- notifiable animal diseases within the European Union;
- animal manure management aspects with regards to the pattern of animal production and transport for landspreading;
- disinfection of infected animal manures on restricted holdings;
- public health aspects including meat plants workers: the matter has been given little attention in various EC Directives which are directly relevant to animal health within the EC, viz. the EC recommendation of 24 July 1989 concerning the rules to be followed for inspection to be carried out in fresh meat establishments approved for the purpose of intra-Community trade (89/214/EEC); the Council Directive of 5 April 1985 on health and animal health problems affecting intra-Community trade in heat treated milk (85/397/EEC) and the Council Directive of 27 November 1990 laying down the veterinary rules for the disposal and processing of animal waste, for the placing on the market and the prevention of pathogens in feedstuffs of animal and fish origin (90/667/EEC).

It is concluded that the co-operation of the animal production industry in the matter of the prevention and control of the animal diseases which are notifiable within the EU for human and/or animal health reasons will be forthcoming in the matter of the control of animal manures and restricted holdings only on the basis that the regulatory measures introduced are scientifically based, economically justified and relate to the control and prevention of a real rather than perceived risk. The identification of the true hazard which infected manures on such holdings requires to be undertaken in the case of each of the diseases currently notifiable or likely to be made notifiable within the EU as a matter of priority.

Agro-industry - Slaughterhouse offal

The disposal of residues from commercial slaughtering must be considered from various points of view: interests of consumers, of the meat processing industry, of the feed industry and producers of organic fertilizers, of other rendering industries, interests of environmental protection, of public health and of veterinary services. In this field are the practices of collection, storage and processing as well as relevant legal regulations. Furthermore, the kind, composition and amount of slaughter offal and by-products vary depending on region and season.

The residues of slaughtering have special properties which have to be observed during handling. They either consist totally or partially of highly perishable material rich in protein or during slaughtering were mixed with or contaminated by such material. This results in a considerable development of odors caused by microbial degradation which is the stronger the higher the storage temperature is. Furthermore, the fact that a high percentage of clinically healthy animals may carry pathogenic agents in their digestive tract has to be reckoned with. These pathogens, as a rule, reach the collected residues via fecal contamination and find there ideal conditions for multiplication. For reasons of both environmental protection and public health, therefore, storage of residues and wastes of commercial slaughtering has to be carried out at temperatures which prevent the growth of putrefactive bacteria and pathogenic microorganisms. Also, other proven conservation technologies may be utilized and rapid processing must be achieved.

In many slaughterhouses much offal is channelled into the sewerage system or in some regions still, usually illegally, washed down into receiving waters, which are heavily contaminated by this practice. Also, the municipal sewage treatment plants are thus increasingly loaded with unwanted organic solids and the raw sludge of the sedimentation tanks is additionally enriched with saprophytic and pathogenic agents. Therefore, these sludges are considered to be infectious from a hygienic point of view and must be treated with consideration for their relevance to public health.

In most countries legislation for waste disposal, disposal of dead animals and of slaughterhouse offal (= animal rendering) exists. The Council Directive 90/667/EEC of 27 November 1990, which is addressed to the Member States distinguishes between „high-risk material“ which must be processed in a high-risk processing plant with a sterilization temperature of 133 °C at 3 bars pressure for 20 minutes. „Low-risk Material“ does not present serious health risks of spreading communicable diseases to animals or man. The amounts of slaughter offal and by-products differ from country to country, depending on the kind and number of slaughtered animals as well as regional and local practices. The primary offals and by-products are utilized in animal rendering plants and processing industries for technical products, animal feed, organic fertilizers, and food. The secondary offals reclaimed from wastewater can only be utilized for technical purposes, disposed of as waste (e.g. sanitary landfill), or under special conditions, also in animal rendering plants.

Collection, storage and, in some cases, processing cause environmental pollution by malodours, organic and inorganic contents of sewage, insect and rodent pests as well as

pathogenic agents, which, as a rule, all comprise hygienic risks. From an epidemiological point of view, the storage and utilization of fecal matter such as paunch contents and intestine contents are very often unsatisfactory. Before utilization in agriculture these materials should be sanitized to interrupt infection chains and to protect surface and ground waters from microbial pollution. Since the microbial pollution of receiving waters by wastewater effluents is at present the focus of public attention, the disinfection of such effluents is seriously discussed and it can be assumed that sooner or later their disinfection will be demanded to remove the microbial load from public waters.

Agro-industry - Animal Rendering

At all stages of animal production, including slaughtering and meat processing, several kinds of materials are obtained as residues, by-products of wastes, e.g. cadavers of animals, slaughter offal, parts of carcasses, hatchery waste, processed meat, milk and egg products condemned by veterinary inspection. These residues present a great veterinary public health problem because they are usually contaminated with all kinds of microbes, in many cases pathogenic ones, for animals as well as for humans. Therefore, these materials have to be collected and sanitized properly to protect human and animal health as well as the environment from contamination. The utilization of such material without a preceding treatment is forbidden in most countries.

Rendering of animal residues, their further processing and marketing are all connected with a host of hygienic problems. It is essential to refer to a publication which was initiated by the Department of Veterinary Public Health in the Division of Communicable Diseases of the World Health Organisation, Geneva: Guidelines on the hygienic disposal and rendering of dead animals and animal wastes to protect human and animal health. This publication contains contributions of experts from five European countries and the USA and reflects the state of knowledge in this field until 1985. After that the Commission of the European Communities enacted the Directive (90/667/EEC) which regulates veterinary legislation in the Member States for the disposal, processing and marketing of animal residues and for the protection of feed of animal origin, also from fish, against pathogenic agents. This directive covers handling and treatment of high-risk and low-risk materials, control of rendering plants and processing plants for by-products. In two annexes, special hygienic regulations for collection and transport of animal residues, for the authorization of rendering and processing plants, for their operation and their products are described (see also the previous chapter).

These regulations came into being after long and difficult negotiations and they are a victory for common sense and scientific rigor over commercial interests.

Identification of gaps in scientific knowledge

a) There is a need for further research on the survival of viral pathogens in slurries and the epidemiological importance of viruses in animal wastes.

b) Experiments on pathogens survival should be carried out under field conditions rather than in the laboratory, so that account can be taken of the activities of soil fauna and wildlife upon the rates of decay of pathogens. Levels of contamination of pasture must be determined.

c) More experience is needed on the problems of disinfection of large volumes of animal slurries and on ways of ensuring sufficient mixing of the disinfectant with the slurry; evaluation of methods for the disinfection of slurry, without decreasing its value as a fertilizer, must be made.

d) There is now a pressing need for a re-appraisal of the scientific data relating to the epi-demiology of the diseases caused by various protozoan and metazoan parasitic agents which are **infectious to animals and associated with human diseases** so giving rise to considerable illness-related costs. Such an appraisal should be made in the context of animal manure management and utilization practices, the survival rates of the agents in animal manures and the effectiveness of existing control measure, including heat and chemical treatments.

   On such information could then be based a rational approach to the management of manures associated with infected animal populations, in anticipation of the introduction of regulatory control measures at a later date.

   If is proposed that, as part of this approach, a critical evaluation of published data relating to the survival and transmission of infective agents during storage, and at/or following landspreading or transportation, which give rise to diseases in animals and/or man which are notifiable, or likely to become notifiable within the EU, be undertaken as a matter of priority, in order to quantify the relative risks involved.

   The need for such an appraisal of the relative importance of animal manures as a source of such agents has assumed a certain urgency in view of the extent to which the profitability, if not the viability, of animal production enterprises may now be jeopardised through a lack of essential measures for disease control when dealing with animal manures.

   An important unsolved problem in this context is the use of common slurry storage tanks which are supplied by more than one farm. This mixture of slurries from different livestock

such as cattle, pigs, poultry, may contain various pathogens which are excreted during the incubation period of an infectious disease, while the animals still appear to be clinically healthy. Since the farmer does not yet know that his animals are excreting pathogenic microorganisms into the slurry, infected slurry continues to be put into the common storage tank, thus contaminating the whole contents. This may result in a serious hazard for all the connected farms and, after spreading of this slurry on the fields, also for all other farms in the surroundings. Therefore, rapid and intensive research to evaluate the epidemiological importance of that storage method is urgently needed. Also because the collection of slurries in common facilities is favoured and subsidised by several EU member states.

e) Survival of pathogens during storage of manure and slurry needs further research to evaluate the process of "self-disinfection" during storage and possibly to use it as a type of disinfection method to save chemical disinfectants and thus to protect the environment.

f) Further research is also needed into the disinfection of manure and slurry by biological-technical and chemical methods, as well as of meso- and thermophilic anaerobic digestion of slurry alone or in co-fermentation with food scraps from restaurants, contents of grease traps, municipal biowastes etc.

The Commission of the European Communities held a European Conference on „Environment, Agriculture and Stock Farming in Europe“, which took place in Mantova/Italy in 1991. The main objective of the conference was to identify problems arising from activities related to livestock farming and their impact on the environment on the basis of existing scientific knowledge. This paper is based on the report of the Working Group No. 4 „Related Hygienic Aspects“ which has been completely published by D. Strauch and G. Ballarini (see „Literature“).

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Hygienic problems in anaerobic fermentation of slurry together with different industrial and municipal wastes in rural aereas (Co-fermentation)

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Summary

In order to safely destroy pathogens which may be existing in liquid manure or food scraps (e.g. salmonella) by anaerobic treatment, it is necessary that the mesophilic fermentation is preceeded by a pasteurisation of the feeding substrates. A temperature of 70° C for one is considered to be sufficient.

By means of thermophilic laboratory and large scale fermenters it was possible to prove that, due to temperature effects of approx. 55° C, salmonellas, *E. coli* and fecal streptococci were reduced by 4-5 logs minimum, down to their detection limit, within only a few hours up to 1 day maximum (Fig 1; laboratory fermenters).

Fecal streptococci proved to be particulary resistent to heat. While during the lab test they were reduced by approx. 3-4 logs after 2 hours, a reduction of only approx. 1 log could be achieved after 4 hours in the full-scale biogas plant.

Comparing the results achieved by germ carrier tests in large scale plants to the stipulations of the Danish authorities for the sanitary veterinary supervision of biogas plants (Bendixen and Ammendrup, 1992), which demand a reduction of fecal streptococci by three to four logs and a maximum content of fecal streptococci in the discharge substrate of 100 cfu/g , it was shown that the thermophilic biogas plant examined can meet these requirements in practice.

Key words: Anaerobic fermentation, tenacity of salmonellas, animal slurries, industrial and municipal wastes, co-fermentation

Introduction

A new Waste Disposal Law in Germany demands the responsible recycling of organic waste including nutrients into the agricultural circulation of material and the utilization of the energy potential available in the waste at the same time. An adequate possibility is given by the so-called co-fermentation, i.e. the combined fermentation (anaerobic treatment) of liquid manure together with biogenic waste by producing biogas.

Unfortunately, wastes-particularly food scraps-, do have a great pathogenic significance as far as viruses causing epidemic animal diseases like e. g. European and African Swine Fever; Aujeszky's Disease; Foot-and-Mouth Disease are concerned (Ahl 1994, Becker 1987, Blaha 1988, Röhrer et al. 1980).

The anaerobic plants therefore must be capable to safely destroy all relevant pathogens causing animal diseases and zoonoses, either by the microbicidal process as such or by means of installed ahead of or after the anaerobic process.

The results of bacteriological investigations with salmonellas, streptococci an *E. coli* in bench-scale and large-scale biogas plants are described.

Description of the Anaerobic Plants

Bench-scale Plants

The laboratory unit consists of 12 horizontal fermenters with a able volume of 16 liters each. The plants were continuosly operated by adding substrate only once a day. The hygienic

and bacteriological examinations were run with several mixtures consisting of cattle manure and food scraps. One variant consisted of pure cattle manure, whereas the other variants consisted of 25 % and 50 % food scraps. Six fermenters each were operated under mesophilic (35° C) and thermophilic (55° C) conditions. For a precise description of the experimental arrangement and further technical details of the set-up see Grunwald and Kraschinski (1995).

Large-scale Plant

The plant is operated in a single-stage process under thermophilic (55° C) conditions. The food scraps supplied from several canteen kitchens are ground in a mill. Obstructive material is removed mechanically, then the substrate is homogenized. In a ratio of 1:1, food scraps are added to the fermenter every 2, and liquid manure every 12 hours. Due to a specially designed agitator and baffle plates built into the bottom of the fermenter the substrates are mixed without causing a fast passage through the fermenter according to the manufacturer's statement (Bauer 1995).

Testing method in Anaerobic Plants

Bench-scale Plants

To test the reaction of *Salmonella schleißheim. E. coli* and *Streptococcus faecium* ATCC 6057 when fermenters are heavily loaded with these microorganisms, 10 ml of the corresponding germ suspensions were added once to the original substrate in a total of 6 fermenters (3 mesophilic and 3 thermophilic). After 2 and after 24 hours as well as after 3, 6 and 8 days tenacity tests were carried out by taking samples of the test germs that had been added.

Large-scale Plant

In that plant a direct contamination of the substrate with salmonellas was not possible due to the proximity of the plant to the pig stock of the farm and the large requirement of salmonella suspension. Therefore suspensions of 13,5 ml of slurry + 1,5 ml of S. senftenberg ($2{,}4 \times 10^7$ cfu/ml) and Str. faecium ($9{,}3 \times 10^7$ cfu/ml), respectively, were filled into germ-carriers, modified by Rapp (1995). These were fastened to rods of structural steel which ten were fixed in the fermenters through the outlet.

The test had been arranged for 3 parallel samples each and 4 sampling times (30, 60, 120 and 240 minutes).

Test results

In this context these will be only listed in a summary. For further details see Grunwald (1995) and Philipp (1996).

Bench-scale Plants

After experimental contamination of the fermenters with salmonellas, *E. coli* and *Streptococcus faecium* it turned out that in mesophilic fermenters the number of the microorganisms which had been added was continuously reduced. After 6 and 8 days no salmonellas were detectable.

The number of fecal streptococci (*Str. faecium*) however was only reduced by ca. 1-2 logs, with no considerable differences between the three substrate mixtures (100 % liquid manure, 75 % liquid manure/25 % food scraps, 50 % liquid manure/50 % food scraps).

In samples taken from thermophilic fermenters salmonellas and *E. coli* were not detectable after 2 hours. Fecal streptococci were reduced by 4 and 5 logs within 2 to 24 hours. By the 3rd day only a very small concentration detection limit could be found (Fig. 1).

Large-scale Plant

During tenacity tests of *S. senftenberg* on the practical plant it was found, that these were reduced by 5-6 powers of ten within 30 minutes. After 1 hour they were not longer detectable in any of the germ carrier samples.

The number of *Streptococci faecium* was reduced by about 1.5 powers of ten after 4 hours in the thermophilic plant.

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Fig. 1: Tenacity of salmonellas (sa; S. scheißheim) E. coli and fecal strepotococci (fc; Str. faecium) in mesophilic bench scale biogas plants (Grunwald 1995).

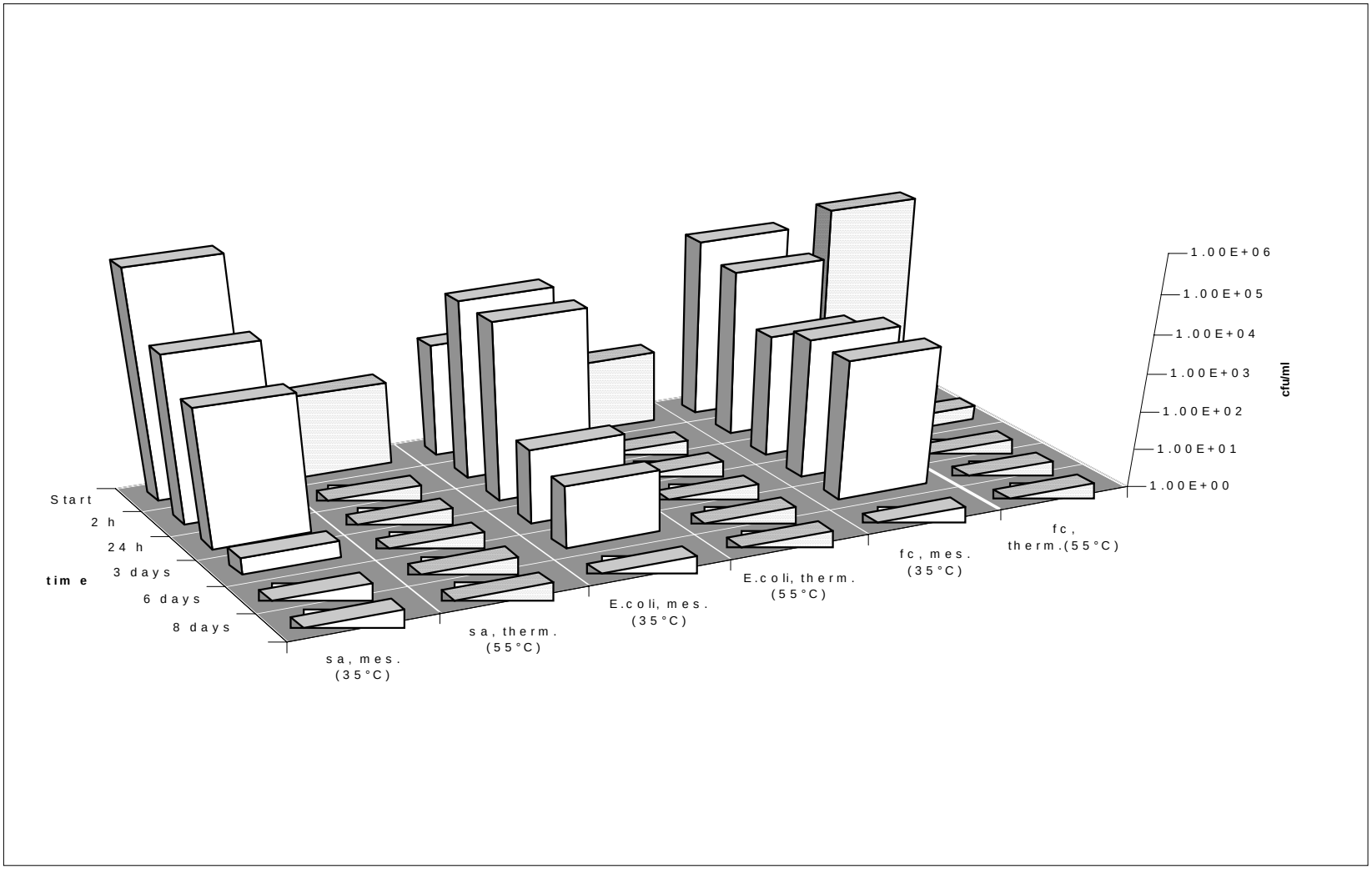

Fig. 1: Tenacity of salmonellas (sa; S. scheißheim) E. coli and fecal strepotococci (fc; Str. faecium) in mesophilic bench scale biogas plants (Grunwald 1995).

Treatment of pig feedlot excrement in Slavakia

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Summary

Bacteriological, helminthological and physical-chemical examinations were carried out to evaluate the technological procedure in a water treatment plant typical for Slovakia, processing pig slurry produced by a large-capacity pig farm. The results obtained revealed that the treatment meets the requirements placed on the effluent which is discharged into the recipient. However, the solid fraction is highly contaminated with bacteria and parasites (Ascaris suum, Trichuris sius, Isospora sp. Eimeria sp.) most of which exhibit high tenacity in the environment. Therefore this fraction should be composted before it is applied to land. The concentrations of heavy metals and other selected elements in sludge do not pose a risk when applied to agricultural land.

Key words: pig slurry, wastewater treatment, bacteria, helminths, heavy metals

Introduction

It is relatively difficult to assess the contribution of agriculture to environmental pollution however it is evident that it belongs to the biggest sources of pollution (Pokorný, 1994). The problems arising from the treatment and utilization of animal excrements have been more adequately dealt with only in the recent period. The improper handling of animal wastes may cause epidemiological problems because all the pathogens eliminated by animals housed in enclosed buildings end eventually in the manure (Strauch et al. 1980, Vasiľ 1992). Thus the manure and slurry produced by large production units must be considered as infected and strict hygienic measures must be taken at its handling and utilization (Ondrašovič, 1996). Some problems concerning the hygienic utilization and disposal of pig slurry may be solved by biological treatment of this waste in a mechanical - chemical - biological water treatment plant.

Material and methods

Investigations were carried out in a mechanical - chemical - biological wastewater treatment plant typical for Slovakia, processing slurry produced by a feedlot for 20 000 fattening pigs. In this plant slurry is subjected to mechanical pretreatment on vibrating screens and the collected solid fraction is applied to the land after some period of storage on heaps. Lime milk and ferric chloride is added in the chemical stage.

The aerobic biological treatment is ensured by activated sludge. The excess secondary sludge is thickened by means of a belt press and used for manuring. Effluent from this water treatment is discharged into a river.

Our investigations included microbiological parasitological and physical-chemical examination. Microbiological examination consisted of determination of plate counts of psychrophilic, mesophilic, coliform and fecal coliform microorganisms using solid cultivation media (MPA and Endo agar) and the procedure according to the valid standard (ČSN 83 0531). Special selective media (Christensen agar, XLD agar and SS agar) were used to detect the presence of Salmonella organisms.

Endoparasite eggs and oocysts were determined in individual stages of the treatment according to the method of Čerepanov (1982).

Physical-chemical examination of wastewater from the pig farm mentioned was carried out according to ČSN 83 0530 and ČSN 83 0540. Analyses were carried out on homogenized samples.The content of heavy metals was determined by the AAS method.

Results and discussion

The results obtained are shown in tables No. 1 - 6

Tab. 1.: Water treatment plant in Košická Polianka - microbiological examination of wastewater liquid and solid fraction (in 100 ml or 10g)

	Mesophilic	Psychrophilic	Coliform	Fecal coliform
Influent	4,86E+9 (1,2E+8 - 7,9E+9)	3,79E+9 (1,2E+10 - 2,0E+8)	8,63E+7 (2,7E+6 - 3,2E+8)	2,59E+7 (9,4E+6 - 8,3E+7)
Effluent	2,36E+7 (5,6E+4 - 8,6E+7)	1,6E+7 (1,0E+5 - 4,9E+7)	4,1E+5 (1,4E+5 - 1,5E+6)	2,8E+5 (2,8E+5 - 8,4E+5)
Effectiveness in %	99,51	99,58	99,52	98,92
Solid fraction	5,0E+8 (1,0E+8 - 1,9E+9)	5,8E+7 (7,4E+7 - 1,6E+8)	4,2E+7 (1,6E+6 - 1,6E+8)	1,9E+5 (1,2E+3 - 2,4E+6)

Tab. 2.: Water treatment plant in Košická Polianka - parasitological examination (number in 1000 ml or 100g)

	A. suum	Oesophago-stomum sp.	Trichuris sp.	Hymenolepis sp.	Isospora sp.	Eimeria sp.
Influent	28 -89	5 -19	1 - 3	0 - 5	0 - 9	6 - 34
Effluent	0,00	0,00	0,00	0,00	0,00	0,00
Solid fraction	12 -35	2 - 6	1 - 2	0,00	0,00	2 - 12

Tab. 3.: Water treatment plant in Košická Polianka - Physico - chemical analysis

	pH	DS	SS	$N\text{-}NH_4$	BOD_5	COD_{Cr}	N_{tot}	P_{tot}	Dry matter %
Influent	7,53 (6,81-7,98)	5165 (3345-8049)	3314 (280-9880)	848 (605-1139)	3656 (1930-5101)	13536 (6700-28200)	1320 (682-1854)	235 (34,5-601)	0,85 (0,49-1,79)
Solid fraction	8,00 (7,73-8,52)	2513 (2141-3112)	47,91 (12,9-136)	199,8 (42-582,7)	87,8 (24,7-167)	810,8 (266 - 2340)	241 (28-777)	27,3 (21-33,3)	0,25 (0,21-0,33)
Effectiveness in %			98,50	76,40	97,60	94,01	81,74	88,37	

Tab. 4.: Heavy metals - influent mg/l wastewater

Cd	Pb	Hg	Cu	Co	Mn	Zn
0,27 (0,02-1,02)	7,05 (0,04-27,6)	0,001 (<1-0,002)	2,895 (1,91-3,42)	0,132 (0,03-0,19)	4,253 (3,69-4,93)	11,63 (0,32-26,92)

Ca	K	Na	Mg	Fe	Dry matter %
335,79 (290,8-421,1)	391,04 (212,3-501,2)	146,78 (125,2-162,3)	154,02 (111,9-189,2)	53,66 (15,39-91,92)	1,019 (0,96-1,34)

Tab. 5.: Heavy metals - effluent mg/l wastewater

Cd	Pb	Hg	Cu	Co	Mn	Zn
0,005 (0,004-0,008)	0,22 (0,02-0,45)	0,002 (0,001-0,002)	0,168 (0,08-0,35)	0,063 (0,03-0,12)	0,113 (0,04-0,22)	0,633 (0,18-1,19)

Ca	K	Na	Mg	Fe	Dry matter %
91,7 (50-161,4)	282 (195,8-409,2)	120,09 (93,84-135)	45,35 (16,54-55,23)	10,24 (0,61-26,15)	0,199 (0,015-0,39)

Tab. 6.: Heavy metals - solid fraction mg/kg

Cd	Pb	Hg	Cu	Co	Mn	Zn
0,156 (0,09-0,344)	1,81 (0,48-4,13)	0,002 (0,001-0,002)	38,14 (7,32-88,79)	2,22 (0,38-5,5)	66,98 (17,8-152,1)	278,11 (20,53-761)

Ca	K	Na	Mg	Fe	Dry matter %
4960,4 (2132-9630)	1 674,82 (509,9-3950)	454,44 (239,57-860)	931,43 (311-1960)	1057,16 (776,9-1377)	34,12 (13,13-71,48)

The results of physical-chemical examination of influent and effluent show good effectiveness of removal with regard to BOD_5 (97,6%) and COD_{Cr} (94,1%) and 98.5% reduction of suspended solids (SS). The removal of N_{tot}, P_{tot} and $N\text{-}NH_4$ was low ,however it corresponds to the effectiveness of the system used.

The nubers of mesophilic, psychrophilic, coliform and fecal coliform bacteria decreased by about two orders of magnitude and Salmonella organisms were not detected. The numbers of microorganisms in the solid fraction were only slightly decreased in comparison with the slurry.

Parasitological examinations revealed that neither helminth eggs nor coccidia oocysts were present in the effluent, however the solid fraction contained vital propagative stages (eggs, oocysts) of endoparasites.

It can be concluded, that from the veterinary - hygiene point of view the solid fraction of slurry poses a risk, mainly when it is applied to land without further treatment. in the environment, e.g. in the form of organic manure.

The content of heavy metals and selected elements in this fraction showed that Cd and Pb values were higher in some samples. This requires additional monitoring. As stated previously (Juriš et al., 1991) water treatment plants processing slurry from large-capacity pig feedlots should be supplemented with an effective equipment for the treatment of the solid fraction obtained by mechanical pretreatment on vibration screen separators, gravity screens, or belt presses. The most suitable method of processing of this fraction is composting by biothermic processes in the thermophilic region (Jonáš and Petříková 1988, Plachý and Juriš, 1993, Novák et al., 1994). The final product of this process is a high quality organic manure.

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On the veterinary-hygienic assement of poultry manure in the light of the further usse in agriculture

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Summary

Our investigations aimed at determining the composition of layer faeces from different kinds of rearing with bacteriological and physicochemical methods and at assessing the results from a veterinary-hygienic point of view. The comparison shows that ventilated faeces from conveyor belts with significantly higher values of the autochthonous faecal flora is least suited from an epidemiological point of view. Salmonellae occurred very frequently both in fresh faeces (in 76,3 % of all samples) and in ventilated faeces from conveyor belts (in 83,9 % of all samples) whereas they were detected in only 1,9 % of all samples from floor management in the same poultry rearing station. There are no objections to the direct, immediate use of faeces from floor management as secondary raw fertilizers from an epidemiological-hygienic point of view. Ventilated faeces, however, should not be directly, immediately used for hygienic reasons. After air-drying in henhouses, the faeces should be stored / composted. It is sufficient to compost them for at least 12 weeks in the open air without shifting the clamps.

Key words: laying hens, autochthonous faecal flora, risk of infection, indicators, salmonellae, clamp composting

Introction

During the last years, the change to procedures with dry faeces has been intensively advertized in poultry rearing in the Federal Republic of Germany. The ventilation of faeces in henhouses reduces stench emission, improves the general climate in the henhouses, the air-dried faeces have a better nutrient content, they need not be stored for a long time and can be spread mechanically (among others Fuhrken, 1988, Roesicke, 1991; Rüppich, 1991). Since the excrements from large poultry rearing stations must be generally regarded as infected (Strauch and Phillipp, 1992), germ-containing layer faeces must be assumed to be a health risk to humans and animals. Therefore, procedures for treating poultry faeces should contain steps which inactivate germs reliably.

Our investigations aimed at determining the composition of layer faeces from different kinds by means of bacteriological and physicochemical methods and at assessing the results from a veterinary-hygienic point of view.

Material and methods

The samples of fresh layer faeces and of those from conveyor belts were collected at a poultry rearing station with 70.000 laying hens kept in 3-storeyed cages and dungcleaning out of the conveyor belts. The faeces were dried in the henhouses with a Big Dutchman Ventilation System during 7 days. The fresh faeces were collected within 2 hours after defaecation. Samples of the total heap of faeces from the intensive floor management of laying hens (with boxes for faeces and littered space for scratching) at the same station were investigated during two rearing periods (from September 1992 to October 1993 and from February 1994 to February 1995). Two trapeziform clamps (3,8 m long; 1,7 m wide; 0,5 m high) of layer faeces from the conveyor belts of the same station were built in the open air between June and September 1994 an another two between January and April 1995. 3 measuring points were both on the edge and in the centre of each clamp. The temperature was continuously measured at these points. Furthermore, 9 little gauze sacs with 500 g layer faeces from the conveyor belts of the same station were laid into the

clamps. They were mixed in a 1: 100 ratio with a test germ suspension of S. Enteritidis resistant to nalidixic acid. The dry substance, the pH value as well as the germ groups, the aerobic total bacterial count, Enterobacteriaceae, E.coli, faecal streptococci and salmonellae (native and test salmonellae) were investigated in the test germ and control samples on days O, 4, 7, 11, 14, 18, 25, 32, 46 and 88. The quantitative determination of the germ groups of the faecal flora was based on the modified method for the determination of the intestinal and faecal flora elaborated in Potsdam-Rehbrücke (Haenel, 1960; Haenel and Müller-Benthow, 1965; Pioch and Spieler, 1993; Spieler, 1995).

Results

The composition of fresh layer faeces and of those from conveyor belts as well as of faeces from floor management are presented in table 1. The faeces from conveyor belts contained higher concentrations of the autochthonous faecal flora and of salmonellae than the faeces from floor management. The comparison of fresh layer faeces and those from conveyor belts shows that, apart from the frequency of detecting salmonellae, they differ significantly in all other investigated parameters. The number of all investigated germ groups decreased during composting. At the end of the experment, E. cold and salmonellae were no longer detectable, neither in the summer nor in the winter clamp.

Conclusion

From a hygienic-epidemiological point of view, there are no objections to the direct, immediate use of faeces from floor management in agriculture. Ventilated faeces, however, should not be directly, immediately used for hygienic reasons.

Since salmonellae and E. cold were no longer detectable and since the number of other autochthonous germs was considerably reduced after composting in summer and in winter, we recommend from an epidemiological-hygienic point of view to only prepare faeces from conveyor belts in a 2-stage system. The air-drying of faeces in henhouses should be followed by an at least 12-week period of composting in the open air,before the faeces are used in agriculture.

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Composition of fresh layer faeces, of faeces from conveyor belts and of faeces from floor management

parameter variant of faeces	dry matter content %	pH value	total bacterial count	endo-germs	coliforme germs	faecal strepto-cocci	fre-quency of de-tecting salmo-nellae %
			Ig CFU/g				
fresh faeces	28,46	6,90	9,17	7,22	7,11	6,45	76,9
faeces from conveyor belts	51,62	7,89	10,11	9,18	9,00	8,40	83,9
faeces from floor manage ment	58,34	9,27	8,39	6,97	6,81	5,79	1,96

A management model for pathogen abatement in animal slurry in view of its agronomic use

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Summary

An important concern connected with the agronomic use of animal slurry is related to hygiene – both animal and human health and it is of paramount importance to adopt techniques and operational criteria that minimise the potential risk of disease transmission. Only information related to the survival of micro-organisms during individual phases of slurry handling (storing, aerobic or anaerobic treamtment, *etc*.) is available.

For this reason, we developed and evaluated a model in which the influence of the slurry management system on pathogen survival is considered (*Salmonella dublin* and *S. typhimurum*).

By using the model, it is possible to evaluate, taking into account the variability, the consequences of different management options. For instance, we determined that for cattle slurry with a total solids concentration of 9%, the storage period required to eliminate *S. dublin* is approximately 150 days, but decreases to 120 days if solid-liquid separation is utilised. Although our preliminary results are very encouraging, the model needs to consider additional information on the behaviour of different micro-organisms and to be calibrated to specific conditions under which it can be utilised.

Key words: manure handling, pathogen control, management models

Introduction

Because of increased societal interest in the relationship between agriculture and the environment, there is an ongoing evolution of practical concerns related to the agronomic use of animal manure. From one perspective, there is a growing demand for new research and scientifically-based advice that is able to concretely guide farmers and agricultural advisors into their manure management choices. From another perspective, lawmakers seek reliable information to help them formulate realistic rules and legislation that can minimise the impact of agriculture on the environment.

One aspect receiving particular attention in this context is the control of hygienic conditions during the management of animal manure.

Currently, information on the behaviour of micro-organisms in the various management stages (storage, aerobic or anaerobic treatment, *etc*.) is available. However, comparable information does not exist from a systems perspective. Therefore, we made an attempt to gather and link experimental data into a system representation. This led to the definition of several models able to identify the main factors that can be modified to control the hygienic aspects of farm manure management.

Material and Methods

We first devised a general model describing the possible stages of manure from the animal to the field, identifying where changes in the bacterial characteristics of the manure are possible. Secondly, we analysed the results obtained in various experiments on micro-organism survival in manure, especially the effect of various treatments on bacterial counts. These data were organised to clearly identify in each study the test conditions and the factors influencing survival time of the micro-organisms. Specifically, we highlighted the initial concentrations of micro-organisms, temperature, total solids content, and pH of the manure. We utilised linear regression analysis in which pathogen survival time was the dependent variable and each survival factor was the independent variable to identify statistically ($P<0.05$) significant relationships. These relationships were then inserted into the management models to facilitate application in practical situations.

Results and Discussion

Our management models are based on the analysis of the different stages of animal manure handling. Within each stage, the initial conditions (*e.g.* the concentration of the micro-organisms) and the values for factors influencing survival time or affecting pathogen concentration in manure (*e.g.* temperature, dry matter content, pH, duration of stage of handling) can be identified. In general terms, the models define an input-output function (IOF) linking the initial conditions and the survival factors in order to evaluate the final conditions.

The final conditions of one stage of handling represents the initial conditions of the subsequent stage. By introducing the IOF's to describe pathogen survival at various stages of manure handling, it is possible to evaluate the hygienic status of the manure when it has to be agronomically utilised, provided that the starting conditions (that is, the infectious state of the herd and the manure handling techniques used) are known.

To illustrate the development and use of our models we give an example based on *S. dublin* and cattle slurry.

During storage, our analysis revealed that the dry matter content had the highest correlation with survival time of the bacterium, in agreement with findings reported by several researchers. The slope of the regression line illustrates that as slurry dry matter content increases, so does the survival time of *S. dublin* (**Figure 1**). On the basis of this relationship, it can be noted, for example, that in diluted slurries (dry matter contents of 1%-2%), the survival time of *S. dublin* during storage is 70-80 days, while in thicker slurries (6%-7% dry matter) survival time is on the order of 120 days.

Figure 1 also contains the regression lines obtained by grouping data into three temperature regimes that are representative of the different seasonal conditions in temperate climates. It can be noted that at higher dry matter contents, survival times during low (1-6 ^{o}C) and moderate (10 ^{o}C) temperatures tend to be similar to each other and to the general regression. Larger deviations are shown at low slurry dry matter contents and for higher temperatures (20-30 ^{o}C).

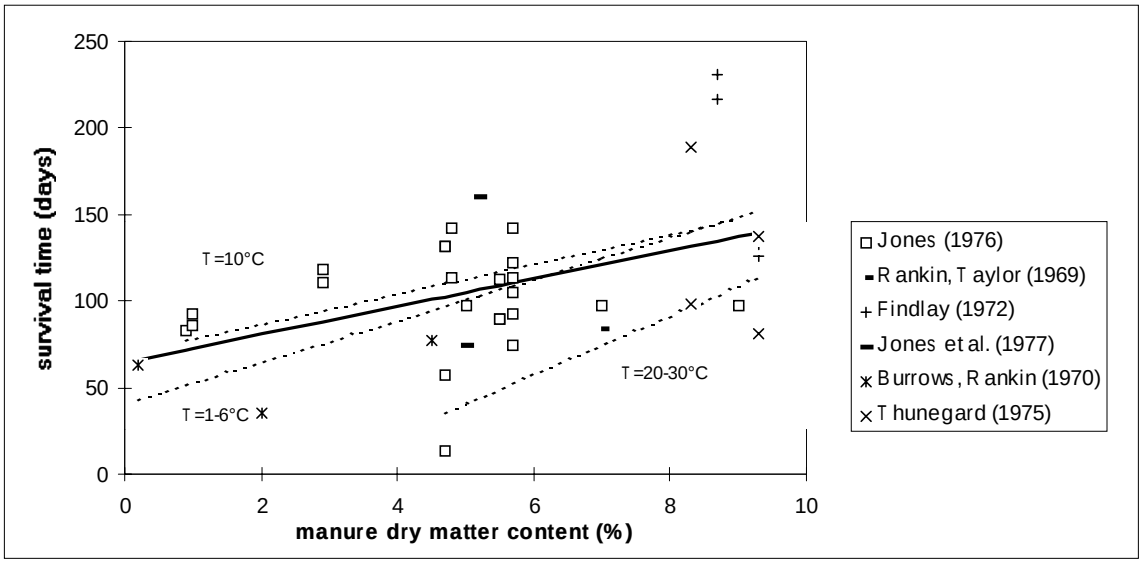

Figure 1 *- Relationship between survival time and manure dry matter content for Salmonella Dublin during storage.*

As an example of how to apply these results to practical situations, consider a manure handling system in which slurry, produced by cattle on slatted floors (dry matter content approximately 9%), flows directly into a storage tank. After storage the slurry is spread on arable land. The hygienic condition of the herd does not exhibit an obvious infection caused by *S. dublin* and does not require a chemical disinfecting treatment of the slurry. Using the relationships we developed, we would recommend that a slurry storage time of 150 days must be provided in order to reduce pathogen concentrations to acceptable levels in winter. However, by using a liquid-solid separator with an efficiency of 30%, the dry matter content could be decreased to 6% and, as a consequence, the storage time required to achieve an acceptable

hygienic standard could be decreased to 120 days. In the summertime, the higher ambient temperatures would halve this value.

These illustrations show clearly how the models we derived can help evaluate different management choices for managing manure according to hygienic considerations. Such choices can have significant economic effects on investment and running costs. Obviously, it would be necessary also to check the compatibility of individual management decisions with the agronomic requirements on slurry spreading. Politicians or others charged with environmental protection could use these results either to develop slurry management criteria, as well as a basis for proposing financial incentives to facilitate adoption of the criteria by farmers.

Conclusions

Despite these encouraging preliminary results, widespread implementation of the proposed management models requires further investigation to incorporate the behaviour of additional micro-organisms and to calibrate the models to the specific conditions in which they would be utilised. An extension of the models to include different kinds of pathogens (*e.g.* viruses, parasites, and mycetes) is also needed.

The inclusion of additional micro-organisms must be done in comparison with the results obtained in this study in order to find management solutions able to give an overall reduction in the pathogenic load, rather than address only a specific agent. To go further in this direction, it is also necessary to consider both the risk (damage in economic terms) posed by a specific agent, and the frequency of infection events in a specific area. By following such an approach, it will be possible to take into real consideration the hygienic problems associated with animal manure management, to evaluate the possible on-site and off-site dangers resulting from the use of this material, and to devise practical methods with which to keep risks within acceptable limits.

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Bacterial microflora in piggery

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Summary

In this study, the level of natural microbiological pollution was evaluated for fattening house with more than 500 piglets in course of their fattening. The bacteriological tests were made on samples taken from the air, drinking water, feed, from the surface of walls and floor, from droppings and from the surface of the skin on the back and belly of 21 piglets. The experiment was made during the 7-months period, since August 1996 until February 1997, and 112 samples were taken for analysis every month. The prepared material was inoculated on: agar, agar with blood and on Mac Conkey's substrate while identifying the matured colonies with the use of the following microtests ID 32 Strep., ID 32 Staph. and ID 32 E.

The flora of investigated habitat was characterized with high number of microbes, which ranged from 13 000 cells in 1 cu.cm of air up to 1,91 billion colonies in 1 gram of droppings, and at the same time 27 isolates were obtained, which belonged to 10 genera of bacteria.

Key words: fattening house microflora, piglets

Introduction

The environment of livestock compartments is conductive to higher survivability of microbes, especially when sanitary-hygienic conditions are deteriorated (**Norpoth**, **Peterson** 1990, **Strauch**, **Ballarini** 1994, **Kluczek** 1996). The aim of this research was to evaluate the biocenosis of the habitat designed for piglets in course of their fattening.

Materials and methods

The research was performed in the fattening house with more than 500 piglets at the age between 6 weeks and 3 months, which were fed according to Polish standard and kept on a bedding-less concrete floor. The samples for qualitative and quantitative bacteriological investigations were taken from the air, drinking water (automatic drinkers), feed, from the surface of walls, floor, from the droppings and from the skin on the back and belly of 21 piglets. The research period lasted for 7 months, since August 1996 until February 1997, where the above mentioned samples were analyzed every month (112 samples every month). Agar, agar with

blood and Mac Conkey's substrate were used, while individual species of bacteria were identified using microtests ID 32 Strep., ID 32 Staph. and ID 32 E, and the result were read with ATB microbiological analyzer.

Results

As it appears from table 1, the number of microbes in livestock compartment depended on material and the place where the sample was taken. With the use of a tampon method, 3,45 x 10^6 to 6,83 x 10^7 microbe cells were found on 1 sq.cm of the utility surface (walls), and 6,68 x 10^7 to 5,57 x 10^9 colonies on 1 sq.cm of the floor, i.e. depending on the type of substrate. The tampon method used to identify the natural pollution with bacteria on the skin of animals gave the following results: 6,65 - 10,13 x 10^6 microbe colonies on the back compared with 3,78 - 9,34 x 10^7 cells on the belly, both per sq.cm. Many microbe cells were also detected in the droppings (range 8,48 x 10^7 - 8,91 x 10^9 colonies in 1 g), and two or three order lower quantities of cells were obtained in the feed (3,56 - 9,82 x 10^4 colonies in 1 g) and in the air (1,30 - 8,10 x 10^4 cells in 1 cu.cm), while the lowest values were obtained in drinking water (5,50 x 10^2 - 9,98 x 10^4 colonies in 1 ml). Specification presented in table 1 also points out the differences in identified species of bacteria.

Discussion

The bacteriological results obtained in the habitat of piglets provide the confirmation of observations on ecological status of microbes, and they indicate for a stimulating influence of a breeding environment to the natural pollution with microbes. 37 isolates were obtained in course of microbiological analyses, and they belonged to 10 genera of bacteria. Distinct quantitative and qualitative variations of bacterial flora isolated from investigated locations in the breeding environment enhance the importance of bacteriological analyses inside the livestock compartments. Although, in most cases the bacteria of Enterobacteriaceae family are the steady element of microflora in the livestock compartment, more an more often it is said, that they are the essential etiological factor in infection of the animals (**Strauch, Ballarini** 1994). The presence of these microbes with high level of pollution in a breeding environment was found in the droppings and on the floor.

Conclusion

1. The appearance of microbes of distinctively diversified composition as well as their quantity in a livestock compartment can impose the real and potential threat to the health of animals.

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Table 1. Microbiological evaluation of piglets breeding environment

Table 1. Microbiological evaluation of piglets breeding environment

Specification	August	September	October	November	December	January	February
AIR in 1 m^3 6,53 x 10^4	Gemella haemolysans Vibrio metochnikovii Hafnia alvei	Escherichia coli K. ornithinolytica Aero.viridans Staph.capitis	Ent.gallinarum Aero.viridans Staph.xylosus,	Aero.viridans Leukonostoc spp. Staph.simulans	Erwinia nigrifluens Serratia plymuthica Staph. lentus Staph. xylosus	Erwinia nigrifluens Serratia plymuthica Ent.faecium	Staph.lentus, Staph.aureus
DRINKING WATER in 1 ml 8,32 x 10^3	Aerococcus viridans, Staph. aureus K. ornithinolytica	Serratia liquefaciens Aero. viridans Staph.cohnii	Ent. gallinarum Aero.viridans Staph.xylosus	Aero.viridans Staph.lentus	Acinetobacter spp Aero. salmonicida Str.sanguis Str.acidominimus Staph.lentus,	Pseudomonas cepacia Gemella morbillorum	Staph.xylosus
FEED in 1 g 6,84 x 10^4	Str. adjacens Staph. xylosus Hafnia alvei	Aero.viridans Staph. cohnii	Ent.casseliflavus Staph.caprae	Aero.viridans Staph.hominis Staph. caprae	Erwinia nigrifluens Erwinia spp. Aero. viridans Staph. lentus Staph. xylosus	Erwinia nigrifluens Erwinia spp. Str.acidominimus	Staph. xylosus
FLOOR in 1cm^2 6,95 x 10^8	Str. sanguis Staph. epidermidis	Ser.liquefaciens Aero.viridans Staph.epidermidis	Aero.viridans Staph.simulans Ent. faecium	Staph. lentus Ent.gallinarum Aero. viridans	E.coli Staph. lentus Staph. xylosus	E.coli Aero. viridans	Staph. xylosus
WALL in 1cm^2 5,55 x 10^6	Aero.viridans Staph. capitis Proteus mirabilis	Hafnia alvei Aero.viridans Staph.capitis	Ent. gallinarum Leuconostoc spp. Staph.lentus	Aero. viridans Leuconostoc spp Staph.xylosus	Ent.agglomerans S.paratyphi Staph. lentus Staph. xylosus Aero. viridans	Ent.agglomerans Erwinia nigrifluens Aero. viridans	Staph. xylosus
DROPPING in 1 g 7,16 x 10^8	Gemella morbillorum Staph. epidermidis	Enterobacter cloacae Str.acidominimus Micrococcus spp.	Ent.viridans Staph. chromogenes	Aero. viridans Staph. chromogenes Staph. aureus Staph. warneri	E. coli Staph. xylosus Staph. lentus	E. coli Staph. xylosus	
BACK in 1cm^2 8,28 x 10^6	Gemella haemolysans Staph. schleiferi Hafnia alvei	Hafnia alvei Aero.viridans Micrococcus spp.	Aero.viridans Staph. lentus	Staph. xylosus Str. sanguis Ent. gallinarum	Erwinia nigrifluens Erwinia spp. Ent. faecium Aero. viridans Staph. saprophyticus	E. coli Ent. faecalis	Staph. haemolyticus
BELLY in 1cm^2 7,15 x 10^7	Aero. viridans Micrococcus roseus Pasteurella spp	Hafnia alvei Aero.viridans Micrococcus spp.	Aero. viridans Staph. xylosus	Staph.xylosus Staph. lentus	Erwinia spp. E. coli Aero. viridans Ent. avium Staph. saprophyticus	Staph. haemolyticus E. coli	Ent. faecalis

DETECTION OF AIRBORNE VIRUSES IN SOLID WASTE TREATMENT PLANTS

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Summary:

Within a study to specify and quantify the microbiological air pollution at different positions and working places in composting facilities, investigations have been made to demonstrate and isolate airborne viruses. Two ”high volume” samplers, the ”special impinger” (Univ. Hohenheim) and the MD 8 (Fa. Sartorius, Germany), were used in parallel for these investigations and the samples were further treated comparatively in two different ways. In the course of the study, airborne human enteric viruses could be detected and isolated several times. Up to now, however, there is no scientifically supported base for a correct risk assessment of these findings. To achieve this aim, more epidemiological and methodological research has to be done.

Introduction:

In Germany approximately 30 million tons of municipal wastes are collected annually (OBERHOLZ, 1996). A growing part of this total amount falls to biogenic residues which are collected separatively as organic waste, so-called "Bioabfall", and led back to the biological circulation mostly by composting.

Apart from a great number of apathogenic microorganisms, also animal and human pathogenic germs can be found in organic wastes, depending on the input material (STRAUCH, 1996). When handling this material, especially when it is moved mechanically (e.g. delivery and tipping of collected organic waste in compost facilities, moving of composting stacks, etc.) these microorganisms can be transferred into the airborne state. These "bioaerosols" may imply a health risk for employees working in composting facilities.

In a cooperative research program supported by the German Federal Foundation of Environment the concentrations of airborne microorganisms at different locations in several composting plants were investigated. Special emphasis was put on the determination of the concentrations of airborne funghi and bacteria. It was also tried to detect and isolate airborne potentially human pathogenic viruses.

Materials and methods:

Sample collection: The samples were taken in summer 1996 in four different composting plants at different locations, over two days. Two volume samplers were used, the MD 8 (Sartorius, Göttingen, Germany) working according to the principle of air filtration, and the "Special" impinger (Imp.), a special construction of the Institute for Environmental and Animal Hygiene, University of Hohenheim.

Samples were collected for ten minutes in both cases, the MD 8 running with an air flow of 6 m^3/h and the Impinger with a pumping capacity of 180 l/min. The Impinger precipitated airborne particles in a collection fluid (1000 ml, 3% w/v BSA, 0,1% v/v olive oil, addition of antibiotics, in PBS, pH 7,5) and the MD 8 at gelatine filters (80 mm diameter), which were put into 10 ml of transport fluid (50% Dulbecco's MEM with 5% FCS and antibiotics, 50% PBS) immediately after collection. All samples were cooled at 4 °C until further treatment.

Sample preparation: From the impinger samples the larger particles were first separated by centrifugation (4200 g /30 min., 1st pellet). The supernatant was precipitated with 8 % PEG 20.000 and centrifugated again (2nd pellet). Both pellets were resuspended in TE-buffer (0,01 M Tris-HCl, 0,003 M EDTA, pH 7,4) and reunited. This suspension was divided as it was done with the gelatine filter samples which were completely solved in the transport fluid after a short warming up at 37 °C. An aliquot was set on a 0,1 % SDS concentration - according to WARD and ASHLEY (1976), the other was adjusted at a 3 % beef extract concentration, pH 9, according to WELLINGS et al. (1976). After treatment in an ultrasonic bath both preparations were centrifugated (4300 g / 30 min.). Supernatants were titrated on cell culture monolayers of Vero (ATCC CCL-1586), Buffalo Green Monkey kidney (ATCC CRL-1688) and A 549 (ATCC CCL-185) cells. At the occurrence of cytopathogenic effects the $TCID_{50}$ (tissue culture infectious dose) was determined according to the Spearman/Kaerber formula and converted to an air sample volume of 1 m^3.

All isolates were cleaned by the plaque technique, increased in cell culture, proved by chloroform treatment and sent to the Institute of Virology, Medical University of Hannover, for further and final classification.

Results:

In two of the composting plants no airborne viruses could be detected on both days of investigation.

In the third plant, a positive result occurred on one of the two days. Viruses could be isolated with all cell lines. At the location delivery a virus amount of 4,5 x 10^3 $TCID_{50}$ (MD8) resp. 1,8 x 10^3 bis 3,2 x 10^3 $TCID_{50}$ (Imp.) was isolated on Vero cells, of 5,8 x 10^3 $TCID_{50}$ (Imp.) on BGM cells and of 8,9 (MD8) resp. 5,2 $TCID_{50}$ (Imp.) with the cell line A 549.

At the location sorting belt 2,5 $TCID_{50}$ (MD8) of virus was isolated on Vero cells and 2,8 (MD8) resp. 4,7 bis 46 $TCID_{50}$ (Imp.) on BGM cells. At the fine sieving in the rotting hall 2,5 (MD8) resp. 1,8 $TCID_{50}$ (Imp.) were detected by Vero cells and 2,5 to 4,5 (MD8) resp. 5,7 x 10^2 $TCID_{50}$ (Imp.) by BGM cells. In the outlet air of the biofilter of this plant no virus was detected.

In the fourth plant, virus could be isolated on one of the two days at the location delivery, 5 to 88 $TCID_{50}$ (MD8) on Vero cells and 5 $TCID_{50}$ (MD8) on the cell line A 549. No viruses were detected at the locations sorting belt, rotting hall and biofilter outlet. However on the second day the location biofilter was the only one with positive result, 5 $TCID_{50}$ on BGM cells in a single probe (MD 8).

All isolates proved to be stable against chloroformic treatment (undeveloped). No Adenoviruses could be detected so far, all isolates belong to the group of human eneric viruses, proven by positive polymerase chain reaction (ADRIAN, 1997). Final classification is still expected.

Discussion:

There is a great number of references wich report investigations about the stability of different viruses in the airborne state by using artificial aerosols, but only few have been described to prove airborne viruses under field conditions.

In earlier investigations we could demonstrate different human enteric viruses in the air of different waste treatment plants mainly by using an aerosol sampler working according to the principle of electric precipitation (PFIRRMANN 1994). The sampler proved to be very suitable for this purpose, but its use is quite difficult under practical field conditions.

In this new study we worked with two high volume samplers which are much more easier to handle. One of them, the special impinger, had been used in the mentioned previous study too, but with it airborne virus could be detected in one single case only. For that reason the method of sample preparation seemed to have further developed to increase the sensitivity of the method. For that, we included some methodical work in sample preparations in this investigation to decrease the sensitivity limit.

We could show that both samplers are suitable for collecting airborne viruses under field conditions. Which of them is more suitable for this purpose cannot be assessed up to now as it is with the two sample preparation techniques we tried in comparison.

Maybe some more of the samples which gave negative results did contain viruses, but only in small amounts. The fact that in some of the positive samples only very few virus was detectable (in the range of 1 to 5 $TCID_{50}$, that means quite near to the detection limit) indicates that more work has to be done to further increase the sensitivity of the detection method.

According to the significance of the results in particular with regard to a potential health risk for the employees in composting plants great care should be taken:

First, the definite classification of the isolates has not yet been done. Therefore, it is not possible at this stage to assess them with regard to their virulence for humans mainly under the aspect of a potential neurotropism. The final classification is still pending.

Second, the real source of the virus aerosol shedding remains uncertain to some point. It should be mentioned that it could have spread by acute excretions of the working personal (PFIRRMANN and VANDEN BOSSCHE 1994), possibly by the investigator himself. Probably it was shed by fresh organic waste materials, because at all locations where airborne virus was detectable fresh organic wastes were moved mechanically to some extent in the nearer surroundings. This had been the case at the locations delivery and sorting belt, and at the location compost fine sieving in the rotting hall of the fourth plant where a nereby conveyor-belt transported the fresh waste to the new rotting stacks. At all locations where no fresh material was treated mechanically no airborne virus could be detected, with one single exception, the isolate from the location biofilter used air in the fourth plant. But as this biofilter is placed in the immediate neighbourhood of a wastewater treatment plant, it is more likely that this was the source rather than the used air of the compost biofilter.

Third, and this is the most important point: the amount of comparable data on occurrence and concentrations of airborne viruses for other indoor and outdoor environmental surroundings is too small to give the possibility for a correct risk assessment.

No epidemiological data or data from working medical studies exist which demonstrate the frequency of possible enteric virus-caused diseases of composting plant workers nor any comparable data referring to other groups of the normal population.

And this leads to the main conclusions: at some working places in composting plants airborne viruses are prevalent and detectable, and the probable source are fresh (maybe fecal contaminated) organic waste materials. Airborne viruses may be present in several different other live surroundings and working place situations (as it could be demonstrated for other locations in waste treatment plants before (PFIRRMANN 1994). More investigations have to be made and some more methodological work has to be done for efficient sampling and demonstration of airborne viruses to give a realistic and scientifically supported base for risk assessment of proved concentrations of airborne viruses.

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Migration of polio virus in soils fertilized with municipal wastes

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Summary

The aim of this research was to define the possibilities of polio enterovirus migration in two soil profiles , which were fertilized with municipal wastes. The physical-chemical analysis of soils was performed. The experimental plots were fertilized using the mixture of wastes and attenuated polio virus. After 28 days, the samples were taken for virology tests. Septic titers of the viruses were determinated according to the commonly used methodology on microplates. Low penetration of viruses into the soil profiles was found. The highest titers were observed in superficial layer (0 - 2,5 cm) - average 5,72 log $TCID_{50}$/g for podzol soil and 5,92 log $TCID_{50}$/g for browned chernozem soil. The polio virus didn't migrate beyond the humus layer, and it was detected up to the depth of 20 - 25 cm. No influence of soil type to the virus infiltration was found. The fluctuations of enterovirus titers, which appeared on individual depths can be a proof of the desorption processes, which take place.

Key words: municipal wastes, polio virus, migration, podzol and browned chernozem soil

Introduction

More than 100 types of viruses can be isolated from municipal wastes, while polio viruses and viruses of infectious hepatitis are found most often. Some part of viruses is not subject to inactivation in waste treatment plants. They can be transferred to the soil, where they become inactivated, and on the other hand they can be absorbed on soil particles. These viruses survive in soil for a longer time, and there is the possibility of migration towards the water-bearing layers [Vaughn, Landry 1983].

The aim of the research was to define the possibility of migration of polio enterovirus in differentiated profiles of soils, which are fertilized with municipal wastes.

Materials and methods

The research was performed on podzol and browned chernozem soils. In the first stage of experiment, the physical-chemical evaluation of selected soil types was made. Among others, the following parameters were determined: pH, content of cations exchange capacity and organic matter, distribution of soil pores size, granulometric composition, bulk density [Lityński et al. 1976]. The penetration of enterovirus into the soil profiles was observed on 2 experimental plots located on podzol and browned chernozem soils, respectively. The dimensions of the plots were

defined with dia. 23 cm metal tubes. The polio virus with titer of 1,5 x $10^{8,0}$ $TCID_{50}$/ml was used for experiments. 1306 ml of the mixture of virus and waste (1:1 ratio) was spread on the plots. The plots were sprinkled with water in weekly intervals using quantity, which corresponded to the average precipitations in that region. After 28 days the samples were taken in sterile spoons, in every 2,5 cm up to the depth of 15 cm, in every 5 cm up to the depth of 30 cm, and in every 10 cm from deeper layers. The samples of soil were frozen in 3 and 2 repetitions. After thawing, the viruses were eluted with 10 % fetus serum. The septic titers of viruses were determinated according to the commonly used methodology in cellular lines on microplates [WHO,EPI 1990,Vaughn, Landry 1983].

Results and discussion

Natural soil structure was maintained in field experiments, while low penetration of virus into the soil profiles was found, see **Fig. 1.** Some authors present the opinion, that most of viruses brought to the soil together with liquid slurry and wastes is kept in a 2 cm deep layer of soil [Reddy et al.1981]. Polio virus was isolated in podzol soil up to the depth of 20 - 25 cm. The highest titers were found in superficial layer of soil (0 - 2,5 cm), and they ranged between 5,27 - 6,27 log $TCID_{50}$ / g of soil. The titer was decreasing together with the depth, thus it reached 2,02 log $TCID_{50}$ / g of soil at the depth of 20 - 25 cm (from 1,77 to 2,27 log $TCID_{50}$ / g of soil). In browned chernozem soil, however, the polio virus migrated down to the depth of 15 - 20 cm, where the average titer was equal to 2,52 log $TCID_{50}$ / g of soil. It should be mentioned, that high titers of polio virus - average 5,92 log $TCID_{50}$ / g, which were observed in superficial layer of this soil (0 - 2,5 cm) - were rapidly decreasing in urther layers of soil, and they reached 1,71 log $TCID_{50}$ / g of soil at the depth of 7,5 - 10,0 cm. Here this relationship was changing faster than in podzol soil. Moreover, no regular decrease in virus titers was observed with increasing depth. The fluctuations, which were found, can be a proof of desorption processes, that take place in the soil. One should also remember, that the desorption processes depend on species features of viruses, though it ought to be mentioned, that washing out of polio virus (Lsc2ab) from the soil is weak [Nasser, Lopez-Pila 1986].

Though in our own research polio virus didn't penetrate beyond the humus layer, there is a rather disquieting fact, that high titers were found in the superficial layer, because this creates the risk of plants' contamination. Also the migration into deeper layers of soil can not be excluded in case of heavy precipitations.

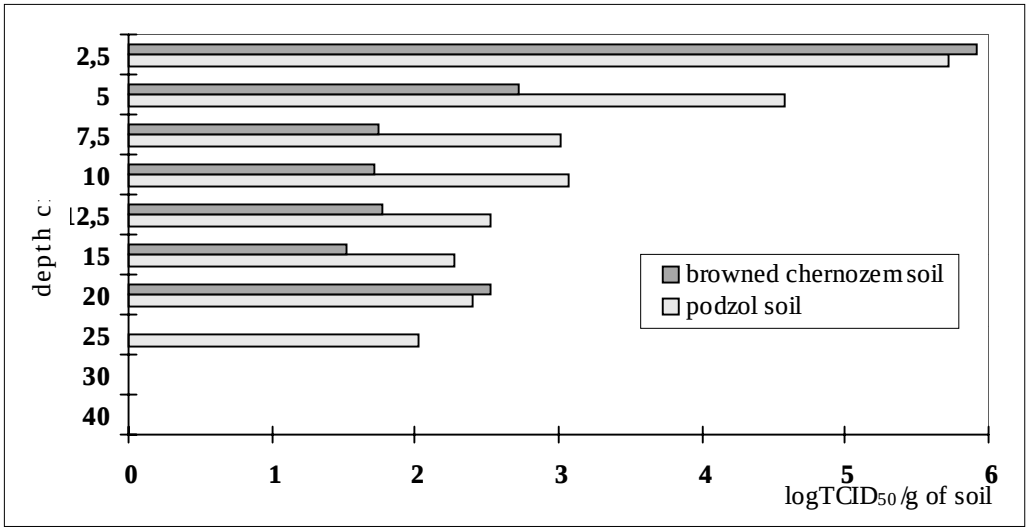

Fig.1 Infiltration of polio virus, suspended in waste, into the investigated soils.

Table 1. Selected physical-chemical indexes of tested soils

Genetic level	Layer cm	Cation exchange capacity	Contents of the with fraction with diameter in mm (%)		% volume of pores with diameter equal µm. (%)		pH KCl	Organic mater
		meq/100 g	< 0,002	< 0,02	> 300	300- 30		(%)
podzol soil								
Ap	0 - 30	7,00	1	5	3,3	25,3	6,7	1,7
C	> 30	2,92	2	4	4,1	27,5	4,4	-
browned chernozem soil								
A	0 - 40	15,28	12	26	3,7	7,7	7,2	3,5
B	40 - 60	27,56	19	35	4,1	4,9	7,7	-
Cca	> 60	32,85	19	37	4,1	5,0	7,8	-

The physical-chemical tests of soil showed, that due to the granulometric composition and low sorptive capacity, the best conditions of migration existed in podzol soil (Table 1). On the other hand, the difference in depth, on which polio viruses penetrated in browned chernozem soil appeared to be smaller than expected. In fact it was equal to some 5 cm. It seems, that low pH of podzol soil, which is conductive to adsorption of viruses, had such a strong influence in field conditions, that it could balance better filtration properties, which appear from the number of macropores. On the other hand, the existence of macropores in browned chernozem soil, which can be the place of fast transportation of microbes into deeper layers of soil [Gisi et al.1990], could limitate the negative influence of sorption complex and higher quantity of

organic matter to the transportation processes. It should also be noted, that many processes take place in the soil (adsorption of viruses together with the loss or maintaining the activity, desorption, migration into the soil), and it is hard to define a priori, which of the processes is the most intensive at a given time, while the viruses can behave in a non-typical manner [Vaughn, Landry 1983].

Conclusions

1. No influence of soil type was found to the penetration of viruses into the soil profiles, though it penetrated a bit deeper in podzol soil.
2. In field conditions, polio virus didn't migrate beyond the humus layer of tested soils.
3. The existence of high titers of viruses in superficial layers of the soils can create the risk of plants' contamination as well as the epizootic and epidemiologic hazards.

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Spores of Bacillus anthracis at former tannery sites - detection methods and risk assessment

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Summary

Former tannery sites are known to generate a certain risk by keeping viable and virulent spores of Bacillus anthracis originating from contaminated raw-materials processed at the beginning of this century. A three step method is described for sensitive detection of such spores by PCR, cultural examination and enrichment in a laboratory animal in soil of such plants. More than 300 samples had been investigated in the last four years the following places have been found to be mainly at risk: places where raw hides had been stored and handled, the waste water treatment system, places where raw materials had been discarded and the banks of former receiving waters.

Key words: Bacillus anthracis, spores tanneries, risk assesment.

Introduction

Former tannery sites are known to represent a certain risk with respect to anthrax spores, heavy metals and organic pollutants. No systematic scientific investigation has been done in Germany until now, either to determine the likelihood of disturbing viable anthrax spores remaining in the soil at these sites, or to determine which factors may influence the actual risk of infection for humans and susceptible animals. The aim of this investigation was to come to a preliminary risk assessment, by systematic sampling of soil from different tannery sites, in a town representative for leather industry in the first half of this century. In the past, several cases of anthrax in tannery workers and in cattle grazing close to wastewater receiving creeks had been reported to the authorities.

Investigated samples

Samples were taken from the soil at several depths, depending on the situation and the intended aim. From core samples, a visual examination was used to determine those parts of the soil columns which indicated the presence either of organic materials, like leather, hair and slurry, or of soil strata generated by the application of organic sludge and wastewater. These were examined for the occurrence of spores of *Bacillus anthracis (B.a.).* A total of 330 samples from different tannery sites was analysed. They originated from parts of the mills where the raw hides had been treated, where the tanning equipment had been located, where leather wastes had been

discarded, from wastewater pipes, pits, and treatment plants, from places where the sewage sludge was stored and disposed, from waste water irrigation areas and from the banks of the waste water receiving river.

Diagnostic procedures

First three identical pooled samples, each of 100 g must be mixed from a maximum of each 10 single samples (10 x 10 g or 5 x 20 g). One of the pooled samples is contaminated with 4 - 10 spores of the reference strain of B.a. one with 40 - 100 spores and one is the investigated sample. Each is mixed with 200 ml sterile water and 60 g of sterile glass beads. The samples are shaked over night (about 15 h) at 6-8 °C. At the next day it is recommended to filtrate the mixtures through sterile analytical metal sieves with mesh sizes of 500 µm at 250 µm. The liquid is heated in a waterbath for 30 min at 70 °C, centrifuged and the pellet is resuspended either in 100 ml Trypone Soya Broth (TSB) or in a small amount (10 ml) of distilled water. This is the base for a further cultural steps leading to the PCR by one of the below described methods.

If the PCR of the pooled sample is positive the single samples have to be investigated by PCR as described above. Cultural techniques will follow in the third step either done in parallel to the PCR or only with the samples which are positive in the PCR. If isolation on PLET-agar fails, enrichment in a laboratory animal must be done.

The PCR is done according to the method described by Beyer et al., 1995 other primers may be used too (Makino et al., 1993; Ramisse et al., 1996).

Cultural examination may be done by several techniques. One way will be selective cultivation on PLET agar.

For cultivation the pellet gained by the above described procedure must be resuspended in 10 ml of sterile destilled water, 1 ml is spread on three agar plates containing PLET and another 1 ml is spread to blood-agar containing either 100.000 units polymyxin B per litre or 16 mg sulfamethoxazole and 3,2 mg trimethoprim per litre.

Another possibility is to prepare a serial dilution in distilled water for enrichment on solid medium and to use more PLET-agar plates or additional blood-agar plates with the above mentioned additives. Suspected colonies of B.a. must be pure cultured and the diagnosis must be confirmed by gamma-phage test or any other approved technique (3) including the PCR.

The enrichtment in mice is done as follows:

Per sample at least three mice must be used which had been previously vaccinated against Clostridial infections (e.g. with Covexin$^{@}$). The sample is processed as described under 2. After centrifugation the pellet is resuspended in the smallest possible amount of sterile saline (0,15

mol/l) this means for 3 mice at least 3-4 ml. 0,5 ml of this suspension is injected subcutaneously in each mouse with a tuberculine syringe carrying liver-lock needle. Spleen, liver, heart and local lessions of dead mice are striked out on blood-agar plates and grown colonies are examined for those of B.a. after over night incubation. After three days the surviving mice are killed and examined as described above. In addition PCR may be carried out with blood or spleen as described by MAKINO et al., (1993) or one of the above mentioned PCR protocols.

The above given description of procedures are using the PCR as diagnostic tool at several steps. The most valuable application of this sensitive method which is capable to detect between 4 and 60 spores of B.a. in 100 g of sample is that in the first step by being helpful in separating the pooled samples in those who are negative and those which may be positive. Because the PCR is not only a helpful tool, it is a sensitive technique too, no PCR should be carried out without spiked samples for the determination of the sensitivity, without a known negative control-soil, without a reference DNA and the obligatory controls for the PCR itself, otherwise the results may be misleading.

Results

In most of the samples investigated, no viable spores of *Bacillus anthracis* could be found (detection limit less than 5 spores/100 g). Most positive samples could be found in one tannery which closed shortly after some spectacular cases of human anthrax. Here, virulent spores could be found at places where the raw hides had been stored and treated, as well as in the waste water system.

The other locations where positive samples could be found were an area where tannery and skin-wool offal had been disposed and sole from the banks of the forrner receiving water area, in the layer above the peat stratum.

Since the sampling density was relatively low in the irrigation and waste disposal areas, no final conclusions could be made. However, the results support the following risk assessment:

All areas where raw hides had been handled and processed carry an increased risk for the occurrence of spores of *B.a.* The risk seems to be greater if a factory ceased operation shortly after an anthrax event.

All places where raw hides and wool-wastes had been disposed of are at risk. A sole close to meandering creeks and rivers is at risk, both in and below the layer of ancient sediments.

A risk of infection therefore exists for humans and more so for susceptible animals at the time earth is being moved in high risk areas. As long as the soil remains undisturbed, the risk both for humane and for animals can be considered to be extremely low.

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Survival and transport of E.coli and Salmonella spp. in soils fertilized with slurry.

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Summary

The aim research undertaken was to define the survival and infiltration of E.coli and Salmonella spp. in the soil of two different types: rusty soil and meadow-forest chernozem. It was proved, that there is the influence of weather conditions and physical-chemical properties of the soil to survival and transport of bacteria. More favourable conditions of existence of the fecal bacteria were found in chernozem (survival of E.coli 23,1 weeks and Salmonella spp. 14,6 weeks) when compared to the rusty soil (15,2 and 8,7 weeks respectively). During wet season Salmonella and E.coli was isolated in longer time. Migration was more effective in rusty soil (90cm) than in chernozem and it depended on the amount of precipitations.

Key words: Salmonella senftenberg, E.coli, soil, liquid manure

Introduction

Rational use of liquid manure is one of the most important tasks faced by ecology nowadays (Paluszak and Olszewska 1996). When used as the organic fertilizer in too high quantities, it can lead to excessive pollution of the soil, underground water, and it can also cause the deterioration of the phyto-sanitary condition of plants grown (Kluczek 1994). Particular importance is assigned to the pathogenic bacteria, which in consequence of spreading the liquid manure on croplands can, in some cases, reach underground water resources (Strauch 1990). To define the level of hazard for environment, the possibility of migration and the survival rate of selected faecal bacteria was monitored in soils fertilized with liquid manure.

Material and methods

The research was performed in two cycles, 4 months each. Liquid manure in quantity of 72 liters(with addition of 1 litreof suspension containig 10 8-9/ ml E.coli and 10 6-7/ml Salmonella senftenberg) was spread on experimental plots (24 m 2), which were located on the rusty sol and on forest-meadow chernozem. The samples of soil from various depths were taken before liquid manure was spread, one week after that, and then 4 times in one month intervals. The number of E.coli in investigated soils was determinated using in sequence McConkey's

bouillon (24 h, 43 deg.C) and Tergitol-agar with addition of TTC. In case of doubts, the test for detection of glutamic acid decarboxylase was used. The content of Salmonella was determinated using in sequence poptone water (24 h, 37 deg.C), Rappaport medium (24 h, 43 deg.C) and BPLA (37 deg.C, 24 h). The quantitative investigations were made using MPN method with appropriate dilutions in the preliminary phase. Simultaneously, climatic conditions in the region of experiment were monitored.

Results and discussion

The research was made on two soil t, which differed in granulometric composition, filtration coefficient and chemical composition (**Table 1**).

Table 1. Selected physical-chemical indexes of tested soils.

Genetic level	The contents of the fraction with the diameter in mm (%0		Filtration coefficient K 10 (m/d)	pH KCl	C mg/100	N mg/100
	1,0 - 0,1	<0,02				
Forest meadow charnozem						
Ap	54	22	0,27	7,3	1298	109
(B)	48	27	0,39	7,3	385	54
C	42	32	0,027	7,4	170	28
Rusty soil						
Ap	75	8	1,50	6,3	748	59
C	89	0	2,97	6,2	102	22

The first investigated period was characterized with drought (precipitations 98 mm, mean day temperature 19 deg.C), while in second one higher precipitations (286 mm) and lower air temperature (15,6 deg.C) occurred. During the first period, the microbes appeared primarily in 12 cm deep top layer of both investigated types of soil. Up to 2,5 x 10^5 E.coli and 2,5 x 10^4 Salmonella microbes occurred in 100 g sample of rusty soil. 2,5 x 10^2 E.coli and 4,5 x 10^1 Salmonella were found at the depth of 25 cm. Deeper however (43 cm) the investigated bacteria occurred in minute quantities only. The occurrence of microbes in forest-meadow chernozem was similar. 7,5 x 10^3 E.coli and 2,5 x 10^4 Salmonella were isolated in 100 g of soil samples taken at the depth of 25 cm, while in case of samples taken in the layer of 43 this count was equal to 1,5 x 10^1 in 100 g of soil. The microbes migrated much deeper during the period of higher

moisture and lower temperatures. In rusty soil, microbes reached the depth of even 90 cm (2,7 x 10^1 Salmonella and 4,0 x 10^3 E.coli in 100 g of soil), but in forest-meadow chernozem they reached the depth of 43 cm (4 x 10^1 E.coli and 9,0 x 10^3 Salmonella). Physical-chemical properties of the soils influenced the process of migration of faecal bacteria especially during moist summer season. This process ran much easier in rusty soil due to higher number of macropores and due to higher coefficient of filtration.

Faecal bacteria in the soil fertilized with liquid manure are isolated at the depths between few and tens of cm (Krannich 1990). As the faecal microbes are subject to adsorption and filtration inside the soil profile, so the most of population occurs in its superficial layers. As appeared from research conducted, a part of population may be become remobilised and migrate into deeper layers, thus creating the hazard to underground water. As the time passed, faecal bacteria were gradually eliminated (**Table 2**).

Table 2. Regression lines for mean survival of investigated bacteria in the whole profile of rusty soil and forest meadow chernozem soils during moist summer L_2 and dry summer L_1 (fields E).

Period	Soil type	E. coli	Salmonella
L_2	Rusty	-0,57x + 12,88	-0,71x + 11,43
	Forest meadow chernozem	-0,43x + 11,81	-0,46x + 10,04
L_1	Rusty	-0,84x + 12,80	-1,22x + 10,60
	Forest meadow chernozem	-0,49x + 11,30	-0,65x + 9,51

The worst living conditions for faecal bacteria existed in rusty soil during the drought. The survival rate was 8,7 weeks in case of Salmonella and 15,2 weeks in case of E.coli. In forest-meadow chernozem Salmonella survived for 14,6 weeks, while E.coli for 23,1 weeks. Investigated microbes adopted easier to the soil environment during moist summer. In rusty soil E.coli survived for 22,6 weeks, while in chernozem for 27,5 weeks. The survival rate of Salmonella was a little shorter, and it was equal to 16,1 and 21,8 weeks, respectively. The research showed the influence of soil and climatic conditions to the survival rate of faecal microbes. The forest-meadow chernozem was the environment of more favourable granulometric

structure and better nutritional properties, which created the promoting conditions for existence of these microbes.

Conclusions

1. The faecal microbes occurring in soil are subject to continuous process of elimination.
2. E.coli microbes were the more resistant against the influence of soil environment.
3. The survival rate of microbes was clearly shorter during the drought.
4. The migration of microbes in rusty soil was easier when compared with that in forest-meadow chernozem.

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Fungal spores and actinomycetes in the working environment of biocompost plants.

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Summary

Health risks at the work place in biocompost plants by microorganisms are mainly originating from new strategies in waste disposal and recycling industry. This is particularly true in biocompost plants where the abundant growth of potentially hazardous microorganisms such as fungi and actinomycetes forms the basis of the biotechnological process. The results of this study show that the airborne emission of potentially hazardous microorganisms is closely related to the maturity (state of digestion) of the composted material when using personal air sampling. The workers were equipped with a complete set of a battery powered pump sucking continuously the sample air through polycarbonate membrane filters (1.0 µm) for 90 to 120 min during the shift. Highest numbers of thermophilic moulds and actinomycetes were found in the areas close to the main waste piles where most of the degradation of the biowastes takes place. The numbers of mesophilic microorganisms were down to the detection limit in these areas. Important factors for the emission and distribution (immission) of microorganisms are the process-related dust generation, seasonal influences, constructional details and the size of the plant.

Keywords: Biocompost plants, actinomycetes, fungi, working environment

Introduction

There is at present no commonly accepted scheme for the evaluation of the health risk workers are exposed in biocompost plants by microorganisms in respect to type of agent, concentration and exposure time. This is mainly due to the fact that the distribution of microorganisms at these work places is inhomogeneous and can vary tremendously according to the maturity of the compost, to working processes and to the composition and history of the raw material. High expositions to dustborne microorganisms are always to expect when the material is turned over and aerated mechanically. Because of the numerous factors which can produce prominent peak contaminations it is obvious that short term sampling can’t deliver representative results for a realistic risk assessment. Therefore it is necessary to develop measuring programs which consider all or most relevant emission factors.

This paper reports on first results of a current research project investigating the working environment and the health of the workers in about 40 biocompost plants in North West

Germany. The paper concentrates on air quality aspects and the influence of the maturity of the biocompost material on the amount of moulds and actinomycetes in four typical working areas of different plants.

Material and Methods

The personal sampler type PGP (Personengetragenes-Gefahrstoff-Probenahmesystem, Ströhlein, Kaarst, Germany) was used with Isopore Polycarbonate mambrane filters (1.0 µm, Millipore, Aschborn, Germany) for sampling airborne microorganisms. The sampling time was 90 to 120 min. The concentration of microorganisms was assessed by the indirect method as described elsewhere (Technische Regel biologische Arbeitsstoffe 430, Bundesarbeitsblatt, 1997). The following nutrient media were used: DG 18 - Agar (Oxoid, Wesel, Germany) for moulds at incubation temperatures of 25° C and 40° C; thermopilic actinomycetes were grown at 50° C on Glycerin-Arginin medium (Kommission für Arbeitsschutz und Normung, 1997).

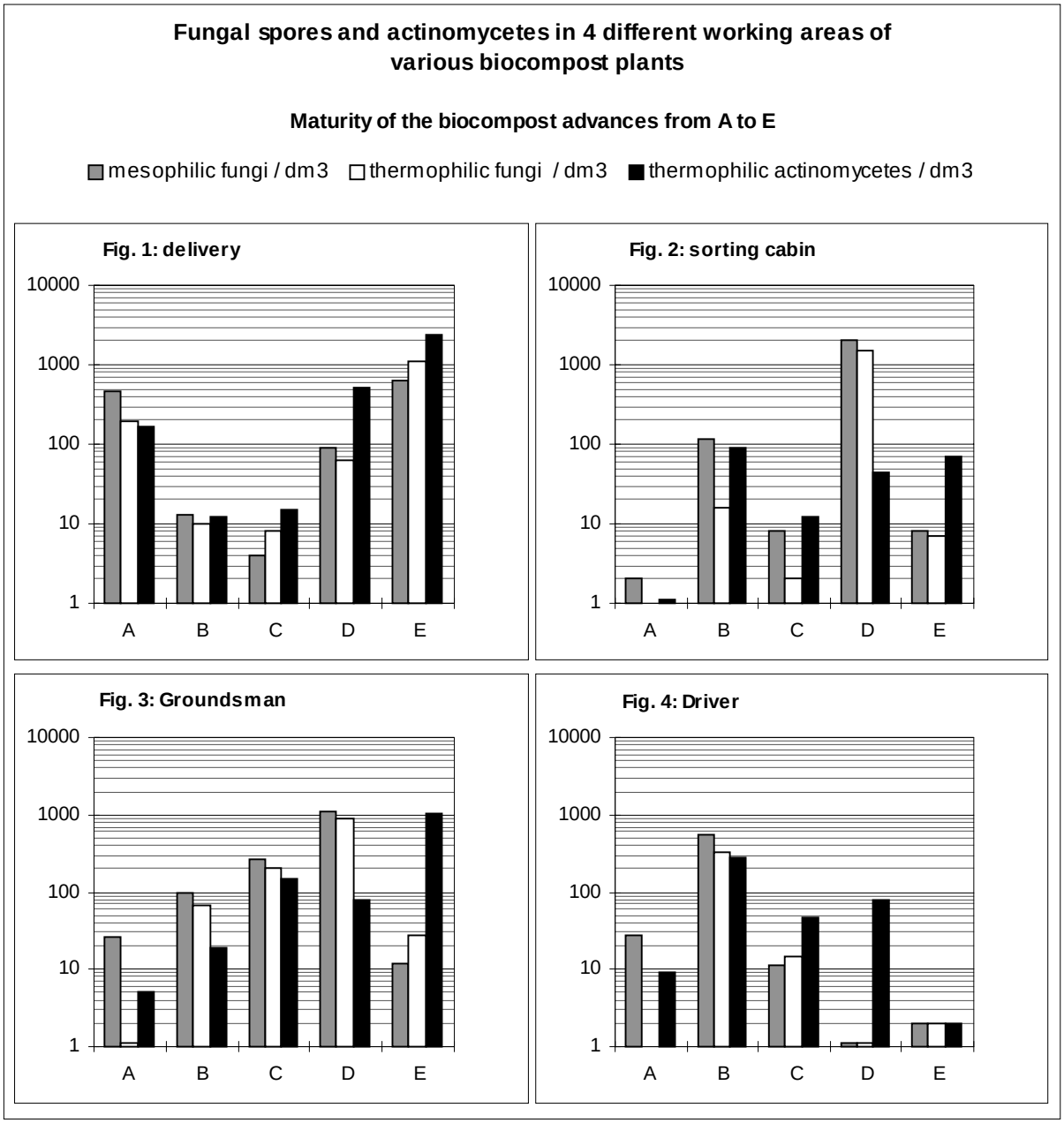
Results

Figures 1 to 4 show the results of five samplings each in four working areas (**Fig. 1**: delivery; **Fig. 2**: sorting cabin; **Fig. 3**: groundsman; **Fig. 4**: driver). The maturity of the biocompost advances in all four Figures from A to E. The concentrations of thermophilic fungis and actinomycetes increases in all four working areas according to the maturity of the waste material. This is particularly clear in Figures 3 and 4. The concentration of actinomycetes is distinctly reduced in the matured composted (Fig. 4, E) because of the self heating process.

Discussion

The concentration of airborne / dustborne microorganisms in the air of biocompost plants is directly related to the content of microorganisms in the composted material. The problem is that thermophilic microorganisms such as aspergillus fumigatus and actinomycetes which are potentially hazardous for human health are necessary for the composting process. The release of hazardous microorganisms, carried by dust particles is higher from compost piles which are under high maturing activity. Towards the end of the composting process a shift from mesophilic to thermophilic fungi and actinomycetes can be observed.

High ambient temperatures and prolonged storage times also contribute to an increased emission of potentially pathogen microorganisms. This can be seen in the delivery area and in the sorting cabin, too. Low ambient temperatures in wintertime are associated with low concentrations of thermophilic microorganisms in the freshly delivered biowaste.



The emission of microorganisms from biocompost plants is also influenced by constructional characteristics. The conditions in open plants, also addressed as biocompost places, depend very much on windspeed and air movement which can cause significant dilutions of the airborne microorganisms. On the other hand, closed plants are prone to high concentrations of microorganisms if the ventilation system does not work properly. Seasonal temperature differences influence the emission of microorganisms in biocompost places, in particular. The outer layers of these compost piles don't reach sufficient temperatures when the ambient temperatures are low, and therefore the emissions are reduced. The survival of microorganisms in the air is also influenced by humidity.

High emissions of microorganisms are to expect when fresh material is shred or the composting piles are turned mechanically. Therefore it is necessary to provide both air cleaning facilities and personal protection measures. The results show that effective measures were realised already for these areas in the investigated plants. This seems particularly true for the filters in driver cabins. The greatest risk for the workers exists in areas such as the manual sorting where the direct contact to the compost material can't be avoided. The investigations continue.

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Acknowledgment: This work was funded mainly by the Bundesanstalt für Arbeitsmedizin (Federal Office for Occupational Medicine), Berlin

Phosphine emissions from animal housing

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Summary

Toxic phosphine gas (phosphane, PH_3) was found to be emitted from animal housing facilities. High concentrations of matrix-bound phosphine were detected in manure from cattle and swine what can cause elevated phosphine concentrations in animal stables. The path of phosphine-loaded faecals ends up in the biogas of anaerobic manure and sewage treatment technologies. Additionally, the feed of cattle and swine was found to contain phosphine at varying concentrations, potentially as a result of grain fumigation.

Key words: phosphine, animal housing, manure, manure treatment, phosphine toxicity

Introduction

Acute toxicity of phosphine was observed in plants, insects, rodents, humans and microorganisms. In this context the detection of phosphine in anaerobic environments such as river and sea sediments (Gassmann, 1994), the faeces of humans, ruminants and swine (Gassmann and Glindemann, 1993) and biogas from manure and wastewater treatment plants (Devai et al., 1988; Glindemann and Bergmann, 1995; Glindemann et al., 1996) is of public health interest.

We investigated phosphine concentrations in animal stables, process gases of three manure treatment plants in Germany and in the feed of cattle and swine by seasonal sampling and analyses.

Materials and Methods

Gas and manure samples from different process stages of three anaerobic manure treatment plants, cattle and swine stables as well as from feed samples were seasonally transferred to the laboratory and analysed within a few hours.

Plant 1: Manure of cattle (100 m^3/d) and biogas processing: The manure is collected in an open tank and subsequently treated in two serial biogas fermenters. The digested material is

separated into a solid and a liquid phase using a centrifuge. The solid undergoes anaerobic rotting.

Plant 2: Manure of swine (200 m^3/d) and biogas processing: After removal of solid material by sedimentation and centrifuging, the liquid manure is digested in an anaerobic biogas process and additionally in an aerobic fermenter.

Plant 3: Manure of swine (40 m^3/d) in simple storage technology: The manure is stored at first in a smaller collection basin and is than transferred to a larger digestion basin.

Gaseous phosphine was measured by gas chromatographic analysis in accordance with Glindemann et al. (1996). Matrix-bound phosphine in manure and feed was released to the gas phase by boiling 1 ml of the sample in 1 M H_2SO_4 for 5 minutes.

All of the phosphine analyses in manure, feed and in gas phases are supported by three samples taken on three subsequent days in every season.

Results and Discussion

Table 1. Atmospheric phosphine concentrations in and around animal housing.

	Phosphine (ng/m^3)			
	Spring	Summer	Autumn	Winter
Plant 1				
cattle stable	0,34	0,16	0,25	0,10
500 m Luv	0,11	0,03	0,43	0,04
500 m Lee	0,09	0,12	0,76	0,04
Plant 2				
swine stable	**3,76**	**1,09**	0,28	
500 m Luv	0,18	0,17	0,15	
500 m Lee	0,40	0,08	0,25	
Plant 3				
swine stable	0,15	0,26	0,10	**23,79**
500 m Luv	0,42	0,42	0,11	1,17
500 m Lee	0,54	0,24	0,12	1,23

Although phosphine concentrations in the air of animal stables are low, in some cases phosphine levels were detected that are about one order of magnitude higher than the background atmospheric levels (**table 1**). The source of these enhanced values can be seen in the phosphine content of fresh faecals. The primary putrefaction of these faecals liberates relatively high

phosphine loads during the short-term processes of manure transport and collection in comparison to the long-lasting following stages in manure treatment (**table 2**).

Table 2. Phosphine concentrations in process gas samples from manure treatment plants.

	Phosphine (ng/m^3)			
	Spring	Summer	Autumn	Winter
Plant 1				
Putrefaction gas in manure sampler	7661	1305	5461	4112
Biogas fermenter 1	8	10	73	48
Biogas fermenter 2	1	212	245	119
Plant 2				
Putrefaction gas manure sampler	327	17	4805	
Biogas anaerobic fermenter	191	4	241	
Putrefaction gas long-term storage	665	16	3177	
Gas aerobic fermenter	2	53	2	
Plant 3				
Putrefaction gas collection basin	2056	1296	25615	35133
Putrefaction gas digestion basin	2660	151	47060	13058

The feed of cattle and swine can contain considerable phosphine loads (table 3). This feed-bound phosphine is potentially a product of grain fumigation and varies dramatically during the year. Phosphine residues in feed may thus represent one possible source of phosphine emissions from animal housing.

Table 3. Concentrations of phosphine bound in feed.

	Phosphine (ng/kg)			
	Spring	Summer	Autumn	Winter
plant 1 feed for cattle	83	16	6	88
plant 2 feed for swine	33184	665	53	
plant 3 feed for swine	1306	236	546	70

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The effect of slurry from pig-farm on the nearby river

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Summary

Limiting parameters of keeping and housing of animals on slatted floor result in great amounts of slurry. It is a mixture of feces, urine and water, and its environmental disposition is ecologically questioned.

Intensive swine production is often accompanied by various environmental problems, the major one being the removal of slurry.

The study was conducted on a large pig-breeding farm with all production phases included. The breeding herd, consisting of 1,600 sows, has a total of 30,000 weaned piglets and 24,000 fatterings. The unessential product of this pig breeding farm is about 20,000 tons slurry *per* year. The procedure of wastewater purification is based removing the settleable solids in settling basins, wherefrom the liquid phase is being pumped into the system of three flow-through lagoons. From the last lagoon, the effluent is discharged into the reclamation channel where the dilution occurs. After two kilometers, it flows into the nearby river.

Investigations were carried out monthly during the current year at several characteristic sites. Physical and chemical parameters relevant for surface water categorization were investigated.

Key words: slurry, pig farm, environmental, free waterflow

Introduction

Slurry is a mixture of feces, urine, spilled feed and water used for housing cleansing. This animal fecal mixture is produced in intensive animal, especially pig feedlots. From the epizootiologic point of view, slurry is a potential source of infectious diseases, primarily because in such a substrate the desirable processes of biosterilization do not occur and the probability for the infectious agents to survive is increased (Strauch 1991). In addition, intensive pig-breeding has also been criticized from the ecologic point of view, emphasizing the problem of feces removal.

In the present study, the removal of slurry from a pig-breeding farm was assessed by the follow-up of physico-chemical parameters of liquid manure from settling basins, lagoons and

reclamation channels, and of a free waterflow categorized as class II-III according to organic pollution.

Material and Methods

The farm observed in the study is located in east Slavonia, at a 2-6 km distance from the nearby settlements. The farm herd consists of 1,600 sows, about 30,000 weaned piglets and 24,000 fatterings *per* year. The slurry produced on the farm is collected in a sewer by the farm sewage system, wherefrom it is pumped into one of 10 settling basins. Upon settling of the solids, the liquid phase flows slowly from one lagoon to anothear. From the last, third lagoon the effluent is discharged into the reclamation channel, wherefrom it merges with fresh water from the river, then flows for about 2 km and empties into a free waterflow (serving as an outlet of the reclamation water). The solid phase from the settling basins and partially from the lagoons is transported to the adjacent plowland in autumn.

During 1996, liquid manure samples were taken once a month from the settling basin (sample 1), lagoon (sample 2), reclamation channel after emptying of the wastewater from the last lagoon (sample 3), and on the reclamation channel (sample 4), free waterflow upstream from the reclamation channel emptying (sample 5), and free waterflow downstream from the reclamation channel emptying (sample 6).

Standard physico-chemical methods (Standard Methods for the Examination of Water and Wastewater,1975) were used to determine suspended matter (mg/L), organic matter (%), electric conductivity (μS/cm), total disolved solids TDS (mg/L), ammonium (mg N/L), nitrites (mg N/L), chlorides (mg Cl/L), potassium permanganate consumption (mg O_2/L) and biochemical oxygen demand BOD_5 (mg O_2/L).

Results and Discussion

From the economic point of view, intensive stock/pig breeding is advantageous, because intensive concentration of animals held in a relatively small area decreases the costs of manpower, infrastructure, etc. However, a number of problems arise when observing it from the ethologic and ecologic standpoints. Keeping animals on a slatted-floor results in the production of large amounts of slurry. Various methods are used for its storage and disposal, usually a system of settling basins and lagoons.

The main purpose of slurry treatment is degradation of the organic substrate and its mineralization. In these processes, humidity, pH, temperature and presence of oxygen are of utmost importance. Various products of the organic substrate degradation thus produced,

depending on the levels of the above factors present, may cause environmental contamination, first of all involving the soil, waters and air. Hadžiosmanović et al. (1994) studied the effect of slurry on the hygienic-sanitary quality of natural waterflow and found it, processed or unprocessed, to be an environmental burden and hygienic problem.

Intensive stock/pig breeding is used in countries with a low farming-active population percentage, to ensure an adequate amount of animal products as well as to capitalize new technologies and breeding methods. Disadvantages of such technologies, however, have soon emerged in the form of air contamination with ammonium and unpleasant smell, and soil and water contamination with nitrites and nitrates (Owen, 1994).

On the farm under study, the problem of slurry disposal has been solved by the system of settling basin and lagoons, utilizing the solid phase of fecal mass for fertilization of about 1,500 ha of the adjacent plowpland. Instead of being directly emptied into the free waterflow, the separated liquid phase is emptied only after dilution with water from a reclamation channel and flow of about 2 km. The physico-chemical parameters of the studied samples indicated gradual occurrence of the biochemical processes of water purification (**Table 1**). This was especially pronounced in the findings of ammonium, nitrites, chlorides, potassium permanganate consumption and BOD_5 in samples 4, 5 and 6. Results of samples 5 and 6 suggested the waterflow to be classified as class II-III, pointing to a conclusion that eventually, the free waterflow must not be considerably contaminated by the farm wastewater.

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Table 1. Physico-chemical parameters determined in slurry and free waterflow during 1996 (mean; N = 12)

Table 1. Physico-chemical parameters determined in slurry and free waterflow during 1996 (mean; N = 12)

Parameter	Sample 1.	2.	3.	4.	5.	6.
Suspended matter (mg/L)	4261	1590	385	330	268	264
Organic matter (%)	72,2	66,3	63,2	57,1	51,2	46,1
pH	7,9	7,2	7,4	7,6	8,1	7,0
Electric conductivity (μS/cm)	6330	1705	886	611	555	536
TDS (mg/L)	3260	908	440	308	277	272
Ammonium-N (mg/L)	3,51	3,45	2,50	2,30	0,41	0,45
Nitrite -N(mg/L)	4,1	3,8	2,0	0,8	0,18	0,15
Chloride -Cl (mg/L)	2300	2200	70	62	40	29
$KMnO_4$ consuming capacity (mg O_2/L)	9160	3954	35,92	20,64	10,54	14,76
BOD_5 (mg O_2/L)	4826	1416	18,3	9,5	7,8	3,9

Farm wastes as a pollutant of water in salmonide fish farms in the view of fish health risk

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Summary

Dissolved oxygen (O_2), COD, NH_3, nitrites, nitrates, pH, Fe, Cu in water of salmonide fish farmes were investigated as to the impact on incidence of fish diseases such us red mouth disease, columnaris, bacterial gill disease and others, of which the erruption is the result of a stress situation. The investigation was made at four salmonide hatcheries in western part of Slovenia. We obtained some results which exceed the standards of the mentioned parametres which were seen most frequently in ammonia NH_3. We established that disease incidence and quality of water are somewhat correlated.

Key words: salmonide fish farmes, dissolved oxygen (O_2), COD, NH_3, nitrites, nitrates, pH, Fe, Cu, fish disease

Introduction

Slovenia (20.000 km^2) is a medium-watered area with 10.000 ha covered by running or stagnant water. Due to large hilly regions a lot of running waters contain plenty of dissolved oxygen which for salmonides is one of most convenient habitat conditions. This means good possibilities for salmonide hatchering in Slovenia which has an over a hundred-year old tradition. In Slovenia there are many small fish farms the number of which is still on the increase. The capacity of most of them does not exceed 5 tons per year. Salmonide fish farmes are mainly located in agricultural areas where the soil and ground water are filled with concentrations of ammonia, pottasium and phosphorus in spring and autumn, when fertilizing with manure and artificial fertilizers is common and there is always a possibility of their denudation into running waters. However, there is always a possibility of environmental pollution caused by fish farming. In our experiment four small typical extensive salmonide farmes were chosen for water analysis. The main aim of our work was to examine the impact of fertilizing and proximity of agricultural areas (meadows, pastures, fields) on the water inlet at the very entrance to the fish farms and eventual impact on health status of fishes (1, 2, 3, 4, 5).

Material and methods

During our study (winter, spring 1997) we made 432 water quality analysis from samples collected from 4 salmonide fish farms. Weekly we collected water samples at the spring, and from the fish farm and at it's outlet, the site where the water flows out from the fish farm. On the way to the objects the water stream flows through meadow and agricultural land and reaches the object with average flow rate of 10 - 20 L per second. Production capacity is approximately 5 tones per year. Fish farm number 1 is supplied with water from the spring 200 m distant from the fish farm. Water resource for fish farm number 2 is 500 m away and runs through woody gorge. The next two fish farms, number 3 and number 4, have the same water resource. Farm number 4 is very close (200 m) to the spring which recieves may effluents to the farm number 3 which is 800 m away.

Certain parameters such as dissolved oxygen, temperature and pH, ammonia were measured in the field (oxygen meter Iskra MA 5485, portable pH meter Iskra, portable spectrophotometer Hach DR/3 with accompanied prepared reagents). In the laboratory we used Hach′s spectrophotometer DR 4000/U. Measured parametes were: ammonia, salycilate method, copper, bicinchoninate method, chemical oxygen demand, COD reactor digestion method and colorimetric determination, nitrate, cadmium reduction method, nitrite, diazotization method and total iron, FerroVer method.

Results and discussion

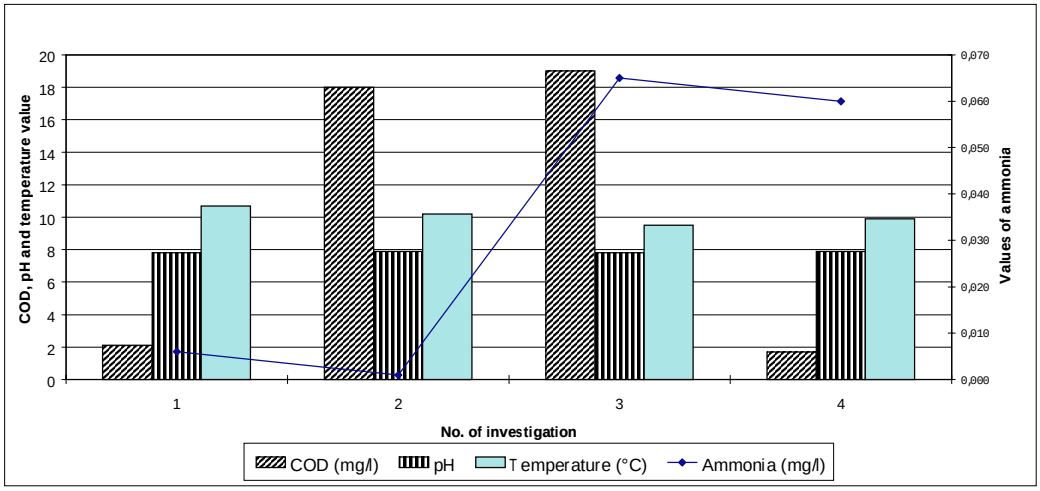
The average values of dissolved oxygene at the inlets in the four fish farms were very high and amounted to between 12,9 and 14,2 mg/kg (**Tab. 1**). The average values of ammonia (NH_3) at the inlet of fish farm number 3 in last two measurements were high (0,014-0,065) and exceed normative values between 14-23 % ($P>0.05$)(**Tab. 1, Graph 1**). The obtained average values of nitrates and nitrites were below the allowed standards for fish breeding. Average chemical demand of oxygen did not exceed the standards (between 5,25 and 11.4 mg/l). The values of cooper (Cu) was extremely low, but the average concentrations of the total iron (Fe) were slightly under the standards (1,62-3,2mg/l)(Tab.1).These results show us that measurements of the mentioned parameterers neither at the inlets nor at the outlets exceeded the allowed values. The only exception was the fish farm number 3. In this farm the value of amonia (NH_3) of the last two measurements exceeded the allowed values (Tab. 1, Graph 1). High concetrations of ammonia (NH_3) at the inlet in fish farm number 3 might be due to the following facts: 1. 800 m above the fish farm number 3 the fish farm number 4 is situated which might be the potential

source of the ammonia. 2. On the both sides of the water stream there are fertilized fields and pastures 3. During the last two measurement it was raining and snowing. The snow was melting, and there is the possibility that the ammonia in the water stream was the result of rinsing the ferilized soil. In farm 3 the health status of the fish of all cathegories was bad. During our visits of this fish farm the one-year -old fish were dark-coloured, they had one or two side exophtalmus and their swimming was uncoordinated. The fish farmer told us that for a long time the growth of fish of this cathegory had been unsatisfactory and they had also been sporadically dying. In the laboratory we have diagnosed red mouth disease (*Yersiniosis*) and have also put the persumptive diagnosis of viral hemorhagic septicaemia (*Viraemia haemorrhagica salmonis*). In spite of high level of ammonia before the inlet of water in the fish farm number 3 levels of nitrites and nitrates were not high. The reason for that was probably the fact that the ammonia needs some time to oxydise and the distance of 800 m from the source of the stream to the inlet into the fish farm is too short.

Table 1: *Average values (x) of measured parameters*

No.of investigation	Ammonia (mg/l)	Cooper (mg/l)	COD (mg/l)	Diss.oxygen (mg/l)	Nitrates (mg/l)	Nitrites (mg/l)	pH	Temperature (°C)	Tot.iron (mg/l)
1	0,003	0,010	3,475	12,375	2,600	0,004	7,875	8,850	2,700
2	0,005	0,013	1,975	13,990	1,625	0,011	8,083	6,375	1,750
3	0,033	0,012	10,200	13,220	1,975	0,004	7,850	10,075	2,700
4	0,005	0,005	5,300	11,810	1,925	0,004	7,475	7,875	2,225

Graph 1: *Water inlet of fish farm No. 3*



The increase of the ammonia in the water which serves the fish farm number 3 is obviously connected with atmospheric precipitations which rinsed the soil. In summer and autumn there are a lot of atmospheric precipitations and it is at this time that the ferilization of agricultural aeras is most intensive. Consequently, there is also the possibility of such a situation in other fish farms

at present as well as in the future. The sensitivity of fish population to the concetrations of ammonia (NH_3) is increasing with the increasing of water temperature, which was noticed in our work. We noticed also incresing the pH levels which is an extra factor for the toxicity of ammonia. Besides the incidence of fish diesease the inappropriate water quality indicates that many infectious diseases occur in a more serious form. Ammonia and its products have toxic and mechanic influence on gill epitelium, the result of which is proliferation of gill epitelium and increased levels of ammonia in the blood and tissue (5).

Conclusions

1. The values of ammonia (NH_3) at the inlet of fish farm number 3 in last two measurements were high (0,014-0,065) and exceed normatives for 14-23 % ($P>0.05$). 2. The increase of the ammonia in the water which serves the fish farm number 3 is obviously connected with atmospheric precipitations which rinsed the soil. 3. In experiment the increasing of tendencies to higher sensitivity to red mouth disease (*Yersiniosis*) and viral hemorhagic septicaemia (*Viraemia haemorrhagica salmonis*)) of fish population to the concetrations of ammonia (NH_3) were appear with water temperature and pH.

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Effect of forage composition on nitrogen excretion in two Italian dairy cow breeds

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Summary

The aim of the trial was to verify the effects of protein solubility and degradability on N use in two breeds of Italian dairy cows. In the two studies (high producing Italian Friesian, avg. 16,7 kg DMI/16,1% CP and Valdostana Pied Red, avg. 11,8 kg DMI/14,0 CP) two diets were fed to groups of animals in early lactation (0-15 weeks). Protein degradability (DIP) was 70% CP and 65% CP respectively while solubility was similar 35% CP (LS : low solubility) and 45% CP (HS : high solubility). The N balance was determined as a combined excreta result.

As a global result it appears that a high protein solubility level in the diet (e.g. large amounts of maize and alfalfa silage) can reach a good compromise between dairy production and N excretion losses only if accompanied by an adequate level of fermentable energy sources (e.g. barley, molasses, beet pulp), but only for high producing dairy cows.

Key words : protein solubility, alfalfa silage, N balance, milk composition

Introduction

In north-western Italy, two different kinds of environments can be distinguished, the Po plain and the mountains. Furthermore, these environments require different types of animal housing. In the plain, there are mainly large farms, while in the mountains the farms are notably smaller. These different kinds of animal housing have consequences on the choice of breed farmed and the destination of milk. In the plain, most farms have Italian Friesian (high producing) cows, whose milk is mainly destined for direct consumption or industrial cheese-making. In the mountainous environments more robust breeds are used, mainly the Valdostana whose milk is used for quality cheese-making (DOP products such as Fontina).

Also in this Italian area the growing concern for the environment has led to more research into the N metabolism in dairy cows. As previously documented, animal husbandry may contribute to the deterioration of the environment by the excretion of considerable amounts of non-digested or non-utilised feed residues, such as N. Legumes, in particular alfalfa silage, have a high crude, degradable and soluble protein content. Utilising this fodder one of the measures that can be taken to obtain a maximum rumen protein synthesis is to supply the ration with more soluble carbohydrates (e.g. wheat, barley) in order to maintain the balance between the degradation rate

of protein and carbohydrates (Broderick 1991, Bianchi et al. 1996). Since rumen degradable protein (DIP) as well as rumen degradable carbohydrates are available, alfalfa silage could contribute to a higher rumen fermentation level as a result of a better synchronisation of protein and carbohydrates and thus determining less N excretion and more milk protein synthesis (Nocek and Russell 1988, Nelson and Satter 1990). This study tried to verify in two Italian dairy breeds the effects of diet protein solubility and degradability on N balance.

Material and methods

The experiments were conducted in two farms near Torino (NW Italy). Based on previous lactation controls, two groups of 6 dairy cows, one for each farm (Italian Friesian - IF : 550 kg LW, Valdostana Red Pied - VPR : 450 kg LW) during early lactation (0-15 weeks) were fed with two different grass-based rations; one consisting of hay, maize silage and concentrate (low protein solubility - LS), while in the alternative diet, hay and concentrate have partly been substituted by alfalfa silage (high protein solubility - HS). The periods, during which the tests were conducted, were two months during the winter 1995 (IF) and two months during the winter 1996 (VPR).

Table 1 *- Diets composition and coefficients*

Dairy breed	IF		VPR	
Diet	LS	HS	LS	HS
As fed				
Hay (kg)	3,5	0	5	1
Maize silage (kg)	18	18	15	15
Alfalfa silage (kg)	0	7	0	10
Maize gluten feed (kg)	2,5	3,5	0	0
Beet pulp dehydrated (kg)	1,5	1	0	0
Concentrate * (kg)	8	6,5	4	2
Maize grain (kg)	0	0	2,5	3
Diet coefficients				
Crude Protein/kg DM	16,1		14,0	
Degradable Protein (% CP)	70		65	
Soluble protein (% CP)	35	45	36	46
Soluble protein (% DIP)	50	64	55	71
NDF (%)	39		42	
ENl (Mcal/kg DM)	1,51		1,50	

** Concentrate composition : soybean meal, maize cracked, barley, soybean extruded, rape seed meal, molasses, min-vit mix : 24% CP on DM (IF)/ 34% CP on DM (VPR)*

DMI, water intake, milk yield, milk composition and combined excreta (faeces and urine) of each cow were weighed three times a week for the 15 weeks. Chemical composition and

characteristics of the rations are shown in table 1. For each diet and from every cow, milk samples were taken and analysed. N analysis on combined excreta were also executed. All parameters were analysed by the analysis of variance.

Results

Table 2 shows the results about intake, milk yield and protein, N balance. Proportions are shown because interest is mainly focused on N utilisation.

***Table 2** – Intake, milk yield/protein and N balance (ratio in brackets)*

Dairy breed	IF		VPR	
Diet	LS	HS	LS	HS
Intake				
DM (kg)	16,4	17,0	11,6	11,9
Water (l)	74,6	72,5	65,7	62,5
Milk				
Yield (kg)	23,9	24,8	18,6	16,3
Protein (g/kg)	33,2	33,1	30,8	31,7
N balance				
N ingested (g)	402 ^{A}	425 ^{B}	263	277
N milk (g)	117 ^{A}	128 ^{B}	89	80
[/ N ingested (%)]	[29]	[30]	[34]	[29]
N combined *excreta* (g)	235	237	139	168
[/ N ingested (%)]	[58]	[56]	[53]	[60]
N total *excreta* (g)	352	365	228	248
[/ N ingested (%)]	[88]	[86]	[87]	[89]
N retained (g)	50	60	35	29
[/ N ingested (%)]	[12]	[14]	[13]	[11]

Means with different superscripts differ: A,B : p<0,01

The forage quality has a great importance affecting DMI (0,3 to 0,6 kg DM more in HS diets) in this particular stage (early lactation). Though almost all results are non-significant, trends can be observed. Regarding combined N excreta with respect to ingested N, in the Friesian breed, results showed no differences between the two diets for N balance (combined excreta N=57%): these results agreed with Crish et al. (1986) demonstrating that a high level in soluble N does not necessarily affect combined N. In VPR the HS diet shows an increase of N combined excreta (53% to 60% of ingested N) that could be explained by a minor efficiency of this breed. Nevertheless, we must also take into account the higher level of soluble protein (71% of DIP) adopted in this case.

Conclusions

The choice of a non specialised breed such as the VPR could find nowadays some limits due to environmental concerns caused by faecal and urinary N losses when animal are bred with intensive systems. For VPR cows the trial showed that using maize and alfalfa silage can lead to higher N losses than Italian Friesian ones. This lower efficiency of N utilisation may be reduced increasing the amount of fermentable energy in the diet.

A good compromise taking into account environmental concern, animal welfare and an adequate milk yield and protein could be achieved for this non specialised dairy breed when using livestock systems based mainly on grazing.

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Various carriers for test bacteria in compost hygienization

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Summary

It is a known fact that open exposure of the test bacteria *(E.coli, S.scheissheim, E.faecalis)* enables adequate assessment of cumulative bactericidal activity of compost. A comparative laboratory study was carried out with the closed broth cultures at 22°C, 37°C and 55°C to find out the most suitable commercial bacterial carrier between the porous beads, bacto blanks disks and dental rolls in perforated plastic probe tubes. During the pilot study of composting the results have shown that the suitable survival time was achieved with porous beads. The system was feasible in the perforated plastic probe tubes in which the survival time of the test bacteria was shorter. Additionally, their response to the raise in temperature was more adequate than those of the broth culture in the closed tubes.

Key words: Test bacterial carriers, perforated probe tubes, faecal poultry compost.

Introduction

Monitoring composting hygienization of the faecal animal mater and of biological byproducts by means of survival time of the openly exposed selected test bacteria and with the use of a standard carrier, is a big challenge. Unlike indirect procedures run under closed conditions, where everything comes to thermal effect, this represents a combination of physical, chemical and biological effects of a substrate, comprising also antibiotic and antagonistic action (Strauch 1993).

The report presents the results of the comparative studies using various carriers openly exposed under laboratory conditions and of a pilot field trial applying a small garden composter and controlled composting.

Material and methods

Standard test micro-organisms *E.coli* (DSM 4779), *S.scheissheim* and *E.faecalis* (DSM 2918) were kept in the Microbank™ system (porous beads) at -60°C and multiplied, when required, on solid or broth media (XLD, Becton Dickinson 4311838; Columbia agar, BBL 11059 and Brain heart infusion, BioMerieux 51281).*[1]

Plastic tubes (Micro test tubes, Ependorf 298) were perforated on four points under 90° and sterilised in a microwave oven for 2 minutes at maximal potency. Microbank™-system was inoculated with 1 ml of bacterial suspension containing on an average 7.5 x 10^8 CFU/ml (approx. OD 0.0225 at 450 nm). After 30 minutes each individual beads was transferred separately into the perforated probe tubes. Each dental roll was imbibed with 50 mcl broth culture each and each bacto disk (Bacto-concentration disks, sterile blanks, Difco 1599-33) with 10 mcl. Broth cultures in the volume of 1.5 ml were spilled and tightly closed in micro test tubes. Laboratory tests were carried out at 22°C, 37°C and 55°C.

For hygienization of chick litter in a closed system, in pilot validation test, a small garden composter (Zgo-Mulaža, polyester 400 1) was positioned in a partly air-conditioned room. Various carriers of bacterial cultures were placed parallel in the central part of the compost mass (at approximately 50 cm height); Survival time of the test bacteria was controlled by determination of CFU/ml applying standard technique of serial dilution's. Broth cultures were

*[1] By cortesy of Institut für Tiermedicin und Tierhygiene, Universität Hohenheim.

directly transferred dropwise into a diluent and various carriers were shaken beforehand at room temperature in 10 ml peptone water at 300 RPM/min for 30 min.

Discussion and results

Laboratory studies have confirmed the longest survival time of the test strains on broth cultures tightly closed in tubes at all test temperatures. Survival times of *E.faecalis, E.coli and S.schleissheim* at 55°C was 9 days, 24 hours and 3 hours respectively. This was in accordance with the known thermoresistance and tenacity of these bacteria (Holt et al., 1994). On Microbank™-system in the perforated plastic probe tubes at 55°C survival time of *E.faecalis* was 11 days, of *E.coli* and *S.scheissheim* almost 24 hours; at 22°C and 37°C it was most commonly between 14 and 22 days.

The results of composting (Fig. 1-3) show that survival time of the test bacteria in Microbank™-system in the perforated plastic tubes was shorter under temperature increase, than of the broth cultures kept in tightly closed tubes.

Similar results had been achieved earlier by direct inoculation of bacterial suspension into the compost mass (Platz 1976., Roth 1994.) and with the use of aiding carriers, fibre rags (Schumann et al., 1994.) and perlon gauze (Roth 1994). It is still necessary to check if the stability of the bacterial test cultures on bacto disks would be satisfactory in pilot studies. Dental rolls are inadequate due to a short survival time.

Conclusions

For evaluation of composting hygienization of deep chick litter and analogously of biowaste in general, an open exposure of the test bacteria on the porous beads carrier in perforated plastic probe tubes gives more reliable results than the known procedures involving the exposure of broth cultures under closed conditions. The achieved survival time is more realistic, involving both temperature effect and the entire antibiotic and/or antagonistic, i.e. athermic, substrate complex. The use of the commercial carrier, suitable for long-time storage of the test strains, allows procedure standardisation and the comparison of composting hygienization results.

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We would like to extend our gratitude to Mrs. Branka Kostenjak for technical assistance.

Survival time of the test bacteria on various carriers during composting

Figure 1. ***Escherichia coli***
Figure 2. ***Salmonella schleissheim***
Figure3. ***Enterococcus faecalis***

Survival time of the test bacteria on various carriers during composting
Figure 1. ***Escherichia coli***

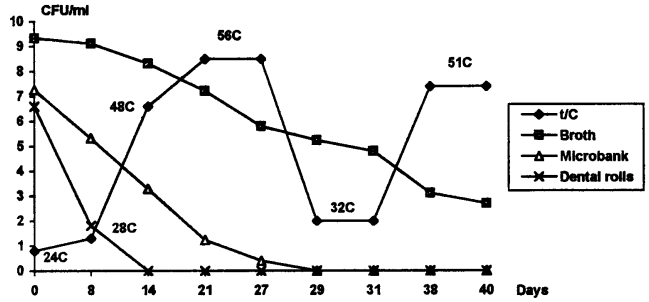


Figure 2. ***Salmonella schleissheim***

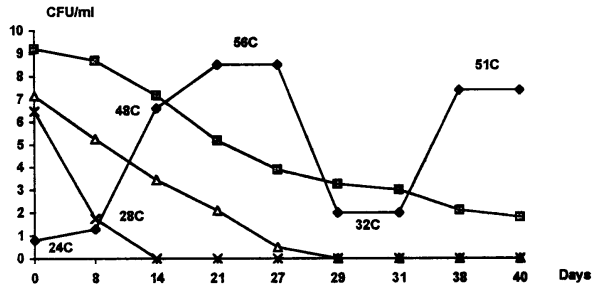
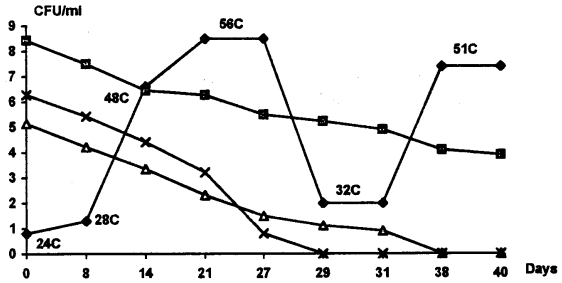


Figure 3. ***Enterococcus faecalis***



The content of nitrogen compounds in surface and ground water for drinking and watering as a consequence of soil fertilizing by animal wastes

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Summary

Studies were undertaken of three types of water sources for drinking and watering. An uncovered 10-m deep well situated in a rural area, a closed 50-m deep borehole of a small town waterwork, and a brook for livestock watering, which flows all along the grassland, all of them adjacent to the soil fertilized with slurry or animal manure, were included.

Study results pointed to a deteriorated bacteriologic and physico-chemical quality of the well water throughout the year, being worse during the wet period (NO_3-N mean, 19.8 mg/L), whereas the water from the waterworks borehole was found to be correct, with the exception of nitrogen compounds, especially nitrates (NO_3-N mean, 12.9 mg/L), which was also more pronounced during the wet period. The quality of brook water was hygienically correct, with elevated nitrate values (NO_3-N mean ,11.9 mg/L) before, and heavily deteriorated after spreading pig slurry over the nearby grassland. After two months, the quality of brook water improved, but still did not reach the initial quality.

Key words: water pollution, nitrogen compounds, soil fertilizing, animal waste

Introduction

Pollution of the ground and surface water is an event which inevitably accompanies urbanization, industrialization and especially intensive agricultural production. Studies performed in numerous countries have shown the quality of drinking water to deteriorate with high increase in the levels of nitrates. The main factors responsible for this trend are increased use of fertilizers and disposal of waste from intensive animal farming (WHO 1985; WHO 1987; Crathorne and Dobbs 1995).

The Republic of Croatia as a whole has plenty of good quality water according to the climatic, hydrologic, hydrogeologic and demographic conditions and agricultural production.The problems of so called "water crisis" have been observed to emerge in the areas with more

intensive agricultural production and higher concentrations of animals, as the one described in the study (Mayer 1996).

The aim of the study was to investigate the quality of drinking water from water sources located close to the soil fertilized with slurry.

Material and Methods

Studies were undertaken on three water sources. Grab bottle water samples were taken monthly from an uncovered well and a borehole of townwater supply, from October 1995 till December 1996 (N=15). Wet period lasted from October till May and dry period from June till December. From the third source, a brook used for watering, samples were taken from February till April 1997, before spreading the pig slurry on the nearby grassland, and then after 7, 14, 30 and 60 days. On each occasion, the physical, chemical and bacteriologic parameters were analyzed in accordance with standard methods (Anonymous 1975), using titration and photometric procedures on a HACH Drel 2000 chemistry/apparatus module.

Results

All parameters were determined using standard methods. Results are presented **in Tables 1- 3** as mean values, and compared with the Croatian guideline and WHO recommended values (Anonymous 1993)

Discusion and Conclusions

Water pollution, although inter related with the pollution of other environmental media, is specifically associated with the pollution of land and soil. Problems have arisen in some areas mainly due to run-off from agricultural land fertilized with animal manure and slurry (Webb and Archer 1994). There has been a marked increase in the nitrate content of both ground and surface waters over the last years. There are implications for human health arising from the presence of high nitrate levels in drinking water. The maximal conncentrations of 10 mg/L for nitrate-N and 1 mg/L for nitrite-N have been proposed. In Croatia, the proposed level of nitrate is the same, and that of nitrite it is lower 0.03 mg/L.

Concerning water protection against pollution from agriculture sources the Code of Good Agriculture Practice has been introduced. It implies a fertiliztation program determining the quantity, timing and distribution of fertilizers depending on the characteristics of the soil, climate and crop.

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Table 1.: Physical, chemical and bacteriologic parameters determined in drinking water from the well and waterworks borehole (mean ; N=15)

Parameter	Well	Waterwork
Color (mg/l PtCo)	12	2
Temperature (°C)	9.6	11.8
pH	7.1	7.3
Electric conductivity (μS/cm)	588	540
$KMnO_4$ consuming capacity mgO_2/L)	3.78	1.20
Chloride Cl^- (mg/L)	35	18
Mesophilic bacteria (cfu/ml)	119	4
Coliform bacteria (MPN/100 ml)	20	0

Table 2.: Nitrogen compounds determined in drinking water from the well and waterworks borehole during the wet (mean; N=9) and dry period (mean; N=6)

	Well		Waterwork	
Parameter	wet	dry	wet	dry
Ammonium NH_4-N (mg/L)	0.26	0.19	0.14	0.10
Nitrite NO_2-N (mg/L)	0.18	0.09	0.04	0.03
Nitrate NO_3-N (mg/L)	19.8	16.5	12.9	11.7

Table 3.: Physical, chemical and bacteriologic parameters determined in the brook water before and after spreading the slurry on the nearby grassland

Table 3.: Physical, chemical and bacteriologic parameters determined in the brook water before and after spreading the slurry on the nearby grassland

Parameter	Slurry spreading				
	before	after days			
		7	14	30	60
Color (mg/L PTCo)	2	54	49	48	27
Temperature (°C)	7	9.0	9.0	9.5	10.5
pH	7.3	7.3	7.1	8.1	8.0
Electric conductivity (µS/cm)	432	965	984	920	730
$KMnO_4$ consuming capacity (mg O_2/L)					
Chloride Cl^- (mg/L)	2.1	10.2	9.8	8.0	3.6
Mesophilic bacteria (cfu/ml)	18	55	41	30	21
Coliform bacteria (N/1000ml)	18	1900	1900	1800	55
Ammonium NH_4-N (mg/L)	27	>24000	>24000	>24000	38000
Nitrite NO_2-N(mg/L)	0.08	0.32	0.30	0.25	0.15
Nitrate NO_3-N (mg/L)	0.03	0.29	0.21	0.18	0.11
	11.9	31.6	28.5	18.7	16.5

Yeast-like in waste products during their storage period

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Summary

The author presented the mycological evaluation of waste products originating from the Utilization Plant during the 0,5 and 1,5 year period of storage in a pile. The determinations were made in two repetitions (1994 and 1995) during 6 months. Microbiological analysis showed, that the defined number of fungi in sediments reached similar level no matter of the storage period. It was found out, that the yeasts and yeast-like fungi were well developing in younger and older sediments, while the most often identified genera included Candida, Cryptococcus and Rhodotorula.

Key words: waste products, storage period, yeast-like fungi

Introduction

The investigations of yeast-like fungi in sewage sediments are important not only from the ecological-biological but also from sanitary point of view (**Kluczek, Kluczek** 1995). Numerous fungi of that group are the forms with potential pathogenic properties (**Strauch** 1993, **Ulfig** 1991). The most dangerous is the direct contact of humans and animals with polluted sediments. Some microbes, which are present in sewage sediments (bacteria, viruses) as well as fungi can probably migrate to the ground and superficial water, and in form of dry and watery bioaerosols they can be transported through the air on various distances. The series of microbiological tests was performed to define the mycological pollution of waste products stored in piles in various periods of their storage.

Materials and methods

The mycological research referred to the waste products stored for a period of one and a half year, which originated from Utilization Plant A, where final products were: meat-bone meal and utility fat. The samples taken at the depth of 30 cm from five defined locations in a pile, were analyzed every month during a half year period, and they were diluted using the sterile physiological fluid with 0,1 % addition of chloramine. Properly prepared material was inoculated on the following substrates: agar and Saboraud's substrate, then grown colonies were counted, while the fungi species identification was based on micro-method API-20C AUX. The

designations were made in two repetitions (1994 and 1995), and the whole was subject to statistical evaluation using the analysis of variance.

Results

The production wastes from Utilization Plant A were characterized with ample growth of fungi belonging to Candida genus and other yeast-like fungi. The growth of these fungi was observed in all samples incubated on pans of sewage sediments. Most of isolated yeast-like fungi belonged to imperfect fungi (Fungi imperfecti). Numbers of growth of the yeast-like fungi in investigated sediments is presented in **table 1**. During the half year period of analyzing the younger sediments, the highest number of colonies of fungi 126 thousand per gram of dry mass ($12,6 \times 10^4$) was obtained in second month of experiment, while the lowest being 69 thousand per gram of dry mass ($6,9 \times 10^4$) in second week. During the 1,5-year period of storage of (older) sediments it was shown, that the minimum number of colonies of these fungi was noted at the level of 37 thousand per gram of dry mass ($3,7 \times 10^4$) in zero sample, while the maximum being 89 thousand per gram of dry mass ($8,9 \times 10^4$) was obtained in third month of experiment. It should be underlined, that yeasts and yeast-like fungi were well growing in both younger and older sediments, and the statistical analysis showed essential differences in quantity of fungi between both sediments ($0,01 < p < 0,05$). The species identification of fungi in sediments showed no essential differences no matter what was the research period

Discussion

From the review of references and from own research (**Kluczek** 1996) it appears, that sewage sediments from Utilization Plants, due to their character, can create a threat to the natural environment, and also some hazard to humans and animals (**Strauch** 1993, **Kluczek** and **Kluczek** 1995). It proves, that the fungi existing in the sediments survived the whole period of storage in piles under atmospheric conditions (**Table 1**), and at the same time the two-factor statistical analysis for two sediments (0,5- and 1,5-year) confirmed the significance for yeast-like fungi with respect to time ($F = 9,48$; $p < 0,01$) and to the research period ($F = 76,69$; $p < 0,05$). Such significance was also confirmed while maintaining the interaction between said two features ($F = 60,91$; $p < 0,01$). Moreover it was showed, that both yeasts and yeast-like fungi in investigated sediments meet favourable biocenotic conditions so that their chance for survival becomes higher (**Kluczek** 1996, **Strauch** 1993, **Ulfig** 1991). It can be assumed, that investigated sewage sediments can not be released for use in agriculture without utilization no matter what is the period of their storage in piles.

Conclusion

1. The yeasts and yeast-like fungi in piled sewage sediments were not reduced in a noticeable way for any period of storage.

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Table 1. Identification of fungi in 0,5 and 1,5 year old sludges during the period of experiment

Sampling time	Yeast-like	
	Sewage sludge 0,5-year	**Sewage sludge 0,5-year**
0 day	Candida ciferrii, Candida parapsilosis, Rhodotorula glutinis, Rhodotorula rubra, $12,0 \times 10^4$	Candida ciferrii, Rhodotorula glutinis, Candida parapsilosis, candida lusitaniae, $3,7 \times 10^4$
14 day	Rhodotorula glutinis, Rhodotorula rubra, $12,6 \times 10^4$	Candida ciferrii, Cryptococcus laurentii, Candida famata, $7,0 \times 10^4$
1 month	Candida ciferrii, Candida lusitaniae, Candida tropicalis, $8,0 \times 10^4$	Candida ciferrii, Candida lusitaniae, Cryptococcus laurentii, $7,2 \times 10^4$
2 month	Candida ciferrii, Candida famata, Candida parapsilosis, $6,9 \times 10^4$	Candida ciferrii, Cryptococcus laurentii, Rhodotorula glutinis, $8,5 \times 10^4$
3 month	Cryptococcus laurentii, Rhodotorula glutinis, Candida famata, $8,1 \times 10^4$	Candida famata, Cryptococcus laurentii, Candida ciferrii, $8,9 \times 10^4$
4 month	Candida parapsilosis, Rhodotorula glutinis, $7,9 \times 10^4$	Candida ciferrii, Rhodotorula glutinis, $8,2 \times 10^4$
5 month	Candida ciferrii, Rhodotorula glutinis, Rhodotorula rubra, $8,0 \times 10^4$	Candida ciferrii, Rhodotorula glutinis, $8,0 \times 10^4$
6 month	Candida ciferrii, Rhodotorula glutinis, Candida parapsilosis, $8,1 \times 10^4$	Candida ciferrii, Cryptococcus laurentii, $8,3 \times 10^4$

Mycological contamination of the piggery during the piglets breeding period

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Summary

The study includes the attempt of evaluation of mycological pollution in breeding environment, in which 450 sows together with piglets of different age were present. Young stock at the age of 3 - 6 weeks was used for research, and the experimental period lasted for 7 months in a autumn-winter season (1994/1995). The samples were taken every month from the surface of wall, trough, floor, from the bedding, feed, drinking water, droppings, air and from the skin on the back and belly of 14 piglets. After appropriate preparation, the suspensions were inoculated on the following substrates: Saboraud's, Czapek's, agar with 4% glucose, potato agar and wort agar. During the mycological analyses 13 isolates were obtained, which belonged to 5 genera of fungi, while dominating ones were: A.flavus, A.carbonarius, A.fumigatus and P.albicans. The appearance of these fungi in large quantities inside the farm building causes the deterioration of sanitary conditions at the farm.

Key words: microflora of the pig rearing house, piglets.

Introduction

In the livestock breeding environment there are sometimes deep and unfavourable changes in the biological equilibrium of compartment biocenosis, especially in scope of microbiology, which lead to changes both in quantitative and qualitative spectrum of microbes (**Kluczek** 1996). The type and quantity of microbiological pollutions appearing inside the breeding compartments belong to the essential indexes of sanitary-hygienic conditions. Among the populations of psychotrophic fungi inside the breeding compartment, more and more often the toxin-generating fungi appear, which belong to the genera like Fusarium, Aspergillus, Penicillium and Candida.

Materials and methods

The research object consisted of the microflora at the Danish-type pig rearing house with 450 sows together with piglets of different age, and the animals at 3 - 6 weeks of age and 7 - 12 kgs of weight were used for experiments. The research period lasted for 7 months during the autumn-winter season (1995/1996), and the samples were taken 7 times from the surface of wall, trough, floor, from the bedding, feed, drinking water, air and from the skin on the back and belly of piglets (from 14 piglets). After appropriate preparation, the suspensions were inoculated on the following substrates: Saboraud's, Czapek's, agar with 4% glucose, potato agar and wort agar; they were incubated at the temperature of 25 deg.C during 4 - 5 days, and the quantitative evaluation of grown colonies was made, while the individual species were identified using the API-20C AUX microsystem as well as using the conventional method. The results obtained were verified statistically.

Results

As it appears from Table 1, during 7 months of research the highest number of fungi was found in the droppings ($4{,}6 \times 10^3$ - $4{,}6 \times 10^4$ colonies in 1 g), then in the bedding ($2{,}9 \times 10^3$ - $5{,}0 \times 10^4$ colonies in 1 g) and in the feed ($2{,}0 \times 10^3$ - $2{,}4 \times 10^4$ colonies in 1 g; $F = 4{,}61$; $p < 0{,}05$). Instead, the quantitative analysis of fungi on the surfaces of the wall, trough and floor presented the same order of magnitude during the whole period of research. The quantity of fungi in the air was maintained within the range between $3{,}2 \times 10^2$ to $3{,}3 \times 10^3$ colonies in 1 cu.cm, average $3{,}0 \times 10^3$ ($F = 15{,}4$; $p < 0{,}05$). When compared with remaining samples, the lowest variations were found here, and this is also related to lower coefficient of variance (11,2 %). Similar stability of moulds also refers to drinking water. Results referring to the mycological pollution of feeds showed, that the numbers of fungi exceeded 10^3 - 10^4 in 1 g of product. Instead, the observations of appearance of fungi on the surface of skin on piglets' back and belly were abundantly increasing during the research period, and they showed the same order of magnitude. Aspergillus flavus, A.carbonarius, A.fumigatus and P.albicans were distinctively dominating among mould fungi and allied species in all samples tested. In mycological analyses 13 isolates were obtained, and they belonged to 5 genera of fungi.

Discussion

From former experiments it appears, that the livestock compartments are the perfect incubators for microbes of various taxonomic affiliation (**Kluczek** 1996). It can be assumed, that the droppings, polluted bedding and the fodder are the natural habitat of yeasts and psychotrophic

moulds. Oligotrophic character of microflora causes, that basically in every breeding environment (compartment) there are optimum thermal-moisture conditions and sufficient quantity of nutrients for its development. When compared with psychotrophic fungi, the pathogenic organisms were not so rare in investigated breeding environment (**Kluczek** and **Kamińska** 1994, **Kluczek** 1996). Pathogenic action of fungi is probably based not only on causing the mycosis of organs but also on producing of mycotoxins (**Betina** 1989), which may show additional immunosuppression and cancerigenic action. It should be admitted, that in microbiologically polluted breeding environment, the piglets didn't show any disease symptoms, and this can be explained by efficient immunological condition of the organism. It is also possible, that there is the fungi infection in animals, which has the asymptomatic or latent course. The study requires to be continued using wider spectrum of experimental material and by observations of mycological pollution of breeding environment in relation to animals' state of health.

Conclusions

1. The composition of microflora in breeding compartment shows close relation to the microflora of the breeding environment as well as to the microbes, which develop on the surface of walls, floor, trough, bedding and in the droppings.

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Table 1. Quantitative characteristics of fungi flora in a compartment for piglets

Table 1. Quantitative characteristics of fungi flora in a compartment for piglets

Specification		September	October	November	January	Febuary	March	April
AIR 10 l		$3,4 \times 10^2$	$3,2 \times 10^2$	$3,1 \times 10^3$	$3,0 \times 10^2$	$3,3 \times 10^3$	$3,3 \times 10^3$	$3,6 \times 10^3$
FEED 1 g		$1,5 \times 10^3$	$2,4 \times 10^4$	$2,0 \times 10^3$	$1,2 \times 10^4$	$4,4 \times 10^3$	$3,5 \times 10^3$	$3,3 \times 10^3$
DRINKING WATER 1 ml		$2,3 \times 10^2$	$2,6 \times 10^3$	4.3×10^3	$3,9 \times 10^2$	$2,0 \times 10^3$	$2,4 \times 10^3$	$2,1 \times 10^3$
WALL 1 cm^2		$1,6 \times 10^3$	$1,9 \times 10^3$	$2,3 \times 10^3$	$3,4 \times 10^3$	$3,1 \times 10^3$	$2,9 \times 10^3$	$2,6 \times 10^3$
FEEDING 1 cm^2		$1,8 \times 10^3$	$2,0 \times 10^3$	$2,5 \times 10^3$	$2,1 \times 10^3$	$3,4 \times 10^3$	$1,6 \times 10^3$	$2,2 \times 10^3$
BEEDING 1 g		$1,8 \times 10^4$	$3,4 \times 10^3$	$4,9 \times 10^4$	$5,0 \times 10^4$	$2,9 \times 10^3$	$2,5 \times 10^4$	$3,0 \times 10^3$
FLOOR 1 cm^2		$2,4 \times 10^3$	$1,7 \times 10^3$	$3,2 \times 10^3$	$2,7 \times 10^3$	$2,7 \times 10^3$	$3,1 \times 10^3$	$2,7 \times 10^3$
DROPPINGS 1 g		$1,8 \times 10^4$	$3,6 \times 10^4$	$4,9 \times 10^3$	$4,6 \times 10^3$	$2,0 \times 10^4$	$4,6 \times 10^4$	$2,9 \times 10^4$
PIGLET	BACK 1 cm^2	$2,6 \times 10^3$	$2,5 \times 10^3$	$3,7 \times 10^3$	$2,8 \times 10^3$	$2,2 \times 10^3$	$1,9 \times 10^3$	$2,2 \times 10^3$
	BELLY 1 cm^2	$2,3 \times 10^3$	$2,2 \times 10^3$	$4,1 \times 10^3$	$3,6 \times 10^3$	$2,1 \times 10^3$	$2,0 \times 10^3$	$2,5 \times 10^3$

SOME PRACTICAL PROBLEMS WITH DETOXICATION OF GAME IN INDUSTRIAL POLLUTED AREAS

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Summary

The detoxication of pheasants organism contaminated by heavy metals were done using mineral-vitamine and aluminiumsilicate-humic feed additivs. In the field experiment pheasants were naturally contaminated by industrial emission of copper industry. During 3 months of feeding of detoxicants in the winter time the real reduction of cadmium and lead concentration in the liver kidneys and muscles and the reduction of mercury in the kidneys of pheasant were obtained. In the cage experiment the optimalization of level of additives in the pheasant food was done. In result it was shown that in the circumstances of high pheasant intoxication the lower levels of additives are also effective.

Key words: game, pheasant, heavy metals, detoxication.

Introduction

In many countries of Europe and also in Poland there are exists heavy polluted regions with a high level of heavy metals contamination (Bodak, Dobrzañski 1997).

Many game species and especially pheasant are exposite to heavy metals contamination (Ciberej at al. 1993). The systems of pheasant feeding during the winter and also sistematical introduction of this species to the hunting areas are a good oportunity to the application of detoxicants to the pheasant food. The positive results in the application of detoxicants in home animals were noticed in the literature (Bodak Dobrzañski 1997).

In this work we tried to use detoxication additives in practical feeding of pheasants in hunting areas.

Material and methods

In field experiment 300 pheasants 12 weeks old were used. Pheasants were introduced to the hunting area in 2 regions- control and experimental. The distans between those regions was about 5 kilometers. Both regions were heavy contaminated by emission from copper industry.

After adaptation period began winter feeding of pheasant. In the control group of pheasants (150 birds) natural corns and bruised grain were used in the 4 feeding places. In the experimental group (150 birds) the mixture of grain food with detoxicant additives in 4 feeding places were

used. Mineral-vitamine additives were used in 3% of vol. of food and contained C, E, D_3 vitamins, methionin, and compounds of selenium and magnesium. Aluminumsilicate-humic additive were used in 10% of vol. food.

After 3 mounth of winter feeding control hunting were done and in 9 pheasants the concentration of cadmium, lead and mercury in liver, kidneys and muscles according ASA method were etimated.

In the cage experiment 100 pheasants (14 weeks old) were used. Birds were divided into 3 experimental groups. After adaptation period experimental food were used. All pheasants were intoxicated with heavy metals and obtained 100mg of lead, 30mg of cadmium and 10mg of mercury in 1kg of food. In the control group any detoxicants were used. The I-st experimental group were feed by food with mineral-vitamine additive (3% of vol.) and aluminiumsilicate-humic (10% of food). In the experimental II-nd group they were the same additives but in the levels 1,5 and 5%.

After 2, 4 and 7 weeks of experiment the concentration of heavy metals in the liver, kidneys and muscles of 10 pheasants in every period were estimated.

Results

During the field experiment the control estimation of heavy metals in pheasants food were done. In the food for control group concentration of cadmium, lead and mercury were respectively: 0,097, 1,00 i 0,0016 mg/kg DM. In the detoxicant food concentrations of this metals were respectively 0,24 , 6,60 i 0,067 mg/kg DM.

After 3 mounth of pheasants feeding in the control group the average concentration of cadmium in liver, kidneys and muscles were respectively: 0,35, 1,30 and 0,029, average concentration of lead - 0,117, 0,465, 0,068 and mercury 0,0014, 0,0039 i 0,0008 mg/kg fresh weight.

The concentration of examinated metals in organs and tissues of pheasants feeded with detoxicant additives was generally lower than in control animals organs and tissues. The reduction of concentration of cadmium in liver, kidneys and muscles were respectively: 62,9, 66,2 i 58,6%, the reduction of lead concentration - 2,6, 54,0 i 38,2% and the reduction of mercury in kidneys was 37,3%.

Althought the short time of experiment, we have found significant lowering of concentration of cadmium and lead in pheasants muscles and organs, and also significant lowering of mercury concentration in kidneys due by influence of used detoxicants.During the field experiment we have noticed some problems connected with intake of experimental food, it was probably connected with specific taste and smell of detoxicant additives. Because of that in

cage experiment we tried to examin effectivity of detoxication of different concentrations of food additives in circumstances of heavy intoxication of pheasants with examinated metals.

The results of cage experiment shows that the procent of reduction of cadmium concentration in liver, kidneys and muscles of pheasants in experimental group I-st was respectively: 18,63 , 26,06 i 60,17%. In the experimental group II-nd it was respectively: 7,62 , 9,35 i 50,42%.

The reduction of level of lead in organism of examinated pheasants can be observed only after 2 weeks of experiment. After this time detoxicants are not so effective. After 2 weeks of experiment the reduction of lead concentration in liver, kidneys and muscles of pheasant from I-st experimental group and was respectively: 15,85 , 3,59 and 8,16%, and in II-nd experimental group of pheasant was noticed respectively: 8,06 , 7,56 i 12,93%.

The detoxication effects of food additives in relationship to pheasant's contamination by mercury in I experimental group are not significant, and in II experimental group was noticed the reduction of mercury concentration in liver, kidneys and muscles respectivelly: 18,4, 27,0 i 24,5%.

The results of cage experiment shows that in circumstances of huge exposition of pheasant organism by heavy metals from the practical reasons it would be well to use the food additives on the lower level.

Conclusions

1. Aluminumsilicate-humic and mineral-vitamine additives have positive influence on detoxication of pheasant's organism contaminated by heavy metals especially by cadmium and lead.

2. Detoxication systems of pheasant's organism reqiure specially doses depends on the level of heavy metals contaminations in polluted organism.

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The effect of Disinfectants on Bacteriophage ∅X 174

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Summary

The kinetics of bacteriophage ∅X 174 inactivation by 20 disinfectants was compared. Experimental groups are halogens (chlorine, iodine), aldehydes, organic acids, alcalines and others (Virkon S, Lechlor). From the results follows, that the most effective desinfection effect has been reached in the organic acid solution (Persteril), followed by the group of others (Virkon S, Lechlor) and the group of aldehydes. the least effective were in our study the iodine compounds.

Introduction

The resistence of viruses is tightly combined with their morphological structure. The resistance of enveloped and nonenveloped viruses varies greatly. „Die Deutsche Veterinarmedizinische Gesellschaft“ (DVG) recommends to test always two representants of nonenveloped viruses (ECBO-virus, Reo-virus) and two representants of enveloped viruses (Newcastle virus a Vaccinia virus).

Disinfectants effective for nonenveloped and enveloped viruses are called - virus killing, disinfectants effective in action only for enveloped viruses are called - limited killing power.

For testing of virus killing effect can be used morfologically similar bacteroiphages with the equal resistance as animal viruses to be tested.

Methods

Virucidal efect is tested on bacteriophage ∅X 174 (ATCCC 13706-B1) which host E.coli (ATCC 13706-1) is characterized by cubic ikosahedral-type symetry of parcicles free of envelope, 27 nm in diameter and contains single-strand cyclic DNA. It is representative type of the Microviridae family of bacterial viruses. By their size, form, and resistence are similar as animal Picorna- and Parvoviridae. We used two experimental methods:

- wood carriers disinficated by plunge into the disinfectants solution
- plastic carriers disinficated by spraying disinfectants solution.

In both methods the virucidal effect is tested under protein loading (meat pepton beef-tea). At the same time we practise control tests of bacteriophage, toxicity, immerging, and plaque medium.

Non-parametric semiquantitive statistical methods has been used for the evaluation of results. For the evaluation of virucidal effect the results from carriers are divided to 6 frequency intervals. In individual frequecy interval the averages are calculated. The graphical expression of results respected the means and the extremal values.

Results and conclusions

The most effective desinfection effect has been reached in the organic acid solution (Persteril), the addition of phosphates as anticorosives substance has only litttle influence on disinfectant effect.

The next group includes the others disinfectants (Virkon S, Lechlor), and the group of aldehydes. Among these compounds the effect was exhibited by formalin. *Neoseptal SDT, Weigosept DF, TAD CID a INTERCID FA*, which contain glutaraldehyde and glyoxal groups with formalin, have had faster virucidal effect in comparision with disinfectatns in this groups wihout formaline substance - Desam G, Incidur Vet, Aldesol.

The virucidal effect of *Natrium hydroxidatum* is fast and reliable.

*Dikonit i*s a representant of chlorine disinfectants with the best effect in this group. *Cl-lime, Stamid, and Stericlean* have slower effect and less reliable. It is interesting, that Savo Prim (NaClO) which has 6 times lower content of active Chlorine is in the outcome comparable with Chloramine B. The least effect was observed in the iodine compounds.

It is interesting, that recomended concentration and exposition time according to our test results apear a less effective as is usually considered. This is the consequence of our stronger measures of testing and evaluating of results.

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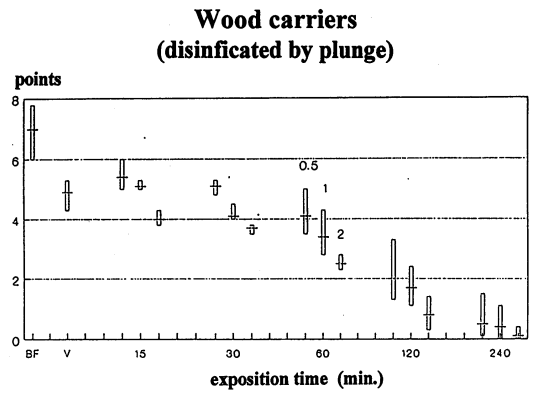
Table 1. Recomended concentration and exposition time (according our results)

Fig. 1 Wood carriers



Note: 0,5 half concentration
1 recommended concentration
2 double concentration

Fig. 2 Plastic carriers

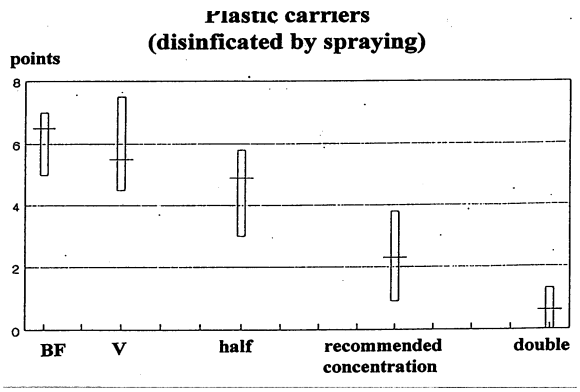


Table 1. Recomended concentration and exposition time (according our results)

Group	Name of Disinfectant	Plunge			Spraying		
		Concentration of (%)		Exposition time (min.)	Concentration of (%)		Exposition time (min.)
		disinfectant	active substance		disinfectant	active substance	
Clorine	Chloramin B	-	-	-	-	-	-
	Dikonit	2	1,2	120	1	0,6	240
		0,5	0,3	240			
	Cl-lime	-	-	-	-	-	-
	Savo Prim	-	-	-	-	-	-
	Stamid	-	-	-	-	-	-
	Stericlean	3	-	120	-	-	-
Aldehydes	Formalin	5	2	15			
		2,5	1	30	-	-	-
		1,25	0,5	120			
	Desam G	4		240	4		240
	Incidur vet	2		240	4		240
	Aldesol	4		240	-	-	-
	Neoseptal SDT	6		15			
		3		60	3		240
		1,5		240			
	Weigosept DF	2		120	2		240
		1		240			
	TAD CID	1		120	1	-	240
		0,5		240			
	INTERCID FA	2		120	2		240
Organic acid	Persteril	2,4	0,72	15			
		1,2	0,36	30	0,6	0,18	240
		0,6	0,18	60			
	Persteril+	2,4	0,72	30			
	+phosphate	1,2	0,36	60	0,6	0,18	240
		0,6	0,18	120			
Alkaline	NaOH	2		60	-	-	-
		1		120			
Iodine	Jodonal A	-	-	-	-	-	-
Others	Lechor	6		60	3		240
		3		120			
	Virkon S	1		120	1		240
		0,5		240			

Note: Recomended procedures for spraying disinfection of stables

- first spraying - 0,25 $l.m^{-2}$
- delay time - 1 hour
- second spraying - 0,25 $l.m^{-2}$
- exposition time - 3 hours (after second spraying)

VIROLOGICAL ASPECTS OF HYGIENIC SAFETY IN SEVERAL LARGE-SCALE SLURRY TREATMENT PLANTS IN GERMANY

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Summary:

Due to the increasing concentration process in animal production in the Federal Republic of Germany a regional surplus of animal slurry must be expected. Slurry from large units of farm animals can be contaminated with obligatory or facultatively pathogenic germs. Therefore, epidemiological risks and risks for environmental hygiene cannot be excluded if the slurry remains untreated.

In an extensive investigation program, six different slurry treatment plants were investigated comparatively with regard to the occurrence and survival of indigenous fecal bacteria and artificially added pathogens like bacteria and viruses. The tenacity of the microorganisms, bacteria and viruses (esp. ECBO-, bov. Parvo- and equ. Rhino-virus) in the plants and treatment processes has been investigated comparatively with ”germ-carrier” methods, which were developed for bacterial and viral tenacity studies under practical field conditions.

Results show that under ordinary conditions thermophilic anaerobioc treatment guarantees a probable hygienisation of the process end product, whereas mesophilic anaerobic processes, although microbicidal in principle, remain uncertain over all and demand an individual risk assessment for the single case.

Bovine Parvovirus proved to be a suitable test germ to investigate hygienisation effects of slurry treatment processes in which the microbicidal component is mainly based on temperature influence.

Introduction:

Increasing concentration processes in animal husbandry lead to regional increasing amounts of animal wastes. This may end in increased environment burden and pollution caused by chemical substances like ammonia gazes (air) and salts (drinking water) as well as microbiological pollutants (BÖHM, 1995). Intensive, spreadless farming in particular leads to regional increased amounts of slurry. It comprises a hygienic risk too, as slurry may contain a great number of obligate or facultative pathogenic microorganisms which can be spread in the environment (STRAUCH, 1990) and which may remain infective for a long time in this material (STRAUCH and BALLARINI, 1994).

The German Ministry of Research and Technology therefore initiated an extensive investigation program about technical treatment of slurry to transform it into environmentally harmless and usefull secondary products like composts and ammonia fertilizers. In addition, aspects of substrate hygienisation were investigated too in several technical pilot slurry treatment plants all over the country. Most of these plants produce energy in the form of biogas as a product of anaerobic slurry digestion processes. Construction and operation principles of these plants have been given in PHILIPP et al. (1997).

Most investigations concerning tenacity of pathogens under different environmental conditions have been made by using bacteria. But in slurry, also several viral pathogens may occur (STRAUCH and BALLARINI, 1994) including the classical swine fever virus, the Aujeszky disease virus and the foot and mouth disease virus, causes of economically important animal epidemics.

In this context, the question if and how viral pathogens in slurry treatment plants are inactivated (e.g., during mesophilic and thermophilic anaerobic treatment) is of great interest, as is the question if there exist correlations between the inactivation of bacteriological and viral pathogens by anaerobic treatment.

Since it is not admissible to perform investigations in real plants using agents of notifiable diseases, representative germs of an environmentally harmless character had to be used instead. These viruses were brought into the treatment processes of the plants by means of a filter sandwich germ-carrier technique developed (TRAUB et al., 1986, 1988) and modified (WINTER et al., 1995) for viral tenacity studies. By this technique contamination of the investigated substrates is avoided.

Material und methods:

Viruses und cell culture: **ECBO** virus (Enteric Cytopatogenic Bovine Orphan), strain LCR 4, cultivated and detected in MDBK cells (Madin and Darby bovine kidney). **ERV 1**-Virus (Equine Rhinovirus), in cell line RK13 (Rabbit kidney). **BPV** (Bovine Parvovirus), strain Haden, in primary BEL cells (Bovine embryonic lung).

Germ carrier technique: The carrier cases are 25 mm membrane filter carriers (Sartorius, Göttingen, Germany) whose inlet and outlet openings are widened to 15 mm diameter. In a carrier a carrier membrane is loaded (ZETA PLUS-Virosorb1- MDS, AMF, Cuno Div., Meriden, Conneticut.) on which a specified amount of virus is adsorbed. To the inlet and outlet this membrane is covered with two additional polycarbonate membranes with a defined pore size of 10 nm diameter (Infiltec, Speyer, Germany). Due to the small size no virus can pass through to contaminate the surroundings, whereas all low molecular components of the surrounding may pass the membranes which means inactivating or stabilisating influences from the surrounding media may effect the encovered test virus (pH value, NH_3, temperature etc.).

For tenacity studies several germ carriers were brought into the medium (or process) of question and removed successively in specified time intervals. By determining of the remaining infectivity by titration on permissive cell cultures the virus inactivation kinetics in the individual process were determined.

Results:

In thermophilic processes at temperatures above 55 °C logarithmic reduction (log. red.) of the tested Picorna viruses (ECBO-Virus resp. ERV) took place within a few hours. Nearly in parallel, even a little faster, all tested Salmonellae were inactivated. As expected fecal streptococci proved to be more thermoresistant, but within 24 hours a titer reduction of more than 4 powers of ten (p.o.t.) took place in the native germs of the substrate as well as in those added artificially to the process. The Parvo virus proved to be the most stable, its titer was reduced under 3,5 p.o.t. sometimes, and in all cases it remained detectable up to the test ends.

In most cases of anaerobic digestion at mesophilic temperatures also a titer reduction of all types of test germs took place. however in a time range of days and weeks. A certain similarity of the inactivation kinetics of *S.Senftenberg* and ECBO virus resp. ERV can be stated. In some cases a log. red. of more than 4 p.o.t. took place, sometimes under the detection limits. Fecal streptococci and BPV, however, were reduced in this period of time mostly by 1 to 2 p.o.t. only and could be detected at the end of all tests. The inactivation kinetics of both germs took place nearly in parallel in most cases.

In some tests with reactor temperatures below 30 °C none of the test germs were inactivated. Even after periods of several weeks, also Salmonellae could be detected in nearly unchanged titer size.

Discussion:

In slurry a great number of pathogenic microorganisms may occur and remain infective for a long time (STRAUCH, 1990; STRAUCH und BALLARINI, 1994). For slurry treatment plants where the end products are used for agricultural purposes the demand should be that the substrate is sufficiently hygienisized during treatment. Otherwise new infection cycles may be created and infection chains closed in the environment.

Our studies proved that adequate anaerobic digestion of slurry under field conditions has microbicidal effects in principle which vary depending on the process parameters. As shown before (HAAS et al., 1995; LUND et al., 1996; PESARO et al., 1995) process temperatures are a decisive element: the higher the temperature, the faster the inactivation of microorganisms.

Testing of disinfectants according to the instructions of the German Veterinary Medical Association demands for the usage of a test germ that a log. red. of 4 p.o.t. can be proven (DVG, 1988). In analogy, the results of our studies proved such reduction for all test germs except BPV. This reveals that a properly operated thermophilic anaerobic reactor guarantees a log. red. of 4 p.o.t. in a reaction time of 24 hours in nearly all cases which strictly reduces (if not eliminates)

the risk of pathogenic germ spreading through the end products. However, this applies only to the fermentation of pure slurry at this stage, not necessarily to the end products of "cofermentation" plants. In cofermentation processes, where other animal residues like fat or meat resp. bone tissue containing wastes are digested together with slurry, pathogens may be introduced into the process in a special protected form. Thus, by fat and in tissue residues they may be protected against inactivating influences of the process and show longer survival time. This question has to be the subject of future investigations.

Also by mesophilic anaerobic treatment inactivation normally takes place, but a log. red. of 4 p.o.t. does not occur regulary, and the process is not sufficient to hygienise the substrate completely in general. Evaluation has to be done for each individual case, among other things taking in account the technical substrate reveal time in the process and, even more important, the real mininal reveal time (risk of short-circuits). For cofermentation plants a pre-treatment of other animal wastes (fat and tissues) the demand of a previous hygienisation of the co-substrates seems to be indispensable, for the named point.

In accordance with our results pevious studies (HAAS et al., 1995; PESARO et al., 1995) showed that in mesophilic anaerobic processes temperature is only one inactivating component among others. According to the microbicidal influences the effective sum of these other components often exceeds the influence of pure temperature/time degree for germ inactivation (data not shown). Such components may be e.g. the pH value, the redox potential and the NH_3 concentration. In our studies we did not succeed in ensuring correlations between germ inactivation and such other factors up to now. To achieve this, complex and systematic research is necessary which may be hard to acchieve and only to a limited extend under conditions of a real working plant.

In our studies we also investigated possible correlations of inactivation kinetics between some bacteria and virus groups often used as test germs for tenacity studies in the environment. It could be shown that the inactivation kinetics of *S.Senftenberg* in the investigated processes nearly correspond to those of the two picorna viruses used. Viruses of this group are known to be comparatively stable in the environment, especially against chemical influences (ECBO virus, for instance, is a limiting germ to test virucidal effects for disinfectants according to the regulations of the DVG (DVG, 1988)). Our results allow the conclusion that inactivation studies by using sufficient amounts of *S.Senftenberg* in anaerobic treatment plants (as may be demanded by law in the future) indicates the inactivation of Picorna viruses or other environmentally lesser stable viral pathogens which may occur in slurry.

However, this applies not to the Parvo viruses which proved to be limiting in other tenacity studies during anaerobic treatment (BØTNER, 1990; LUND et al., 1996; PESARO et al., 1995). In our studies BPV proved to be clearly more thermostable than fecal streptococci: in mesophilic processes where inactivating effects may not predominately be ruled by thermic inactivation both agents proved quite similar tenacity. But with rising temperature, as the results of thermophilic

treatment plants and pre-hygienisation steps of some cofermentation plants show (data not shown), fecal streptococci were inactivated faster than BPV. This is in accordance with LUND et al. (1996) who compared the tenacity of a bovine enteric virus, fecal streptococci and porcine parvo virus in anaerobic processes.

BPV - being easy to handle in a virological laboratory and environmentally save - might be a suitable test germ for hygienisation effects of treatment processes like thermophilic anaerobic digestion in which thermic influence is the main component of germ inactivation. The aim of it should not be a complete inactivation of this virus (because for farming animals this virus group is not of epidemiologic importance, except the porcine parvo virus) but by log. red. of it statements could be made which are applicable to nearly all specific pathogens, esp. to the economically main important causes of animal epidemics that are much more instable against thermic influences than BPV is. Future studies related to this topic are necessary and in preparation.

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Possibility of sanitizing the waste waters of a rendering plant by means of the peracetic acid

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Summary

Research into the possibility of sanitizing the waste waters of a rendering plant by means of the peracetic acid was carried out in 1990. The experimental data reveal that the plant waste waters are intensely contaminated by bacteria, particularly by enterobacteria, faecal streptococci, coliform bacteria and salmonellae. Besides, the chemical analysis shows a high level of chemical pollution of the waste waters. The hygienic conditions of the plant were also evaluated, so that constructional, technological and organizational impairments were established. The author proposes possible improvements with respect to them. Testing of the waste waters revealed 29 different salmonella serovars, and R-form of salmonella was discovered in 7 cases. The 1% concentration of peracetic acid rendered satisfactory results, both with respect to microbiological and chemical sanitation of the plant waste waters. The decrease in pH value as a consequence of the peracetic acid application was the only unfavorable effect established during the investigation.

Key words: sanitizing the waste water, salmonella, peracetic acid, rendering plant

Introduction

Research into the possibility of sanitizing the waste waters of a rendering plant was carried out at the public rendering plant. The row materials for a rendering plant are carcasses, animal wastes from meat industry, slaughter houses, farms and from public areas (Asaj, 1974; Centar, 1979; Strauch, 1975). A rendering plant has a large importance in preventing the environmental pollution. However, because of the concentration of waste and polluted materials from a wide area, rendering plants can be a big danger for the environment, too. The row materials for the rendering plant were collected from the area of the northwest and north Croatia. Mentioned rendering plant has the negative influence on the environment by disposing the waste waters without any kind of sanitizing. The possibility of sanitizing the waste waters of the rendering plant by means of the peracetic acid was carried out because the high costs of building the cleansing equipment. As *Salmonella spp.* are well known as a dangerous and wide spread organism, salmonellosis become a great problem through the world, even in the countries with high hygienic standards (Weise, 1978; Kane, 1979; Weise, 1981), so *Salmonella* monitoring was under special consideration

Material and methods

Standard equipment for a bacteriological laboratory is used. As all the details cannot be given here, only those materials and methods that are of special importance will be described.

The samples of the waste water were taken in the 1500 ml plastic containers, washed, rinsed in the purified water and dried in the warm air every time before use. The quantity of the samples was 1000 ml, and the samples were taken every Monday through 1990., except at the time of state holidays and through the July, when the plant was in smaller reconstruction. Samples were taken at the open flow where the waste waters have been disposed to the environment.

In laboratory, the number of different bacteria was determined by the "surface method" on agar plates. For determining the total No. of Mesofilic bacteria the agar plates were prepared with the Standard I Nährmadium, Merck Nr. 7881 (Merck, 1992), the No. of Enterobacteria the agar plates were prepared with MacConkey Agar Nr. 3 (Merck Nr. 5465), the No. of Faecal Strptococci the agar plates were prepared with Slanetz & Bartley Medium (Merck Nr. 5262) and for the determination the No. of Coliforms the agar plates were prepared with ENDO C Agar (Merck Nr. 4044). In addition the most probable number (MPV) of *Salmonella spp.* as well as the serovars of *Salmonella* was determined. The methods for determining *Salmonella spp.* is described by Narančić (1995.). The number of viable cells is expressed as cfu per ml.

The determination of pH, oxygen content, biochemical oxygen demand, ammonium content (NH_3), nitrite content (NO_2) and potassium permanganate ($KMnO_4$) demand was performed by the methods described by Asaj (1974).

The evaluation of sanitizing effect of peracetic acid (CH_3COOH) - PAA was performed as follows: In each 1000 ml sample was added 15% PAA in quantity that mixed with 1 l of sample gives required final concentration of PAA in the waste water. Mixed solution was left at the room temperature for 30 min. In the following step, from each mixed solution was taken 10 ml and added in 90 ml of sterile 0,95% NaCl solution + 0,7% of Sodium Thiosulphate ($Na_2S_2O_3$). Than the determination of the No. of survived total mesofils, enterobacteria, feacel strptococci, coliforms by the surface method on agar plates or and MPV *Salmonella* was carried out as described above. The same procedure was made with the different concentrations of Potassium Permanganate solution as the control and without adding any chemical as the blind control. Also, there were determined the changes in content of above mentioned chemicals.

The hygienic conditions of the plant were evaluated by the "Guidelines for evaluation of the conditions at e rendering plants" made by Biesinger & others (1979).

Results

Average contents in the waste waters of the rendering plant were 2.77x107 cfu of total mesofils, 4.72x106 cfu of enterobacteria, 1.52x106 cfu of faecal streptococci, 3.09x106 cfu of coliforms and *Salmonella* MPV were 9340.37 in ml. After exposure to PAA and to Potassium permanganate in different concentrations the reductions of bacterial content were as follows:

Concentration of		Average reduction of the No. of									
PAA	$KMnO_4$	Total Mesofils		Enterobacte-riaceae		Feacal Streptococci		Coliforms		Salmonella MPV	
in %		in %									
0.1	1.0	73.79	48.73	83.05	83.08	82.68	82.73	82.23	82.1	100	100
0.05	0.75	53.9	11.46	80.98	16.24	82.68	21.39	82.23	27.58	99.90	80.72
0.01	0.50	7.21	12.74	9.99	19.08	17.45	22.86	10.95	26.85	69.96	39.76

There were, also, the difference in investigated chemical indicators as follows:

Concentration of		Average difference of the							
PAA	$KMnO_4$	pH		Oxygen Content		Ammonium Content		Nitrite Content	
in %		in %							
0.1	1.0	-2.32	0.2984	5870.4	3709.1	-92.3	-87.5	105	76
0.05	0.75	-1.75	0.1628	2338.2	2431.7	-84.9	-80.8	6.03	65.9
0.01	0.50	-0.99	0.1558	1010.6	1336.8	-66.2	-73.0	7.09	48.4

Salmonella spp. were isolated 255 times from total of 248 sampling. It was found 29 different serovars, plus *Salmonella Subgenus I. "R" (rough) form* in 7 occasions. The most common serovar was *S. virchow* (17,25%), than *S. agona* (10,98%) and *S. anatum* (10,59%). *S. virchow* were present in samples through 10 of 11 months of sampling. There were no primoisolations.

Conclusions

The constructional, technological and organizational impairments of the rendering plant were established. The main hygienic problems at the mentioned rendering plant were not distinct division between "unclean" and "clean" side in the plant, and disposing the waste water in the environment without any sanitizing.

The experimental data reveal that the plant waste waters are intensely contaminated by bacteria, particularly by enterobacteria, faecal streptococci, coliform bacteria and salmonellae. Besides, the chemical analysis shows a high level of chemical pollution of the waste waters. 1 g

per liter of PAA in the waste water rendered satisfactory results, both with respect to microbiological and chemical sanitation of the plant waste waters. The decrease in pH value as a consequence of the peracetic acid application was the only unfavorable effect established during the investigation.

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Anaerobic fermentation of liquid manure combined with organic matter from slaughterhouses from the hygienic point of view

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Summary

The objective of this work was to study the dynamics of processing (thermophilic, mesophilic and psychrophilic microorganisms) and hygienic-epizootologic (coliforms, coli-faecalis, enterococci, and moulds) microbial indicators during anaerobic fermentation of animal liquid manure with addition of a small part of gastrointestinal matter from poultry and rabbit slaughterhouses. The dynamics of parasites was also observed.

During fermentation the number of mesophilic and psychrophillic microorganisms, as well as coliforms and faecal coliforms decreased by 2-3 log units. Total devitalization of coliforms and faecal coliforms in most samples was reached during storage in collected tanks. In the liquid manurestuff for spreading to fields the total devitalization of parasitical eggs was finded.

Key words: anaerobic fermentation, process indicators, hygienic-epizootologic indicators

Introduction

Anaerobic fermentation is considered as one of the methods of recycling of organic matter in agroecosystem. The increase of production is ascribed by degradation of compound organic matter and their transformation to simple components.

Various viruses, bacteria, and parasites may persist in solid and liquid manure, by-products, and wastes of animal origin. According to BENDIXEN (1994), many of the pathogens may also survive for longer period in anaerobic fermentors, which operate at a mesophillic temperature level. On the other hand, it is quite typical, that many pathogens are eliminated within some hours at the thermophilic level.

Methods

The technical data of biogas plant : Total volume - 750 m^3

Retention time - 30 days

Operating temperature - 41^oC

Number of collected tanks - 2

Volume of one tank - 1250 m^3

Daily amount of organic matter from slaughterhouses-0,5m^3

During 2 years we collected 60 samples of liquid manure: 20 samples of fresh liquid manure, 20 samples of liquid manure after fermentation, 20 samples of manurestuffs before spreading in the fields (from collected tanks).

The objective of this work was to study the dynamics of processing (thermophilic, mesophilic and psychrophilic microorganisms) and hygienic-epizootologic (coliforms, coli-faecalis, enterococci and moulds) microbial indicators during anaerobic fermentation of animal liquid manure with addition of a small part of gastrointestinal matter from poultry and rabbit slaughterhouses. The dynamics of parasits was also observed.

Results

Microbiological observations

From the dynamics of process indicators ti is clear, that the number of *thermophillic mikcroorganisms* shows only a moderate variation in order 10^5 during the whole process. While the number of *mesophillic microorganisms* in fresh liquid manure was 10^9 per 1 ml, after fermentation dropped by 2 log unit and their content in collected tanks was 10^6 per 1 ml of samples. The number of *psychrophillic microorganisms* decreased during anaerobic fermentaction by 3 log units (from 10^{10} to 10^7 per 1 ml of samples).

As a basic hygienic-epizootologig criterion we observed the dynamics of coliforms and faecal coliforms microorganisms. During fermentation the number of *coliforms* as well as *faecal coliforms* decreased by 2-3 log units. Total devitalization of coliforms and faecal coliforms in most samples was reached during storage in collected tanks. The number of *faecal enterococci* decreased during frementation from 10^5 to 10^2 in 1 ml of samples. Their number in collected tanks increased by 1 log unit (to 10^3).

In *moulds* we proved a great decrease (from 10^7 to 10^4 in l ml), as well as *in other microorganisms growing on Czapek-Dox agar* (from 10^9 to 10^6 in 1 ml of sample).

Salmonella strain we proved only in one sample of fresh liquid manure. All samples of fermentated liquid manure and manure from collected tanks were hygienically safe.

Parasitical observations

Oocysts of genus *Eimeria, Isospora, Cryptosporidium,* and cysts of *Giardia* and *Balantidium* were identified together with eggs of *A.suum, Trichuris spp.,* and *Hymenolepis spp.* in samples of fresh liquid manure by direct methods. In the liquid manurestuff for spreading to fields the total devitalization of parasitical eggs was founded.

By nonparametrical statistical test by Wilcoxon we reached significant difference between samples of fresh liquid manure, manure after fermentation, and also before spreading on fields. Correlation between all three types of liquid manure was proved by Spearman correlation coeficient.

Conclusion

During Anaerobic fermentation the number of mesophilic and psychrophillic microorganisms, as well as coliforms and faecal coliforms decreased by 2-3 log units. Total devitalization of coliforms and faecal coliforms in most samples was reached during storage in collected tanks. In the liquid manurestuff for spreading to fields the total devitalization of parasitical eggs was founded.

Is is necessary to observe

- the process technology with operating temperature 43^oC
- using only the gastrointestinal matter from healthy animal from the clinical point of view
- add this material to fermentor gradually after its decomposition
- from the veterinary point of view store liguid manure after fermentation in self- governing tank for 6 weeks and before landspreading perform micoribioloigical examination.
- the field landspreading made in conformity with Instruction of Ministry of Agriculture for manipulation and landspreading of cattle, pig and poultry liquid manure, which covers hygienic and water protection principles.

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Nutritional value and heavy metals content of fermented broiler poultry litter

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Summary

Macrominerals (Ca, P, Mg, Na, K) and microminerals (Cu. Fe, Zn, Al, Mn, Se, Co, Mo), heavy metals (Pb, As, Hg, Cd), occurence of pathogenic microorganisms, and nutritional parameters (dry matter, nitrogen, crude fibre, ether extract, crude ash) of broiler poultry litter were analysed pre and post six weeks ensiling in polyethylene bags. Nitrogen content of broiler poultry litter was close to some cereals (corn, barley) and was higher than in commonly preserved feeds (corn and grass silage). Minerals and heavy metals are within the tolerance levels stipulated for an individual component of feed in Slovak Republic. Non-pathogenic bacteria were found in the litter after fermentation.In conclusion, fermented broiler poultry litter showed sufficient nutritional value to enable its use as a feedstuff and the concentrations of heavy metals were not risky for animal health.

Key words: broiler, poultry, litter, ensiling, microorganisms, macro and microminerals, heavy metals, nutrient, nutritional values, fermentation.

Introduction

Wastes such as poultry litter can be reutilised considering the enormous quantities produced world wide each year (Wilkinson 1988). According to the reported results, ensiling seems to be the only method which can effectively preserve the nutritional value of poultry waste as well as protect the fed animal from possible infections, because the increased temperature during the fermentation process enhance the destruction of most of the pathogenic organisms (McCaskey et al. 1985). The objective of this study was to evaluate the effect of six weeks ensiling of broiler poultry litter on its nutritional value, macrominerals, heavy metals and occurence of pathogenic bacteria.

Materials and Methods

Broiler poultry litter came from houses in which cereal´s straw was used as bedding material. The litter was processed by ensiling in a transparent polyethylene bag (60 cm x 120 cm) to a weight of 40 kg per bag. Each bag of litter was moistened with 2 litres of water and compressed to eliminate air. The bags were tied with a string as tight as possible, a scotch tape was used to seal the endings and they were placed in a dark room. The litter was left to ferment for six weeks. The broiler poultry litter was analysed pre and post fermentation for its nutritional value, and macro and microminerals contents. Dry matter, nitrogen, crude ash, ether extract, and crude fibre were determined.

Macro and trace minerals were analysed by atomic absorption photometric method (AAS 306 and Zeeman AAS 4100ZL, Perkin Elmer) -- Ca, P, Mg, Na, K, Fe, Cu. Zn, Se,Mn,Co,Mo,Al,Cd,As,Pb,Hg.

Microbiological examination was carried out after fermentation as described by (Mikula et al. 1985).

Results

A decrease in dry matter (51.29 %), nitrogen (8.67 %), crude ash (9.74 %), crude fibre (10.94 %), ether extract (0.7 %) was seen after fermentation (**Table 1**).

Fermentation of broiler poultry litter resulted in a small decreasing concentrations of all elements determined in the present study (**Tables 2 and 3**).

Total number of mesophilic and psychrophilic germs were 5.0 x 10^6, and 8.8 x 10^7 per gram, respectively. Only non-pathogenic organisms were isolated in the fermented poultry litter *(Pseudomonas cepacia, Bacillus cereus, Acinetobacter calcoaceticus, Acinetobacter Iwofii, Pseudomonas pseudoalcaligenes, Staphylococcus epidermidis, Bacillus coagulans, Bacillus polymyxa, Hafnia alvei* and *Flavimonas oriryhabitans).*

Table 1. Nutritional composition of broiler poultry litter pre and post fermentation (% dry matter)

	Dry matter % FM*	Nitrogen	Crude ash	Crude fibre	Ether extract
Pre	68.49	14.07	14.48	12.07	1.17
Post	51.29	8.67	9.74	10.94	0.70

*Fresh matter

Table 2. Macrominerals (g/kg DM*) in broiler poultry litter pre and post fermentation (%dry matter)

Minerals	Ca	P	Mg	Na	K
Pre	7.12	10.57	4.33	5.36	34.19
Post	6.62	10.15	4.14	4.62	31.66

*Dry matter

Table 3. Microminerals (mg/kg DM*) in broiler poultry litter pre and post fermentation

Minerals	Cu	Fe	Zn	Al	Mn	Se	Co	Mo	Pb	As	Hg	Cd
Pre	50.1	860	164	15.1	235	.14	1.39	.46	.43	.53	.06	.40
Post	43.8	810	146	14.3	222	.09	1.10	.33	.34	.44	.05	.38

*Dry matter

Discussion

Broiler litter is of greatest value as a feedstuff for ruminants when fed in a limited amount as a source of supplemental nitrogen. Dry matter, crude ash and crude fibre content of broiler poultry litter used in the present study, corresponded with the reported ranges by Flachowsky (1994). Nutritional value depends on the environment, the management of the poultry houses and the litter. In accordance with our result, Müller (1984) reported that ensiling is a simple process which not only prevents crude protein losses but also converts part of the available non-protein nitrogen into protein-bound nitrogen (true protein). Nitrogen content of broiler poultry litter is closer to certain cereals (corn, barley) and much higher when compared with commonly preserved feeds such as corn and grass silage (Vajda *et al.* 1994).

Broiler litter contains abundant amounts of all minerals. Pb, Hg and Cd are rarely found in excessive levels in poultry waste (Müller 1980). In contrast with our results, Müller (1968) on an earlier survey on broiler poultry litter from farms in CzechoSlovakia reported a higher concentration of Cu (100 ppm) and lower concentrations of Zn and Mn (138 ppm, and 99 ppm, respectively). It seems that over the years, the concentration of Cu in poultry feeds has decreased while that of Zn and Mn probably increased. However, the microminerals studied are within the tolerance levels stipulated for an individual component of feed in Slovak Republic.

Bacteria are one of the sources of danger in refeeding of animal excrete because of reinfection of animals and humans handling it. In the present research work, the litter exhibited non-pathogenic bacteria. *Staphylococcus epidermidis* though isolated occassionally from clinical diseases of domestic animals, but in general it is considered to be non-pathogenic (Scalan 1988). Litter based silages exhibit a negligible coliform population (Harmon *et al.* 1975), and the oxygen-limiting capability of polyethylene sheeting was useful in preserving the nitrogen quality of broiler litter while still providing a safe (pathogen-free) product (Ranking *et al.* 1993).

In conclusion, simple processing of broiler poultry litter by ensiling for a period of six weeks, preserved the nutritients studied. Both the concentrations of heavy metals, which are within the tolerance levels for feed, and the high nutritional value of fermented broiler poultry litter indicate its safe use as an animal feed.

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Bacteriological aspects of hygienic safety in several large scale slurry treatment plants in Germany

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Summary

Microbiological -hygienic investigations at different large-scale slurry treatment systems- described exemplary by the results of one plant (No. 2) - showed that a considerable reduction of the test bacteria could only be achieved with thermophilic treatment. If the solids of the slurry are separated after the thermophilic fermentation and properly composted the finished compost is free of salmonellas and can be used freely in agriculture.

The other plant (No. 1) investigated was so-called "root-area" treatment plant which showed no effect to reduce the numbers of *E. coli* or fecal streptococci.

If substrates like food scrapes and other biogenic material should be treated in anaerobic digestion plants together with slurry it is necessary that these materials are pre-heated before they are added to the slurry in the anaerobic digester because they can be infected with various pathogens.

The poster shows 5 technologically different slurry treatment systems and the results of the microbiological experiments performed each of them.

Key words: water protection areas, agricultural wastes, slurry treatment plants, aerobic and anaerobic treatment plants, tenacity tests with salmonellas and *Streptococcus faecium.*

Introduction

On the occasion of a comprehensive research program for the reduction of environmental affections caused by modern slurry treatment plants, the Institute for Environmental and Animal Hygiene of the University of Hohenheim had the task to cover the hygienic aspects of various slurry treatment plants and their products in the view of epidemiology (Metzger 1994).

In the light of this background each method was examined as to its hygienic efficiency. Input-output analyses and step controls in the individual sections of the process were carried out. Besides the determination of the number of enterobacteria, *E. coli*, fecal streptococci and salmonella, tenacity tests on salmonella (*S. senftenberg* W 775 and *S. enteritidis*) and fecal streptococci (*Streptococcus faecium* ATCC 6057) were carried out, using a germ carrier technique which had been specially further developed by Rapp (1995) and Böhm et al. (1997).

On the poster 5 examples of slurry treatment plants are introduced, each preceeded by a short description of the procedure. A summary of the results of the individual step controls of these plants is given as far as the quantity and reaction of the examined germs *E. coli* and fecal streptococci is concerned, as well as the existence of salmonella in final products.

The slurry treatment plants as a total can technologically be devided into aerobic plants (aeration system) and anaerobic plants (biogas plants with mesophilic (35° C) and thermophilic (55° C) operation, and plants with a separation of solids by means of various types of separators (Böhm et al. 1997, Fink and Winter 1997).

Further expositions of the subject show examplary results of microbiologically-hygienic examinations in a large-scale technical mesophilic anaerobic plant and on a 2-stage mesophilic/thermophilic anaerobic plant.

Characteristics of the Plants

Plant 1

(Anaerobic mesophilic slurry treatment and subsequent biological elimination of nutrients in a "root-areas" treatment plant (reed plant).

- Separation of solids and utilization of the solid as organic humic fertilizer.
- Pre-treatment of the slurry/liquid manure by a special ventilation method.
- Mesophilic anaerobic fermentation of the slurry separated in a biogas reactor.
- Treatment of the discharge of the biogas reactor in a "root-areas" plant purification facility.

Plant 2

(Treatment and utilization of liquid manure/slurry from dairy farming in a biogas plant with ammonia stripping)

- 2-step mesophilic/thermophilic anaerobic treatment of the liquid manure.
- Separation of solids from the liquid manure treated anaerobically and composting of the solids.

Results

Plant 1

Table 1 shows the result of the bacteriological examinations in plant 1. As far as the number of *E. coli* and fecal streptococci is concerned, there is no significance difference between the samples taken from the input and output of the biogas reactor., i.e. there is only a low degree of reduction of the germs examined and no reduction at all of the salmonellas (see **Tab. 2**).

Similar results were obtained from the samples of the input and output of the root digestion plant. Here, the average values of the samples taken from the output showed even higher germ contents in comparison to the input samples (Tab. 1). As against the values found in raw slurry however there is a reduction of *E. coli* and fecal streptococci within a range of approx. 3 logs.

Reeds in the root digestion plant seem to have only a low bactericidal effect on the salmonellas.

While in 2 of 5 input samples salmonellas could be detected, infection-causing agents could be found in 3 out of 5 discharge samples from the root digestion tank planted with reed (Tab. 2).

Moreover, 5 out of 6 samples of the solids separated from the slurry which had not undergone compost-preparation, contained salmonellas.

Table 1: Germ concentration in each processing step of plant 1

Sections examined	Number of controls	*Escherichia coli (E. coli)* [cfu/ml]			Fecal streptococci [cfu/ml]		
		Average	Maximum	Minimum	Average	Maximum	Minimum
Raw slurry	6	$9,7 \times 10^4$	$2,4 \times 10^5$	$1,5 \times 10^3$	$3,6 \times 10^5$	$1,5 \times 10^6$	$4,3 \times 10^4$
Input biogas reactor	6	$8,4 \times 10^3$	$2,4 \times 10^4$	$2,4 \times 10^2$	$4,6 \times 10^4$	$9,3 \times 10^4$	$9,3 \times 10^3$
Output biogas reactor	6	$1,1 \times 10^3$	$4,3 \times 10^3$	$1,5 \times 10^2$	$1,5 \times 10^4$	$3,5 \times 10^4$	$4,3 \times 10^3$
Input root digestion tank	5	$1,3 \times 10^1$	$4,2 \times 10^1$	n.d.	$1,2 \times 10^2$	$2,4 \times 10^2$	$4,3 \times 10^1$
output root digestion tank	5	$5,0 \times 10^1$	$1,5 \times 10^2$	n.d.	$1,4 \times 10^2$	$4,3 \times 10^2$	$4,3 \times 10^1$
Solids	6	$1,1 \times 10^4$	$4,8 \times 10^4$	0,36 in 10g	$9,2 \times 10^3$	$2,1 \times 10^4$	0,92 in 10g

n.d.: not detectable; cfu = colony-forming units

Table 2: Number of native salmonella isolations in the section examined on plant 1

Sections examined	Number of samples	Number of salmonella isolations
Raw slurry	6	n=6
Input biogas reactor	6	n=5
Output biogas reactor	6	n=4
Input root digest. tank	5	n=2
Output root digest. tank	5	n=3
Solids	6	n=5

Plant 2

The results in **Table 3** clearly show that the largest reduction of the number of germs examined (*E. coli* and fecal streptococci) takes place during the thermophilic stage (55° C) of the biogas plant. While on average values slightly above the detection limit could be found for *E. coli*, the fecal streptococci showe a value of $2,1 \times 10^2$ cfu/ml. During the thermophilic stage the number of fecal streptococci natively existant in the slurry was reduced by approx. 4 logs (Tab. 3). Salmonellas were found neither in the sections examined, nor in the composted solids.

Table 3: Concentration of bacteria in each processing step of plant 2

Sections examined	Number of controls	*Escherichia coli (E. coli)* [cfu/ml]			Fecal streptococci [cfu/ml]		
		Average	Maximum	Minimum	Average	Maximum	Minimum
Raw slurry	6	$9{,}1 \times 10^5$	$2{,}4 \times 10^6$	$2{,}4 \times 10^5$	$1{,}0 \times 10^6$	$3{,}8 \times 10^6$	$9{,}3 \times 10^4$
Output mesophilic reactor	6	$7{,}8 \times 10^3$	$2{,}4 \times 10^4$	$4{,}3 \times 10^2$	$1{,}8 \times 10^5$	$4{,}3 \times 10^5$	$2{,}4 \times 10^4$
Output thermoph.reactor	6	$0{,}6 \times 10^0$	$2{,}1 \times 10^0$	n.d.	$2{,}1 \times 10^2$	$9{,}3 \times 10^2$	n.d.
Muddled water	5	$0{,}5 \times 10^0$	$2{,}3 \times 10^0$	n.d.	$1{,}4 \times 10^1$	$4{,}3 \times 10^1$	n.d.
Solids	6	n.d.	n.d.	n.d.	$1{,}2 \times 10^2$	$4{,}3 \times 10^2$	n.d.

n.d.: not detectable; cfu = colony-forming units

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Dynamics of quantitative changes of index bacteria in composts made of municipal wastes

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Summary

Biological wastes designed for composting can contain different microbes including those, which are pathogenic for human beings and animals. The research undertaken allowed to define the survival rate of *Salmonella senftenberg* during the wastes composting process. The research proved, that the highest number of investigated bacteria existed in the side part of the pile during the whole period of research. During the experiments it was proved, that the temperature value, which was required for reduction of *Salmonella senftenberg*, was reached within the second week of composting process.

Key words: *Salmonella senftenberg*, composting, reduction of microbes

Introduction

Considering the hazard to natural environment, which appears from storing of municipal wastes, new technologies are searched for repeatedly to utilize these wastes (Roth 1993). One of such ways is to compost the municipal wastes using "DANO" system (Winiarska and Lekan 1991). The advantages of this method are as follows: the utilization of organic substance contained in the wastes, decrease in volume of useless wastes as well as the recycling of ferrous metals. However the use of final product as the addition for soils is conditioned, among the others, by the sanitary aspects, especially with respect to pathogenic microbes (Strauch et.al. 1993). The aim of this research was to evaluate the survival rate of index bacteria, *Salmonella senftenberg*, during the process of maturing of the compost made of municipal wastes.

Material and methods

To investigate the dynamics of quantitative changes of index bacteria, *Salmonella senftenberg* microbes were introduced into the composting pile, dimensions 15 x 4 x 1,5 m, namely into its top, middle and side parts. Every sample was prepared of 100 g of compost and 10 ml suspension of test microbes. The samples were taken once a week within the period of 14 weeks. Additionally, the measurements of temperature of composted mass were made in locations, where bacteria were introduced, and these measurements were made using the

electronic-digital thermometer, type WB-500. The external temperature was measured using a weekly thermometer, type TZ 18.

From every fresh taken sample, the weight portions of 1 g and 10 g of compost were prepared in 3 repetitions, and they were diluted in 9 ml and 90 ml of pepton water, respectively (Merk, Pepton-Wasser, Art. Nr 7228). From the weighed portions of 1 g, the series of dilutions were prepared in the range of 10^0 up to 10^{-9}. After 24 hours of incubation at 37 deg.C, 0,1 ml of sample was transferred from every dilution to the selective, breeding liquid substrate according to Rappaport (Merk, Salmonella-Anreichungs bouillon nach Rappaport; Art Nr 10236) and then it was incubated for 24 hours at 37 and 43 deg.C. In the last phase of determination, the material was sifted using the sterile aesa on selective agar BPL-A (Merk, Brillant, Phenolrot-Lactose-Agar nach Kaufmann; Art Nr 77236), which was incubated within 24 hours at 37 deg.C. The positive growth was proved by pale-pink colonies. In case of doubts, the colonies were sifted on XLD agar (Merk, Xylose-Lysin-Desoxycholat-Agar; Art Nr 5287), on which Salmonella gives positive result in form of black points, which include ferrous sulfide. In the final stage of analysis, the serologic test was made - the serum to agglutination in a droplet of Salmonella for HM antigen.

Results

The results of investigation on the dynamics of quantitative changes of *Salmonella senftenberg* bacteria are presented in **Table 1.** The number of microbes after two weeks since introduction of the sample ranged between 3,0 x 10^5 in 1 g of compost in upper layer of pile and 2,5 x 10^5 in 1 g of compost in side layer. The highest reduction of *Salmonella senftenberg* microbes was observed in the middle part of pile. When taking samples from three levels, during the whole period of research, the highest reduction of investigated microbes was found in the middle part of pile, where the highest temperature of composted mass existed. Moreover, no index bacteria were found in this part of the pile until 8th week of research, and this indicates faster elimination of *Salmonella senftenberg* bacteria on three levels investigated.

Table 1. Colony count of *Salmonella senftenberg* in 1 g of compost in weeks of experiments.

Sampling place	2 week	4 week	6 week	8 week	10 week	12 week	14 week
Top	3,0 x 10^5	2,5 x 10^3	2,5 x 10^2	1,5 x 10^2	1,1 x 10^2	4,5 x 10^0	n.d.
Middle	4,5 x 10^3	2,0 x 10^1	9,0 x 10^{-1}	n.d.	n.d.	n.d.	n.d.
Side	2,0 x 10^5	1,5 x 10^4	7,5 x 10^2	2,5 x 10^2	4,5 x 10^0	1,1 x 10^0	n.d.

n.d. - not determined

Table 2. Temperature (deg. C) in the weeks of experiments.

Sampling place	2 week	4 week	6 week	8 week	10 week	12 week	14 week
Top	40,6	41,9	49,1	43,2	39,1	35,9	35,9
Middle	42,9	44,1	50,9	45,8	40,1	38,8	38,8
Side	20,1	19,9	25,8	22,7	17,6	14,5	14,5
Outside temperature	+1	+2	+6	-1	-7	-1	-1

Table 2 presents the profile of temperature inside the experimental pile on various levels (top, bottom, side) against the external temperature. As appears from the data, the lowest temperature was noted in the side part of the pile and it was equal to 14,5 - 25,8 deg.C. On the other hand, the highest temperature was observed in the middle of pile, and it ranged between 38,8 and 50,9 deg.C. Distinct increase of temperature inside the clamp appeared in 6th week since the beginning of experiment, which could be caused by the increase of ambient temperature in as much as 5 deg.C. During the consecutive weeks, stabilization of temperature was observed, both inside the pile and in its environment. Barely in 10th week of experiment the external temperature was reduced in 1 to 7 deg.C, which was reflected by slight decrease of temperature inside the compost mass in 4,1 deg.C in upper part of compost, 3,7 deg.C in middle of the pile, and 5,1 deg.C in the side part of the pile.

However, the results obtained allowed to confirm, that assumed processing plants for composting of municipal wastes contribute to the release of pathogenic microbes after 8 weeks of composting. The number of microbes, which remained after the termination of composting in 12th week, was so low, that it can be assumed, that the compost is safe for soil and plants, and it can be used for land reclamation and be useful for agriculture.

Conclusions

1. The survival rate of *Salmonella senftenberg* index microbes depended on maturing time as well as temperature.
2. The reduction of index microbes was the highest in the middle part of the compost pile in the contrary to its side layer.

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The effect of natural zeolites on the release of ammonia from the solid fraction of pig slurry

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Summary

Experiments were carried out using the solid fraction of pig slurry collected on vibrating screens before the treatment of pig excrements in a wastewater treatment plant. Different doses of natural zeolite (clinoptilolite) were added and the mixture, together with the control, was stored at different temperatures. The concentration of ammonium in water extracts was strongly influenced by the zeolites and the storage conditions used.

Key words: natural zeolite, pig slurry treatment, ammonia

Introduction

Pig slurry produced by large-capacity farms poses a serious risk to the environment for its microbial contamination, high concentration of nutrients and gas emissions that arise at manipulation, storage and application of slurry. Moreover, the incorrect handling and utilization of slurry can lead to considerable economical losses.

Because of the large amount of pig slurry produced by large-capacity farms, this waste is frequently subjected to aerobic biological treatment consisting of several stages. In the first stage - mechanical pretreatment - the solid and liquid fractions of slurry are separated using vibrating or gravity screens, belt presses, centrifugation, etc. The solid fraction obtained in this stage contains a large number of bacteria, viruses, protozoa and helminths that can survive for different period of time. For this reason this fraction should be subjected to biothermic treatment before it is used in plant production [1]. In the process of its storage and composting The biological activity of microorganisms during composting of this substrate results in the changes in its physical, chemical and microbiological properties. The changes depend on a number of factors and can be affected by an addition of organic and inorganic materials that can improve the decomposition processes, increase the fertilizing value of the resulting material and decrease the loss of nutrients [2]. Due to ion-exchange and adsorption properties of zeolites and their high

selectivity for ammonium, these materials can contribute to better utilization of nutrients and reduce the environmental pollution resulting from animal production [3].

The aim of our study was to investigate the effect of an addition of natural zeolite (clinoptilolite) to the solid fraction of slurry on the processes that take place during the storage of this substrate at different temperatures.

Material and methods

The solid fraction of pig slurry used in the experiment was obtained by separation on vibrating screens in the first stage of biological treatment. Natural zeolite (clinoptilolite) from Nižný Hrabovec, Slovak Republic, was used in a powder form. The size of powder particles of the main fraction of zeolite (representing approx. 77%) ranged from 0.125 to 0.25 mm and CEC was 0.77 $mol.l^{-1}$. Two different additions of zeolite (1:10 and 1:100) were tested and the mixtures obtained were stored together with the control sample in dark, with the access of air, at 4°C, 20°C and 37°C for 3 months. In this period the content of dry matter (DM) at 105°C was determined after 1 week and 1, 2, and 3 months of storage and the values of pH, ammoniacal nitrogen (steam distillation and titration) and conductivity (HACH Conductivity/TDS meter, model 44600) were determined in water extracts in intervals specified above.

At the same time, the number of mesophilic (37°C, MP agar) and coliform bacteria (37°C, Endo agar) were determined by the common methods .

Results and discussion

The results obtained revealed that the addition of zeolites affected the content of dry matter during the storage of the solid portion of slurry. The content of DM was the highest in the mixture with the higher addition of zeolite at all temperatures tested while for the lower dose the differences were smaller and lasted for limited period of time. This can be explained by the structure and properties of zeolites due to which they can contribute to better utilization of water present in the substrate, and by continuous dehydratation also at temperatures exceeding those of DM determination (105°C).

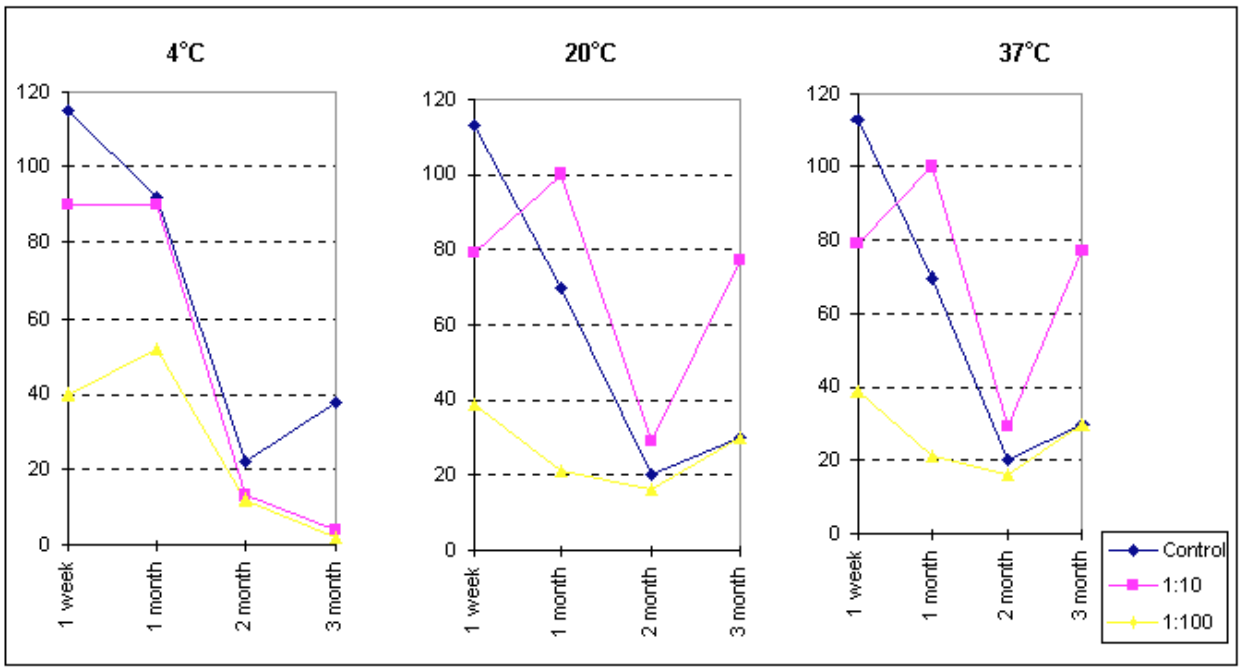
The value of pH in the water extract reflected the concentration of hydrogen ions in the solid fraction of pig slurry which depended on the processes of decomposition of this substrate [4]. The course of changes in pH was similar for all samples at all temperatures tested with the exception of substrates kept at 37°C. An acceleration of the initial decrease in pH and its deceleration later on was observed for the higher dose of zeolite.

Conductivity values of the extract express the total sum of anions and cations and are in direct relationship with the total concentration of dissolved substances. With few exception during the first two months of storage they reached the highest level in the control samples which is in an agreement with the ion exchange and adsorption properties of zeolites.

With regard to the structure and properties of zeolites mentioned a decreased rate of release of ammonia to the water extract was expected. The results obtained confirmed the expectations (see Figure). The course of changes in $N\text{-}NH_3$ concentrations was similar in all the samples, although there were differences in absolute values, in dependence on the zeolite dose, with the exception of lower dose of zeolite stored at 37°C. This sample exhibited the highest concentration of $N\text{-}NH_3$ in the extract almost for the entire experimental period. Whether this results from more favourable conditions for amonification or decreased rate of some other processes must be determined by additional experiments.

The number of mesophilic and coliform bacteria differed only by 1-2 orders of magnitude in the samples examined with no marked trend in the changes. The possibility of some influence of zeolites on the survival of coliform organism at 37°C cannot be excluded.

Concentration of ammonia in water extract in ml. l^{-1}



Conclusion

The use of zeolites in the treatment of pig slurry can contribute to better utilization of ammonium released from pig excrements. Besides alleviating the economic losses the load on the environment can also be decreased.

The results presented in this paper were obtained within the scope of solving the project No. 95/5195/575 and 1096/94.

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Food hygiene and salmonella prevention

THE NATIONAL CONTROL PROGRAMME AND SITUATION OF *SALMONELLA* IN FINLAND

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Abstract

The salmonella situation in live animals and at meat inspection had been very favourable in Finland for more than ten years before joining the EU in 1995. To maintain this situation an agreement was reached with the EU Commission of a comprehensive and active salmonella control programme in Finland and an authority to require guarantees of salmonella-free status of imported commodities. The control programme concerns bovine animals, swine and poultry for breeding, production and slaughter, and beef, pork, poultrymeat and eggs for direct consumption. Faecal samples from live animals, hygiene samples from animal housing, lymph node and carcass surface swab samples from slaughterhouses and samples of crushed meat and trimmings from meat cutting plants are taken according to schemes detailed in the programme. The number of samples is sufficient to detect a prevalence of 0.1 % at the population level (95% confidence). The samples are examined for salmonella accoding to approved methods in certified laboratories and the results are reported to a central register. The programme gives extensive instructions for measures to be taken if salmonella is encountered. The prevalence of salmonella in slaughterhouse samples of bovine animals was 0.8 % in 1995 and 0.5 % in 1996; in pigs 0.3 % both in 1995 and 1996. The poultry hatcheries and breeding and rearing stocks have been virtually free of salmonella; prevalence in egg production units was 0.1 % both in 1995 and in 1996. 3.8 % of broiler production flocks were positive in 1995 and 0.9 % in 1996; the corresponding figures for broiler meat were 3.1 % in 1995 and 0.8 % in 1996.

Introduction

Salmonella infections of domestic animals have been under scrutiny since the 1950's in Finland. A severe epidemic of *Salmonella* Infantis in broiler production in the early 1970's led to regular control and surveillance measures and gave eventually rise to a new concept for prevention of salmonella infections in newly hatched chicks (Nurmi and Rantala, 1973). This 'Nurmi' or 'Competitive Exclusion' concept has since gained worldwide acceptance. The prevalence of salmonella in bovine animals has been low for more than ten years; figures from meat inspection indicate an average level of 0.5 % (0.1 - 1.9 %). The actual prevalence in the cattle as a whole will have been considerably lower since the samples examined for salmonella are taken especially from carcasses originating from herds known to carry salmonella infections, and from sanitary slaughter. The salmonella prevalence in pigs remained below 0.1 % according to the meat inspection data. The percentage of salmonella positive broiler production flocks has been on average 2.0 % (0.5 - 3.8 %) in this decade (**Table 1**). These figures contrast favorably with those obtained from most countries outside Scandinavia. The good situation is also reflected in the low and roughly constant number for more than ten years of 1 000 annually reported human salmonella cases of domestic origin (**Figure 1**).

National control programme

To maintain the good situation even after joining the EU (and free transport of animals, food-stuffs and foods of animal origin) Finland negotiated an agreement with the EU Commission, whereby our commitment to an active salmonella control programme authorizes us to require that imported commodities carry a certificate of salmonella-free status if they come from countries not having a similar control programme. At the moment this means all EU-countries except Sweden.

The programme concerns bovine animals, pigs and poultry for breeding, production and slaughter, beef, pork, poultrymeat and eggs for direct human consumption. Overall strategies of the programme are:

- to prevent salmonella contamination at all stages of the production chain
- to monitor the production chain at critical points
- to activate necessary control measures if salmonella contamination is encountered

The aim of the programme is further to provide reliable surveillance data of the salmonella status especially in breeding animals and products of animal origin. On the slaughterhouse level the prevalence of salmonella positive samples is to be maintained below 5 % whereas on the country level the prevalence of infected animals or contamination of products must not exceed 1 %. The number of samples will be large enough to demonstrate salmonella infection or contamination even below these limits (with 95 % confidence). Bacteriological examination of the samples for salmonella is performed according to the ISO method 6579/1993 or NMKL 71/1991 (or amended editions of the latter, Decision 97/1072/EU).

Control in bovine animals

The control in herds is based on the examination of faecal samples from all clinically suspected animals. Bull calves sent to semen collection centres as well as the originating herds must be examined and found negative before entering the centre. Measures if salmonella is encountered in a herd include official restrictions of selling or transporting any animals and visits to the animal sheds, evaluation of the spread of the infection in the herd and isolation of the infected, cleaning and desinfection of the premises and a follow-up sampling with one month intervals. After two consecutive salmonella negative samples the restrictions can be lifted.

The control at slaughter includes a total of 3 000 lymph node samples and another 3 000 surface swab samples annually, divided between the 21 slaughterhouses according to the annual production.The sampling must be evenly distributed over the working day, week and each quartal of the year. Control at approved meat cutting plants is based on random sampling of crushed meat and trimmings, the frequency of which depends on the capacity (in kg/week) of the plant.

Control in pigs

Control in herds is based on examination of all suspected cases of salmonellosis at the farm or at the meat inspection. All pedigree breeding units and holdings which belong to the national swine health control scheme are tested for salmonella at least once a year. Also all boars sent to semen collection centres must be examined and found negative before entering the centre. Measures if salmonella is encountered in a herd follow the principles outlined above with cattle herds. Restrictions can be lifted after two consecutive salmonella negative samples a month apart. In herds with fattening pigs the restrictions are released when all animals have been slaughtered and the premises have been desinfected.

The control at slaughter includes a total of 3 000 lymph node samples and another 3 000 surface swab samples annually from both sows and fattening pigs, divided between the 18 slaughter-houses according to the annual production volumes. The sampling must be evenly distributed over the working day, week and each quartal of the year. Control at approved meat cutting plants is based on random sampling of crushed meat and trimmings, the frequency of which depends on the capacity (in kg/week) of the plant. Sow and fattening pig production lines are sampled separately.

Control in poultry

All categories of poultry are kept under surveillance. The sampling schemes for breeders, egg laying flocks and meat production flocks are shown in **Table 2** and the faecal sampling design in **Table 3**. Measures taken if salmonella is encountered include official restrictions, both

general and specific to the poultry category, of transporting birds or eggs, of visits to the sheds or houses, of tracing the source of the infection and of examination and/or destruction of eggs and dead birds and desinfection of the premises. Restrictions may be lifted after environmental samples taken by the official veterinarian are found salmonella negative.

The control in poultry meat establishments is based on examination of production hygiene and sampling is focused on cutting plants. Each production line for poultry is sampled separately, randomly during operation and at least once per week.

Isolation of salmonella from slaughterhouse or meat cutting plants causes intensified cleaning and desinfection and increased hygiene sampling until the samples turn negative. Measures are taken to trace back the source of the contaminated carcasses.

Situation of salmonella

The annual incidence of salmonella in cattle herds was 0.7 % in 1995 and 0.2 % in 1996. The higher level in 1995 was due to a spread of *Salmonella* Infantis via feed to cattle farms especially in the western part of Finland. The remarkably rapid decline in the numbers of salmonella-conta-minated cattle herds is a good example of how effective a joint control effort of all the parties from the herd owners to the veterinary officers of the MAFF can be. In swine herds salmonella has appeared only rarely.The numbers of lymph node and carcass surface swab samples taken at slaughter and those found salmonella positive for cattle, sows and fattening pigs in 1995-1996 are shown in **Table 4**.

The poultry hatcheries and breeding stocks have been free of salmonella in 1995-1996. Only one egg layer rearing flock was positive in 1995; no salmonella was detected in this category in 1996. 0.1 % of the egg laying flocks was positive in 1995, including the first appearance of the serotype *S.* Enteritidis in an egg production unit in Finland. The hens of the unit were destroyed and the unit was closed down for the time being. 0.1 % of the egg laying flocks was positive also in 1996. Rearing flocks for broiler production were free of salmonella in 1995; one flock was positive in 1996. This flock was slaughtered before start of the egg production. The numbers of broiler production flocks as well as the percentages of salmonella positive flocks in 1990 - 1996 are shown in **Table 1**. The percentages of salmonella positive broiler meat on kg basis were 3.1 % in 1995 and 0.8 % in 1996.

The main serotype both in cattle and in poultry is *Salmonella* Infantis. *S.* Typhimurium is found much less frequently, and serotypes *S.* Anatum, *S.* Isangi, *S.* Poona and *S.* Thompson are encountered only occasionally.

Conclusions

The laborious and even costly national control programme and painstaking efforts of the industry have enabled us to keep a favourable salmonella situation in domestic animals even as a member of the EU. The control programme is subject to EU legislation and repeated evaluations by the commission and the only justification for it will be our exemplary good salmonella situation.That can be maintained only through an unswerving and constant vigilance of everybody involved.

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Table 1. TOTAL NUMBER OF BROILER FLOCKS 1990 - 1996 AND THE %:s OF Salmonella POSITIVE FLOCKS AT THE AGE OF 4 - 5 WEEKS

Y E A R	NR OF FLOCKS	% POSITIVE
1990	2068	2,6
1991	2201	2,9
1992	2139	1,7
1993	1835	0,5
1994	2432	1,8
1995	2112	3,8
1996	2568	0,9

Table 2. Sampling scheme for breeders and egg layers

Age phase of birds	Time of sampling	Type of sample	Number of samples
BREEDERS			
Rearing	Day old	Chicken box lining Dead chicks	10 10
	4 wk and 2 wk before start of laying	Faecal	See Table 3
Egg production			
hatchery capacity < 1000	Every 2 wk on the holding	Faecal	See Table 3
hatchery capacity > 1000	Every 2 wk through the hatchery	Meconium from 250 or 50 chicks	Pooling done acc. to Community ref. methods
	Every 2 mo on the holding	Faecal	See Table 3
EGG LAYERS			
Rearing	1-2 wk before transport to laying unit	Faecal	See Table 3
Egg production	20-25 and 55-60 wk + 1-2 wk before slaughter	Faecal	See Table 3

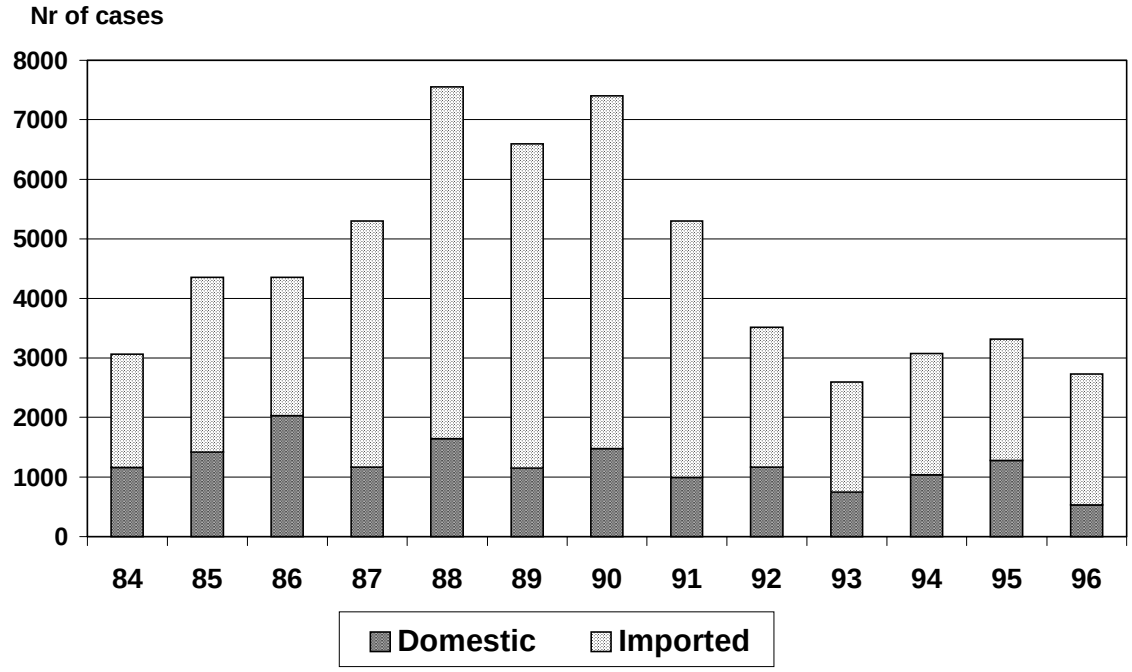
Table 3. Faecal sampling of poultry

Nr of birds kept in a building	Nr of faecal samples from the building or group of buildings on the holding
1 - 24	Nr = nr of birds up to 20
25 - 29	20
30 - 39	25
40 - 49	30
50 - 59	35
60 - 89	40
90 - 199	50
200 - 499	55
500 or more	60

Table 4. Salmonella surveillance at slaughterhouse level

	Lymph node samples			Surface swab samples		
	Number examined	Number positive	pos. %	Number examined	Number positive	pos. %
1995						
Cattle	2728	23	0.84	3208	24	0.75
Sows	2725	9	0.33	2867	4	0.14
Fattening pigs	2787	10	0.36	2993	4	0.13
1996						
Cattle	2550	6	0.24	2781	15	0.54
Sows	2627	8	0.30	2711	6	0.22
Fattening pigs	2683	5	0.19	2964	5	0.17

Figure 1. HUMAN SALMONELLA CASES IN FINLAND, 1984 - 1996



Field experiences in pre-harvest food safety for salmonella prevention

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Summary

The results and conclusions of a 3-year study (1994 to 1996) on monitoring and reducing *Salmonella* in a German poultry meat production chain supplying a fast food chain are presented. The production chain consists of one grand parent flock, several parent flocks with two hatcheries, 35 producers of chicken, one poultry slaughterhouse and one poultry meat processing plant. The aim of the study was to gradually lower the high *Salmonella* contamination rate of the final product toward zero. The study was designed in two phases: the *Salmonella* prevalence in animals and the *Salmonella* presence in and on materials in the environment of the animals were determined during phase I, and, the effectiveness of intervention measures for reducing the *Salmonella* load and cross-contamination on each production level was evaluated during phase II. The paper focuses on the pre-harvest area, i.e. the production steps prior to slaughter, describing the sampling strategy at farm level and the *Salmonella* findings in the animals and their environments. Recommendations for lowering the *Salmonella* prevalence in poultry meat production chains are discussed.

Key words: salmonella in poultry, pre-harvest food safety measures

Introduction

The aim of the study was to determine the reasons for the steady increase of the *Salmonella* contamination in the poultry meat produced at a slaughterhouse in Northern Germany, and, concluding from the results to implement pre-harvest food safety measures to markedly reduce the introduction of *Salmonella* into the prodiction line from broiler flocks supplying the slaughterhouse.

Material and Methods

Bacteriology: For this study, a modification of the ISO 6579 *Salmonella* detection method was developed . The method used consisted of pre-enrichment of the samples in buffered peptone water at 37°C, selective enrichment in tetrathionate brilliant green-broth and Rappaport-Vassiliadis-broth at 42°C, and culturing on Rambach agar and xylose lysine desoxycholate-agar at 37°C (Müller, K. et al. 1997).

Meat production chain*:* The poultry meat production chain consists of one grand parent flock, several parent flocks with two hatcheries, 35 producers of broilers, one poultry slaughterhouse and one poultry meat processing plant. The animals are sexed and kept on floor. Hens are fattened for 45 days, roosters for 59 days.

Sampling strategy: The *Salmonella* prevalence in animals and the *Salmonella* presence in and on materials in the environment of the animals were determined before (phase I = 1994) and after (phase II = 1995 to 1996) implementing intervention measures at farm level using the following standardized sampling plan: The day-old chicks were sampled at the hatchery and at arrival at the farm. During the fattening period, the flocks were investigated at days 14, 35 and 49. At each time, 60 fecal samples and 20 dead animals per flock were bacteriologically examined. Before restocking, each house was checked bacteriologically for the effectiveness of the cleaning and disinfection, and various samples from the environment of the farm were taken to estimate its *Salmonella* contamination

Results

Phase I: The *Salmonella* prevalence in the animals and in the environment of the animals before implementing intervention measures was: day-old chicks from grant parent flock 0%, day-old chicks from parent flocks 1 - 5%, feed 0%, water, straw, tools and the vicinity of the broiler houses 20 - 60%, broilers supplied for slaughter 60 - 80%, cut carcasses 60%, and the meat supplied for processing 30 - 50%.

Control measures according to the results of phase I: All persons were intensively informed on the sources and routes of infection. Technical defects on the farms were removed to ensure proper operating conditions. Hygiene plans referring to the risk factors detected were established. Cleaning and disinfection plans were improved. In all herds, stringent hygienic measures were introduced. These included regularly performed cleaning and disinfection of the houses and their surroundings, hygiene barriers and change of clothes for all persons, rodent and

beetle control. Positive parent flocks were slaughtered. Restocked parent flocks were vaccinated and fed with competitive exclusion flora to lower the risk of a new *Salmonella* infection.

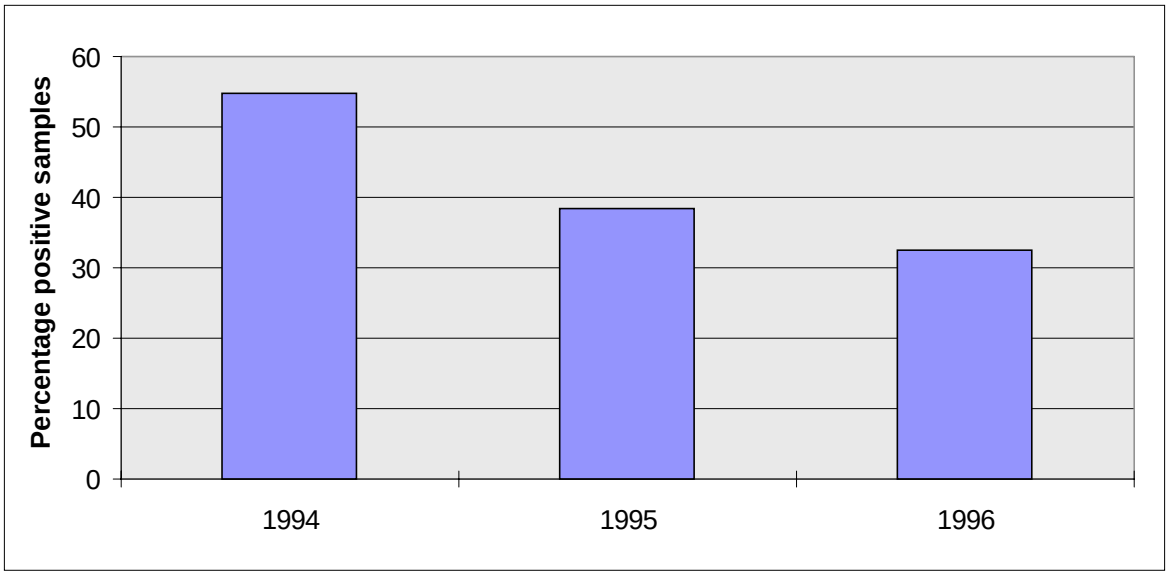
Phase II: The introduction of *Salmonella* via the day-old chicks into the broiler flocks could be reduced to almost 0%. This markedly reduction of the *Salmonella* prevalence in the day-old chicks, however, did not result in the intended reduction of the *Salmonella* prevalence in the broiler flocks at slaughter. Flocks that received *Salmonella* negative day-old chicks showed as high an intra-herd prevalence at 14 days as flocks that introduced *Salmonella* positive chicks.

These experiences showed clearly that control measures in the breeder flocks alone were not sufficient enough to reach the aim of the study. Therefore, the following management and hygiene measures were taken at farm level:

1) Change of shoes and clothing before entering the broiler houses, 2) Keeping the vicinity of the houses clear of waste and plants, 3) Intensive control of rodents and beetles, 4) Storing the litter far away from the flock, 5) Use of water from the community water supply, 6) Use of straw from low risk areas, 7) No use of feedstuffs that had remained from the preceding fattening period, 8) Cleaning and disinfection of the entrance and the surroundings of the broiler houses, 9) Disinfection of drinking water during the fattening period, and many other farm-specific measures more.

These control measures led to a significant reduction of the *Salmonella* findings at farm level and in the animals prior to slaughter. The effect of these pre-harvest measures on the final meat product is shown in Figure 1:

Figure 1. *Salmonella* findings in poultry meat



Conclusions

For successful pre-harvest food safety programs, the control of the vertical transmission of *Salmonella* (as described in the EU zoonosis directive) is not sufficient enough to get rid of *Salmonella* in poultry meat production chains. However, it is possible to reduce the *Salmonella* load in well-organized production chains, if each production level is taking adequate measures. To reach this aim in the pre-harvest area, broiler producers have to adopt *Salmonella*-reducing working procedures every day over a long period of time to reduce the contamination of the animals' environment and to prevent the horizontal infection of their flocks from the still *Salmonella*-contaminated environment.

Control measures such as vaccination or competitive flora are useful in lowering the *Salmonella* prevalence, but they are not able to replace on-farm hygiene and good management procedures.

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Eradication of prolonged bovine salmonellosis on Finnish farms

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Summary

Around 10 per cent of the 243 herds diagnosed with salmonellosis in the provinces of Vaasa, Oulu and Lapland in 1994-96, mainly those infected with S. infantis, were slower than others in eradicating the infection.To break the infectious chain, the critical points in feeding were examined by taking samples of the fodder and swabs of the feeding environment. Cleaning operations, especially feeding hygiene, were intensified through joint efforts by veterinarians and dairy advisors. About 75% of the farms were able to eradicate the infection, while 25% ceased milk production.

Key words: eradication of Salmonella, prolonged salmonellosis, contamination of feeds

The typical progress of Salmonella eradication.

Of the cattle farms diagnosed as having salmonellosis in the Salmonella project of 1994-96, 63.2% of those in the province of Oulu and 52.3% in the province of Vaasa were found to be free of the infection within four months. Experience gained from this project suggests that a target of six months could be set for eradication of salmonellosis. Prolonged problem cases become evident 3-4 months after the introduction of intensified cleaning measures. How soon herds are free of the infection depends on how long the herd had been infected before being diagnosed and on the percentage of cattle infected in the herd.

Cattle are infected with salmonellae by the oral route. The infection dose has been smaller since stress factors have increased, and natural infections have been described where animals are thought to have been infected by very small doses, particularly in their feed (Jones 1992, Selbitz, 1995). Cleaning operations at the farms were based on the assumption that the salmonella infection could be eliminated from the cows through their natural resistance while oral reinfection was being prevented. Stress factors were minimised by improving external conditions.

Salmonellae easily develop resistance to antibiotics (Selbitz 1995),and antibiotic treatment is not used in Finland except for clinically sick animals.

Once infection is detected, the veterinarian must draw up a cleaning and disinfection plan for the farm, including detailed written instructions on practical measures for eliminating the infection.

In the present cases the cleaning process started by reducing bacterial sources in the animal areas. The disease carriers were separated from the healthy animals and the shed was made less crowded by moving animals to other premises or taking them for slaughter. The shed was kept as dry as possible, avoiding the use of water in the animal areas.

The primary target for disinfection was the feeding table, where conditions were rendered adverse for salmonella growth. Contamination of the feeding table by human agency or via

equipment etc. and of the feed store was avoided. Lime was used to disinfect areas contaminated with manure and the manure pits. Further spread to other herds or food processing plants was prevented.

Recognition and investigation of problem farms

About 25 of the 243 salmonella herds diagnosed in the project in 1994-96 did not show much progress in the eradicate salmonella. Either the infection rate in these herds had not decreased as much as was intended or the infection had spread to previously healthy animals once the infected one had recovered.

More efficient cleaning programmes were then recommended jointly by the dairy health service veterinarian, the dairy advisor, the veterinary practitioner and the veterinarians in the community or the province. The reasons fore failure were thoroughly examined and the cleaning programme was renewed. Environmental samples were taken from all critical points in the feeding line in order to detect the route of infection. Samples were taken first from stored feeds, while swabs and samples were taken from uneaten feed all the way from the stores to the feeding table in front of the cow. The hygiene of the drinking troughs and feeding stations was investigated. The practical routines of feeding and the movement of people in the shed were studied. A floor plan of the shed was drawn up showing the respective routes of feed and manure, and areas were designated as being either clean or contaminated. The areas considered clean comprised the entire feeding line from the feed stores to the mouth of the cow and to the milking station, milk parlour and service areas. The dirty areas were all those in contact with manure: stalls, boxes, passages, exit passages for the animals, gutters and manure pits. This was explained to the herdsman, who was told how to work in the shed and in what order and what points were problematic crossroads between clean and contaminated areas where the infection chain had to be broken.

Reasons for failure

About 75% of the problem farms succeeded in eliminating salmonella after the introduction of the intensified cleaning programme. Two of the farms decided to give up dairying before the cleaning programme was estabilished. At three farms milk deliveries to the dairy were stopped because cleaning instructions had not been followed.

The failure of cleaning on the problem farms was to a large extent due to contamination of the fodder line. Concentrates had usually been contaminated, including grain stores and mills. The roughage was not often contaminated, but was occasionally a vector to the feeding table.

The slowest recovery was found on farms where feeds other than acidic AIV silage were used, e.g. TMR, dry hay or silage preserved without acid as roughage. Salmonella proliferates optimally under neutral pH conditions, and bacterial growth can be prevented by adding organic acids such as propionic or formic acid to the environment (Selbitz 1995). Acidic silage and acid disinfectants render conditions on the feeding table adverse to salmonella growth. The acidity of the fodder was adjusted by adding formic acid to TMR rations and to zero grazing feeds. Calves

recovered well after being given colostrum or milk fermented with lactobacilli in combination with strictly hygienic drinking vessels and a diet including acidic silage.

The problem farms were frequently reluctant to slaughter disease carriers. A high incidence of infection and slow recovery on some farms seemed to indicate that salmonellosis had been latent in the herd for a longer period of time. Economic difficulties hampered cleaning operations on a number of farms. A reasonably priced salmonella insurance policy was developed during the project, and this has since become available to producers all over the country via their local dairy or slaughterhouse.

The division of labour proved difficult on many farms, and there were a number of other reasons for failures in taking the appropriate decisions, often social ones. Indifferent attitudes towards cattle management and advisory services were not infrequent. With some exaggeration it could be said that the problem of the prolonged cases was not one of salmonellosis alone.

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SURVIVAL OF SALMONELLAE IN CATTLE SLURRY AND HIS CHEMICAL DISINFECTION

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Summary

The aim of this work is to measure the concentration in salmonellae in contamined cattle slurry, especially effects of compounds for disinfection. This studdy consisted in laboratory trials with contamined slurry with marked strain of *Salmonella typhimurium*, in observations and field trials in 25 infected herds. At 20 °C the concentration decreases quicker than at 15 °C. The others factors have few effect on decreasing, except adding some urea or calcical cyanamide. In the herds which are affected by clinical salmonellosis, acute disease persists around 2 months. The duration of slurry contamination depends on recurrence of the clinical signs. The decontamination of slurry can be obtained in 3 weeks by adding 4,5 to 6 kg of calcical cyanamide per m^3 in the tank. This result is interesting for a secure use of slurry as fertilizer on the pasture.

Key words : *Salmonella*, salmonellosis, slurry, disinfection,calcical cyanamide, decontamination, pasture,fertilizer, hygien, milk quality.

Introduction

Each year around 3 % of french herds are suffering of salmonellosis. Over the disease of the animals, this situation needs to protect the milk and to prevent its spreading in the environment. Till now it is adviced to wait 4 months before to use contamined slurry, most on pasture. The main aim of this studdy is to find others secured middles, easy to use and with an acceptable cost, to avoid all risks. On second level it seems interesting to describe on details the evolution of the disease on a quite big sample of herds.

Material and methods

The aim of all experimentations and observations is to control the variations of one variable, the number of salmonellae per ml of slurry, explained from 3 groups of factors :

physical and chimical characteristics of slurry, infection of herd, treatment of slurry.

Trials in laboratory

Some slurry with pH 7 - 7,3 and 7 - 8 % of dry matter is obtained from heifers'feces. The slurry is placed in columns of 200 liters and contaminated with marked strain of *salmonella typhimurium*, resistant to streptomycine and nalidixic acid, to get a concentration of 10^6 per ml. Different physical factors are applied on the slurry.

Observations in herds

25 herds are observed on the criterary of recent appearing of clinical salmonellosis, confirmed in the laboratory. The herds have a size between 30 to 70 dairy cows, with a level of production of 6 000 to 10 000 Kg of milk per cow and year.

4 types of housing are tested : cubicles or strawed areas, covered or not.

Trials of decontamination

In laboratory many physical and chimical processes found in litterature are done. No one of them appears efficient, easy to use and cheap. But to measure the effect of nitrogen matter on the decreasing of salmonellae, we add some with different matters. From this observation we choose calcical cyanamide (CaCN2) as the use for decontamination.

We find the minimum activ amount of calcical cyanamide in laboratory (4,5 Kg per m^3).

Then we test this method in herds as field trial to check the efficiency and the conditions of practicability (in 9 herds with one control tank of 50 m^3 in experimental station).

Results

Trials in laboratory

The strain of the studdied marked *S. Typhimurium* survives around 70 days at 20 °C (from an initial concentration over 10^6 per ml to a concentration under 10^2 in 7 weeks). At 15 °C we cannot find any salmonella after 4 months from beginning of storage (concentration still over 10^3 at 9 weeks), whereas at 10 °C the survival continues over 154 days. The level of the sample in a column of slurry, the agitation of it, its concentration in dry matter and the pH variations have no influence.

Clinical observations in herds

In 25 observed herds we find 5 serotypes : *typhimurium* (22), *montevideo* (1), *bredeney* (1), *anatum* (1), *infantis* (1) associated with *typhimurium*. The average duration of the first clinical time (succession of salmonellosis cases with less than 2 weeks between two) is 53 days ($\pm$ 25 days).

In 21 herds the first clinical case appears between beginning of July and end of Oktober.

In 2 herds the milk is contaminated during the clinical time. In 6 herds other types of animals are contaminated in the same time (beef cows, males, heifers, goats ...).

52 % of dairy cows have an hyperthermia, 48 % have diarrhea, 23 % have another symptom (especially respiratory), 3 % abort, 4,5 % die (1,8 cow per herd).

In 8 herds the decrease of milk production is important a long time. 54 % of the calves which are born before or after the clinical time have diarrhea and 15 % die.

There is no difference in the prevalence of clinical manifestations, neither depending of housing type, nor prescribed treatment by the veterinary, nor one vaccination done after beginning of disease, nor size of the herd, nor level of production. Hygienic measures takened after beginning of clinical manifestations don't change the prevalence of them.

Contamination of slurry

Compared with the artificial contamination in a laboratory, the time of natural contamination of slurry on the farms is much more variable.

Two main factors are associated to one duration of contamination over 7 months : the first one is the clinical recurrence, that means some new cases again after 2 weeks with no more. In the 11 herds without clinical recurrence the duration of contamination of slurry is under 4 months,whereas in the 14 others it is from 4 to 7 months in 4 of them and more than 7 months for the others ($p<0,01$).

The second factor is the percentage of calving during the clinical time : 35 % when the contamination is under 4 months, 60% when the contamination is over 7 months.

The calves born during the clinical time are more sick and die more ($p<0,01$).

In the herd with higher level of production the duration of contamination of slurry is longer, perhaps depending on more sensibility of the animals ($p<0,05$).

Decontamination of slurry

After adding CaCN2 at 4,5 Kg per m^3 of slurry, the concentration of salmonellae don't vary during one week. On the contrary their decreasing is important from the second week. From 4 weeks after the risks are very limited (concentration < 10^2 salmonellae per ml) and at 7 weeks the concentration is null, whereas in the control tank the concentration is still 9.10^2. It is to say that in the tank with liquid manure the calcical cyanamide is spread at 6 kg per m^3 (the decreasing was slowlier than in the slurry).

The calcical cyanamide is mixed with slurry in different ways : with a specific mixer in the tank, with a mixer on the tractor, with a mixer-crusher, with a strong staff, with suction and force pump. With this last method the homogenization is quick : 5 to 30 minutes when 1,30 to 2 hours

with other way. The farmers consider that there is few contraints for its use, even 3 of them say that they have small irritations.

The average cost of the treatment is 19 FF (3,5 US $) per m^3 or 68 FF (12,5 $) per cow.

Half of the farmers consider that this cost is acceptable compared with the incurred risks, the others prefer to wait the long decontamination and then use it on cultivated areas.

Conclusion

Even the survival time of salmonellae can be very long [2] in the best conditions, their decreasing in a contaminated slurry is quick enough to be under a level of risks (< 10^2 per ml) in some months. But two groups of factors have to be considered to avoid the diffusion of the disease from the use of slurry. The first is recurrence of the disease, the slurry can be contaminated more than 7 months. When the calving takes place most during the first clinical time, the duration of contamination is also long. It seems that a high level of production increases the duration of contamination. The second group is about practical level : the tanks are not always big enough to wait for decontamination. This studdy brings a new point of view instead of the usual advice to wait always some months before to use slurry. Ca CN2 appears for the first time as an efficient and easy way to disinfect the slurry, with lower concentration than in previous studdies [1, 3].

This studdy brings also some precisions about the influence of the chimical and physical factors, that are sometimes considered as responsable of the duration of contamination. Some more details about the disease : the time of appearing in one herd; the duration of the disease in the herd; the excretion due to the recurrence of the disease.It is important to control the slurry contamination for its use on the pasture and for all food chain [4].

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Roles of animals as infection sources of human salmonellosis

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Summary

This study evaluates the relative importance of cattle and poultry as infection sources of human salmonellosis. *Salmonella enterica* serovar Infantis (*S.* Infantis) accounts for the majority of *Salmonella* infections in production animals and belongs to the three most common serovars of endemic human salmonellosis in Finland. *S.* Infantis isolates from broiler chickens and cattle can be differentiated by molecular typing. The isolates from the endemic infections in cattle, poultry and humans in 1995 were analysed. Cattle was the major source of human infection: 90% of the human infections were caused by the *S.* Infantis strains prevalent in cattle. Molecular typing connected only 6% of the human infections to broiler chickens. The results demonstrate that the spread of *Salmonella* from broiler chickens to humans can be efficiently prevented by control programmes, heat-treatment of the contaminated meat and consumer advice.

Key words: *Salmonella*, epidemiology, molecular typing

Introduction

Food of animal origin is regarded as a major source of *Salmonella* infection. *Salmonella enterica* serovar Infantis (*S.* Infantis) is the predominant endemic *Salmonella* serovar in the production animals in Finland. It was introduced into the broiler chicken production more than 25 years ago, and subsequently hundreds of infections occurred in humans, too (Nurmi et Rantala, 1973). The serovar accounts for roughly 80% of all the *Salmonella* infections in poultry ever since. *Salmonella* Infantis spread to cattle farms in the 1990s as well and is at present by far the most common bovine *Salmonella*. The prevalence of *S.* Infantis is, however, not high: acccording to the national surveillance programme, 3% of the broiler chicken flocks and less than 1% of the cattle farms were infected with *S.* Infantis in 1995.

Salmonella Infantis belongs to the three most common endemic *Salmonella* infections in humans with no previous contacts to foreign countries. The incidence rate was 1.8 cases per 100 000 population in 1995. For historical reasons broiler chickens have been regarded as the main

source of human *S.* Infantis infections. A cost-benefit analysis of *Salmonella* control programmes of production animals requires knowledge of the real risk associated with various infection sources. This study evaluates the relative importance of cattle and poultry as infection sources of human salmonellosis in Finland.

Materials and methods

Salmonella Infantis isolates from cattle and poultry sources were obtained from the laboratories of the National Veterinary and Food Research Institute in Helsinki, Kuopio, Oulu and Seinäjoki. The isolates from human infections were from the National Public Health Institute, Laboratory of Enteric Pathogens, Helsinki. Human infections were classified to be endemic if there had been no direct or indirect contact to a foreign country prior to the infection. All the available isolates from the endemic *S.* Infantis infections that occurred in Finland in 1995 were included in the study: 71 out of the 90 human infections, 240 out of the 242 infected cattle farms and 119 isolates from poultry.

Molecular typing was based on pulsed-field gel electrophoresis (PFGE) of bacterial and potential plasmid DNA digested with restriction enzymes *Xba*I, *Sfi*I or *Spe*I for the determination of the macrorestriction profiles, pulsed-field (pf) types (manuscript submitted). The same preparations were digested with S1 nuclease before PFGE for the detection of the plasmid DNA in a linearised form (Barton et al., 1995, manuscript submitted). The pf types could be further subdivided into plasmid types.

Results and discussion

The endemic *S.* Infantis isolates can be divided into two major pf types, pf1 and pf2. The types associate with the production animals in a constant manner (manuscript submitted). The type pf1 was found almost exclusively among cattle, while less than 1% of the broiler isolates were pf1. Broiler isolates were regularly (93%) pf2 (manuscript submitted). Five percent of the cattle isolates were pf2 and 21% other less common types.

Most of the human infections (90%) were caused by the pf-types typical of cattle infections, i.e. had plasmid types of pf1 similar to those in cattle or other less common pf types associated with cattle (manuscript submitted). Based on the name and location of the affected humans and farms we estimated that at least 60 % of the human cases had been contracted either by direct contact or by drinking contaminated, unpasteurised milk. Four human isolates (6%) had the genotype pf2 more prevalent in broiler chickens than cattle. Only one isolate had a particular plasmid type of pf2 that was associated with an outbreak in a broiler chicken company in 1995.

There were three human isolates with strange genotypes that were not likely to originate from the endemic infection. According to the typing results the classification of the human infections into foreign and endemic is reliable: 95 % of the infections classified as endemic had endemic pf types.

The study shows that it is possible to prevent the spread of infection from chickens to humans by having strict in-house control programme at the hatchery and farm level. Further infections at the farm level can be prevented and the broiler carcasses from the contaminated flocks heat-treated before the market. The consumer advice on how to prepare the foodstuffs that may be infected plays an important role besides the preventive procedures of the authorities and producers.

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A METHOD TO APPRECIATE POULTRY HOUSES DECONTAMINATION WITH PARTICULAR ASPECTS ON *SALMONELLA* PREVENTION

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Summary

A count plates method was used to appreciate poultry houses decontamination. This method was carried out in ten broiler houses and ten pullet houses, before entering day-old chicks. Two culture media (VRBG and ENTEROCOUNT) and localisations of samples were compared (feeders and base of walls). Residual contamination of poultry houses appreciated by the count plates method was compared to the *Salmonella* status of the house. Results obtained with both culture media were related and residual contamination observed on feeders and on base of walls were not significantly different. The control of decontamination realised with VRBG count plates was significantly related with the *Salmonella* status.

Key words : decontamination control,count plates, poultry houses, *Salmonella*, broiler, pullet.

Introduction

The persistence of *Salmonella* in poultry environment is a major hazard for the recontamination of successive flocks (Baggesen et al., 1992 ; Davies and Wray, 1996a). In order to recover *Salmonella* in a decontaminated poultry house, it is necessary to associate several samples to increase sensitivity of detection (Caldwell et al., 1994 ; Davies and Wray, 1996c). Count plates method can be used to appreciate decontamination in poultry houses (Drouin et al., 1988) or in pig weaning facilities (Foucher and Madec, 1997). This fast and simple technic could contribute to evaluating hygienic conditions before entering day-old chicks in the house. The aim of this work was to investigate if residual contamination appreciated by the count plate method was related to the *Salmonella* status of the house.

Material and methods

1. Sample

This study was carried out in ten broiler houses and ten pullet houses, in Brittany (France) from november 1995 to july 1996. Samples were taken in the house just before day-old chicks were placed in.

2. Decontamination control

25 cm^2 count plates were used and two culture media were compared : ENTEROCOUNT for thermotolerant streptococci (AEB122710C, AES laboratory, Combourg, France) and VRBG medium for total enterobacteria (AEB153202, AES laboratory, Combourg, France). Each one contained a desinfectant neutralising solution (507114, LCB laboratory, Combourg, France). sixteen count plates of each medium were applicated firmly until crushing in each house for 5 seconds. Sample sites are summed up in **table 1**. After application, count plates were stored at +4°C during transport and incubated at 37°C for 24 hours. After incubation, plates were counted and over 300 colony forming unit (cfu), plates were considered « invaded ». Plates with confluent colonies or invaded by fungi were eliminated.

3. *Salmonella* status of the house

Samples were taken with sterile gauze swabs impregnated with buffered peptone water and a desinfectant neutralising solution (507114, LCB laboratory, Combourg, France). The sampling procedure described in table 1 was repeated in the four quarters of the house. Gauze swabs were incubated in buffered peptone water at 37°C for 24 hours for pre-enrichment. Enrichment was realised with Rappaport-Vassiliadis medium (MSRV) and tetrathionate medium at 42°C for 24 hours. Xylose Lysine Tergitol 4 medium was used for plating (24 hours at 37°C). The poultry house was declared « *Salmonella*-positive » if one of the 16 samples was positive.

4. Statistical analysis

Systat 5.1 software was used. Colony counts led to a four-cluster repartition. Spearman correlation rank test was used to compare ENTEROCOUNT counts and VRBG counts. The Mantel-Haenszel (MH) $\chi 2$ test was used to compare on the one hand, the contamination levels observed with the broiler production and the pullet production, and on the other hand, the contamination levels observed on the feeders and on the base of walls. In order to compare the control of decontamination with the *Salmonella* status of the house, the level of residual contamination of each house was defined by the median of the counts done on the 16 count plates applicated per house. The distribution of the 20 medians was compared to the 20 *Salmonella* status thanks to the Fisher exact test. The level of significance was $p<0.05$.

Results

1. Levels of contamination in the two kinds of production

The 10 broiler houses were globally more contaminated than the 10 pullet houses. The part of count plates with more than 30 cfu was more important for broiler houses than for pullet houses when the analysis was adjusted on the kind of medium ($\chi 2(MH)= 19.3$, $p<0.001$).

2. Comparison between the two culture media

Counts realised with the two media were significantly related (Spearman correlation rank coefficient = 0.56, $p<0.001$).

3. Application sites (feeders or base of walls)

When adjusted on the kind of production (broiler house or pullet house), the levels of contamination of the feeders and of the base of walls were not different ($\chi 2(MH)= 2.3$, NS).

4. Relation with the *Salmonella* status of the house (table 2)

Residual contamination appreciated by VRBG counts was significantly related with *Salmonella* status evaluated by the swab-based procedure ($p=0.02$). No relation was found when residual contamination was appreciated by ENTEROCOUNT counts.

DISCUSSION

Levels of contamination on both sites of application (feeders and base of walls) were slightly different. Thus, it was difficult to define a « limiting factor-site » which could represent a good indicator of the microbial contamination of the house. It has been shown that contamination was more important on rough surface or difficult to desinfect (wall bases) (Drouin et al., 1988). The difference observed according to the kind of production could be explained by the different kinds of materials used. In addition, the presence of feedstuff or dust feedstuff in the feeders made counting be difficult and led to keep only the site « base of walls » as an indicator. VRBG counts were related with *Salmonella* status of the poultry house, even with a small sample of 20 poultry houses. This underlines the importance of decontamination in the control of resident *Salmonella* in poultry houses (Fris and Van Den Bos, 1995 ; Davies and Wray, 1996b). Such a method could be a supplementary predictive method of the *Salmonella* risk before entering day-old chicks in the house. Results obtained with both culture media were related, thus the fact that ENTEROCOUNT counts were not related with *Salmonella* status might be imputed to a lack of power (small sample). Such a comparison realised on a large sample and using only base of walls as an indicator would be worth carrying out.

ACKNOWLEDGEMENTS

The CNEVA Ploufragan and authors thank « Region Bretagne, Direction Economique et de la Recherche » for its financial contribution.

Table 1 : Sampling procedure : sites and quantity

SAMPLES	SITES	Quantity per quarter
ENTEROCOUNT count plates	base of walls	2
	feeders	2
VRBG count plates	base of walls	2
	feeders	2
SWABS	walls (10 m^2)	1
	feeders (10)	1
	air systems (10 m)	1
	drinkers (10)	1

Table 2 : Relation between VRBG counts and *Salmonella* status of the house

		Salmonella	
		Negative	**Positive**
cfu / plate (VRBG)	**< 30**	9	1
	≥ 30	3	7

p = 0.02 (Fisher exact test).

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Salmonella screening in broiler flocks in combination with slaughter ranking - a programme zu reduce the *Salmonella* contamination of poultry carcasses

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Summary

169 broiler flocks were tested for *Salmonella* 2 to 3 weeks before the scheduled slaughter date by taking drag swabs and collective faeces samples (Zoonoses Directive, 92/117/EEC). *Salmonella* were detected in 37% of the samples. By comparison, if the test was performed by 9 cloacal swabs as required by the Poultry Hygiene Regulation (BGBl. 274/1991), only 19% of the samples were positive.

In the abattoir, *Salmonella* negative broiler flocks were ranked first for slaughter in 7 cases. In 6 tests, 0 to 13% of the carcasses were found to be contaminated with *Salmonella* when the whole carcass rinse technique was performed. When the skin test was performed, which involves the removal of a 100cm^2 skin patch, *Salmonella* were found in only 0 to 6% of the carcasses. In one case, however, 56% (rinse sample) and 20% (skin) of the carcasses were contaminated with *Salmonella enteritidis*, respectively. The investigation of *Salmonella* positive broiler flocks revealed a high number of positive results for the skin samples too, in particular for the serovar *S. enteritidis*.

Key words: broiler flocks, *Salmonella* screening, slaughter ranking

Introduction

In central European countries, almost 100% of poultry carcasses are contaminated with Salmonella. Many attempts have been made to reduce the *Salmonella* contamination of poultry meat. These activities ranged from *Salmonella* monitoring and vaccination programmes for the parent flocks to the application of decontamination procedures in the abattoir. Faced with this situation, the government of Austria passed the „Poultry Hygiene Regulation“ (BGBl. 274/1991). According to this regulation, 9 cloacal swabs are to be taken from broiler flocks 3 weeks before the slaughter date. However, due to methodological and biometric weaknesses, these legal measures failed to produce the desired effect. The high degree of contamination of the carcasses with *Salmonella* can be attributed to two factors: firstly the failure to identify infected broiler

flocks, and secondly the cross contamination between positive and negative animals in the abattoir.

The aim of the project was to subject broiler flocks to a careful *Salmonella* examination 2 to 3 weeks before the slaughter date. The allocation of different slaughtering times to positive and negative flocks in a well cleaned and carefully desinfected slaughtering house should reduce the degree of contamination, even in the short term.

Material and methods

2 to 3 weeks before the slaughter date, 169 broiler flocks were tested for *Salmonella* by drag swabs and cloacal swabs. For a period of 5 weeks, the slaughter line and specially the scalding tank were cleaned and desinfected according to a regime of tight controls. Twice weekly, 6 samples of the scalding water (500 ml each) were taken before the slaughter and examined. Then, during a period of 3 months, 7 *Salmonella* negative and 4 *Salmonella* positive flocks were tested to evaluate the efficiency of the programme. In each case, 30 carcasses were analysed after air cooling. 100 cm^2 of skin, taken from the breast and back, were used as samples and introduced in 100 ml buffered peptone water. Then the carcasses were rinsed with 300 ml buffered peptone water. All samples with exception of the cloacal swabs were preenriched for 16 hr at 37°C. 3 drops were then transferred to MSRV medium. The confirmation of positive samples was performed serologically.

Results

37% of the samples taken from the 169 flocks examined by drag swab samples were positive 2 to 3 weeks before the slaughter date. By contrast, only 19% were diagnosed to be *Salmonella* positive when the animals were tested by 9 cloacal swabs per flock. It should be added, however, that the efficiency of the cloacal swab samples as compared to drag swabs improved when there was an occurrence of *S. enteritidis*.

Before the examinations in the abattoir began, the cleaning and desinfection measures were tightened. It took 3 weeks to eradicate *Salmonella* in the scalding tank. The carcasses were examined after a further 2 weeks, as there were now sufficient *Salmonella* negative results for the scalding water.

30 carcasses from 7 *Salmonella* negative flocks were examined for the presence of Salmonella. The percentage of positive carcasses identified by the whole carcass wash technique was 0 to 13%. For skin sample, the comparable figure was 0 to 6% (see **fig. 1**).

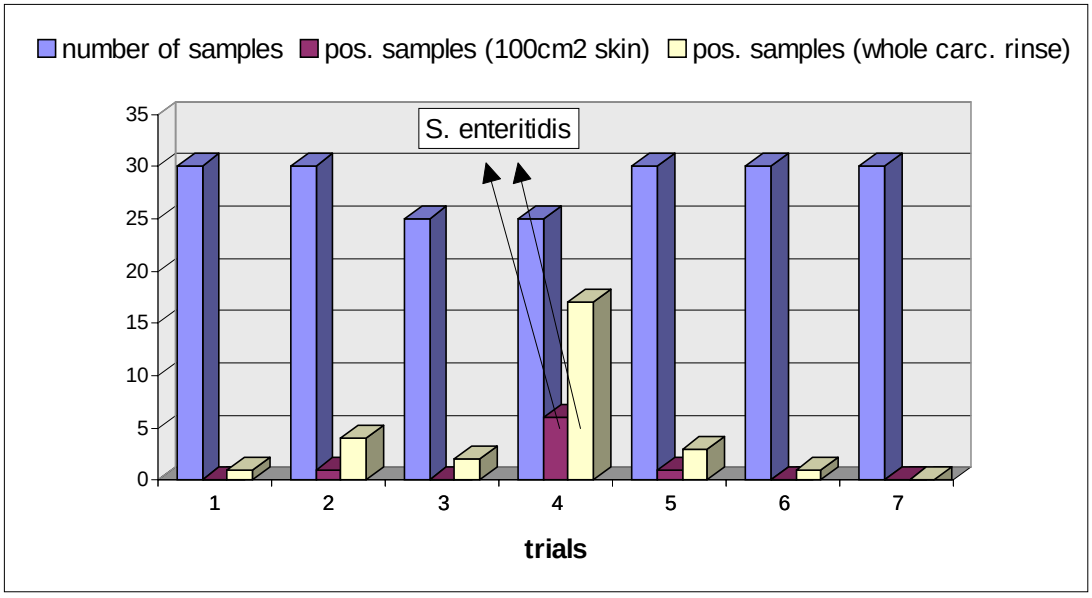

Figure 1: Number of positive carcasses of *Salmonella* negative broiler flocks (rinse and skin samples)

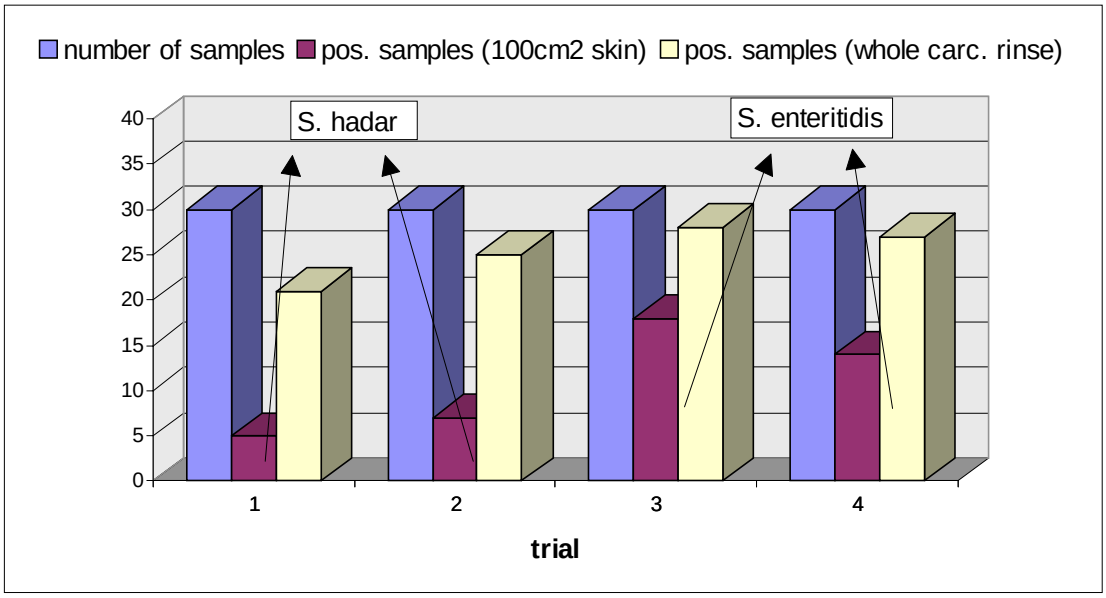

Figure 2: Number of positive carcasses of *Salmonella* positive broiler flocks (rinse and skin samples)

In one case, the degree of contamination was considerably higher, reaching 56% and 20%, respectively . As expected, the examination of *Salmonella* positive flocks resulted in a high percentage of positive *Salmonella* tests. The percentage of positive skin samples was extremely high, in particular when *S. enteritidis* occurred (**fig. 2**).

Conclusions

Our results showed that *Salmonella* screening of the broiler flock and separate slaughter of *Salmonella* positive and negative flocks can considerably reduce the contamination of carcasses with *Salmonella* as cross contamination is prevented. The efficiency of the method, however, greatly depends on the quality of the cleaning and disinfection measures in the abattoir. Examinations by Käsbohrer et al. (1995) showed that even in the case of *Salmonella* negative flocks, the percentage of positive samples after the slaughtering and processing stage is still high, and that intensive cleaning and desinfection measures are indispensable to achieve a noticeable reduction. Our investigation confirms the problems caused by this situation. It took 3 weeks of intensive efforts and targeted cleaning and desinfection measures to eradicate *Salmonella* in the scalding tank. Even though the suggested method did considerably reduce the entry of *Salmonella* into the food chain, priority should be given to the sanitation of the parent and broiler flocks. The differentiation of the individually isolated *Salmonella* strains showed that the infection pressure on broilers is particularly high if *S. enteritidis* occurs.

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The role of competitive exclusion in poultry management

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Summary

Competitive exclusion (CE) or the "Nurmi concept", in combination with conventional hygienic measures, has been shown to be very effective as a preventive measure against *Salmonella* infections in poultry. The main application of the concept is in newly hatched chicks or turkey poults, but it can also be used in older birds after oral antibiotic treatment. In addition to *Salmonella* CE has been shown to be effective against pathogenic *E. coli, Yersinia, Campylobacter* and *Clostridium perfringens*, and claims have also been made that CE treatment enhances the growth and decreases the mortality of birds. In a recent study the commercial CE product BROILACT® was shown to improve nutrient digestibility, and increase the metabolizable energy of the feed, viscosity of the ileal contents, percentage of faecal dry matter and the production of volatile fatty acids.

Key words: competitive exclusion, *Salmonella*, poultry production, growth enhancement

Introduction

Chickens are known to be very sensitive to salmonella infections during the first weeks of life. Resistance to the infection develops with the establishment of adult type intestinal microflora (Milner and Shaffer 1952; Seuna 1979). This progress can be accelerated by exposure of newly-hatched chicks to caecal or faecal bacterial flora from adult fowls (Nurmi and Rantala 1973). The phenomenon by which the normal intestinal microflora protects the host against invading pathogens is called competitive exclusion (CE) or the "Nurmi concept". Protection depends upon the administration of viable anaerobic bacteria, but the mechanism of CE is still poorly understood. The two most often cited in connection with CE are: production of volatile fatty acids in the caeca, and occupation of sites on the mucosa (Rolfe 1991).

There is an increasing interest in CE all over the world and especially in Scandinavia where the standard of hyginene in poultry production is very high and the sales of *Salmonella* contaminated meat is prohibited by regulations of MAFF the concept is succesfully applied to broiler production.

Pathogen and host specificity

The applicability of CE has been tested in numerous laboratory scale trials around the world using *Salmonella enterica* serotypes Infantis, Kedougou, Typhimurium or Enteritidis as the

challenge organism (Schneitz 1993). These studies suggest that the CE concept applies to all serotypes capable of intestinal colonisation.

Although the concept was first tested and designed to control *Salmonella* infections in poultry it has been shown to be effective against *Yersinia* (Soerjadi-Liem 1984), *Campylobacter* (Mead *et al.* 1996), pathogenic *Escherichia coli* (Hakkinen and Schneitz 1996) and *Clostridium perfringens* (Elwinger *et al.* 1992).

Turkey poults are effectively protected with native chicken microflora and *vice versa* (Schneitz 1993). Some protection was afforded to chicks by floras of other bird species (Snoeyenbos *et al.*, 1979) but floras of cow and horse were uniformly ineffective (Rantala and Nurmi 1973).

Field experience

CE preparations have been used in Finland since 1976 and now more than 90% of Finnish broilers are given the treatment. The long term use of CE preparations is considered to have contributed significantly to the favourable *Salmonella* situation in Finland. *E.g.* the percentage of *Salmonella* contaminated broiler flocks in this decade has been on average 2.0 (range 0.5 - 3.8%, Annual Reports of The National Veterinary and Food Research Institute). In 1987 the commercial competitive exclusion product BROILACT® substituted the original CE preparations.

In Sweden, the CE treatment has been used since 1981. During 1981-1990, 179 flocks, involving 3.82 million chicks, were treated and only one of these flocks was found subsequently to be contaminated with *Salmonella* (Wierup *et al.* 1992).

Successfull field studies have also been conducted by Blankenship *et al.* (1993) and Corrier *et al.* (1995).

Because the CE treatment is prophylactic rather than therapeutic, the chicks should be *Salmonella*-free prior to treatment. However, the *Salmonella* reducing effect of CE treatment has also been shown in those flocks that were *Salmonella* positive in the hatchery (Bolder *et al.* 1995).

The main application of CE is in newly-hatched chicks and turkey poults, but it has also been used successfully in older birds after oral antibiotic treatment (Johnson 1992; Reynolds *et al.* 1997).

Other benefits acquired by use of CE

In addition to pathogen control, claims have been made that CE treatment enhances the growth and decreases the mortality of birds. Corrier *et al.* (1995) reported an improvement in the efficiency of feed utilization in broiler flocks that were given CE treatment on the day-of-hatch. Higher bodyweight and lower mortality were also noticed by Bolder *et al.* (1995).

To explain the nutritional effects of the treatment a laboratory scale study was conducted with BROILACT®. Broiler chicks were treated orally on the day-of-hatch and ileal and caecal samples were taken at 12 and 31 days-of-age. The results of the study showed that BROILACT®

decreased the viscosity of the ileal contents and increased the faecal dry matter content. It also improved the ME value of the feed by 1.6%, increased the concentration of propionic acid in the caecal contents and decreased that of butyric acid in the ileal contents (Schneitz *et al.* 1997).

Conclusion

Competitive exclusion or the 'Nurmi concept' has been proven to be very effective as a preventive measure against *Salmonella* and other enteropathogens in poultry. However, it must be remembered that CE treatment is no panacea which compensates unsatisfactory production hygiene but an additional tool in a proper salmonella control program. Improvement of bird performance is also of great importance from the producers' point of view. It may encourage them to use the concept, and thus diminish the spread of poultry-borne pathogenic bacteria, especially *Salmonella* to humans.

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MOLECULAR TYPING OF SALMONELLA SAINT-PAUL RELATED TO AN OUTBREAK IN SOUTHERN GERMANY

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Summary

Three molecular typing methods, a repetitive sequence-based (rep) PCR fingerprinting, plasmid profiling, and arbitrarily primed (AP) PCR fingerprinting, were used to characterise isolates of Salmonella Saintpaul most of which were obtained from epidemic human cases of food-borne salmonellosis, together with some from the suspected food material, and a few from other cases apparently not related to the epidemic. With either of the three methods, the epidemic strain was adequately discriminated from other strains of the serovar. Of the two fingerprinting methods, repPCR fingerprinting was more discriminative.

Introduction

Plasmid profiling, arbitrarily primed (AP) PCR fingerprinting and lately, repetitive sequence based (rep) PCR fingerprinting, have been extensively used to characterise strains of various bacterial species. This study describes the usefulness of the three molecular typing methods to discriminate the epidemic strain of S. Saintpaul from among several isolates of the serovar recovered from human stool samples during the course of an epidemic as well as from sources not related to the epidemic, which was coused by paprica containing food items.

Materials and Methods

The isolates were divided into three groups. Group 1 consisted of five isolates obtained from suspected food material (potato chips); group 2, thirty nine isolates from human cases of salmonellosis; and group 3, seven isolates obtained at other times not temporary related to the epidemic and in one case, from a different source other than man (poultry). Crude DNA extracts used in rep-PCR were prepared by boiling. DNA from isolates which should be used in AP-PCR was purified further by extraction with phenol/chloroform. In the PCR a hot start protocol was used containing a wax bead (Invitrogen) with 3.5 mM $MgCl_2$. The methods will be published completely by Beyer et al. (1). For plasmid profiling about half a loopful of fresh colonies from agar plates were lysed according to Kado and Liu (2) with modifications described by Beyer et al. (1). Visual examination of fingerprint patterns was based on criteria described by Vandamme et al. (3). Statistical comparison of the PCR fingerprints was performed either as unweighted comparison of peak positions or as comparison of peak position and the values of densitometric scan and clustered by the UPGMA, using a computer assisted analytical program, DNA Fingerprint Analyzer (Wincam 2. x Modul, Cybertech, Berlin).

Results

Table 1 summarizes results of rep-PCR fingerprint patterns obtained from isolates of all groups of S. Saintpaul. All the isolates from Group 1, recovered from suspected contaminated human food material (potato chips), yielded identical fingerprints with each primer used (not shown). The fingerprint pattern generated by primer pair REP-D: was designated is Ia and one generated by ERIC1R as Ib. Because all the patterns of fingerprints from Group 1 were identical, only one,

isolate XL4427 was fingerprinted together with every subsequent batches from groups 2 and 3 isolates. It was noted that with either primers, the majority (95 %) of isolates of Group 2 yielded identical fingerprints to the one from isolate XL4427. However, two out of forty (5 %) of the group 2 isolates, (019171, isolated in June, 1993 and 041120 isolated in September, 1993) yielded fingerprints similar to but with notable variations from the most prevalent pattern. The ,,non-typical" fingerprints with REP-Dt and ERIC1R were designated IIa and IIb, respectively. Pattern type denoted by II varied from pattern I, with the respective primer, by either additional, lack of, or variation in intensity of at least two minor bands. Such and more variations in fingerprint patterns were also noted in four out of seven of the Group 3 isolates. Isolates 143095 (from man in Nov., 1995), 41 (from the laboratory's strain stock), and GGD930D (from poultry) yielded fingerprint variations large enough to justify their classification as different from the reference strain, XL 4427. These patterns were designated IIIa and IIIb, respectively.

Table 1: Fingerprint pattern categories obtained from isolates of Salmonella Saintpaul

Isolate No.	Group	Date of isolation	Source	REP-Dt	ERIC1R
XL 4427	1	June, 1993	potato chips	Ia	Ib (reference)
026625	2	July, 1993	feces	Ia	Ib
041120	2	Sept., 1993	feces	IIa	IIb
211054	3	March, 1993	feces	IIa	IIb
143095	3	Nov., 1995	feces	IIIa	IIb
GGD930D	3	Nov., 1995	poultry	IIIa	IIb
140729	3	Dec., 1995	feces	Ia	Ib
206917	3	March, 1996	feces	Ia	Ib
41	3	Unknown	lab. strain	IIa	IIIb

As a second molecular typing method, plasmid profiling was applied to all S. Saintpaul isolates that were discriminated from the epidemic strain by rep-PCR fingerprinting. The results are shown in Table 2.

Table 2. Plasmid profiles from the various isolate groups

Isolat Group	Isolate No.	Date of Isolation	Source			Plasmid	Profile
(MDa)							
3	211054	March, 1993	man	2.7	-	-	-
1	XL 4427	June, 1993	potato chips	-	-	-	-
2	019171	June, 1993	man	2.8	3.0	3.4	-
2	026625	July, 1993	man	-	-	-	-
2	041120	Sept., 1993	man	2.7	-	-	-
3	GGD930D	Nov., 1995	poultry	2.7	3.0	-	-
3	143095	Dec., 1995	man	-	-	-	-
3	206917	March, 1996	man	-	-	3.7	11.0
3	41	unknown	lab. strain	-	-	-	-

AP-PCR fingerprinting with primer AP21 discriminated the strains like ERIC1R fingerprinting. The primer AP22 was the least effective, differentiating only the epidemic strains from the other.

Discussion

In this study we investigated the suitability of rep-PCR fingerprinting with primers REP-Dt and ERIC1R in discriminating between strains of S. Saintpaul and assessed the relative suitability of the method, with two other molecular typing methods, when applied to an epidemiological situation involving this pathogen. While it was within the realms of reason that some strains in Group 3 isolates would be different from the epidemic strain, given their temporal and source diversity, it was interesting that some human cases during the epidemic may have been caused by a different strain which could not have been distinguished apart. Also interesting from the preliminary screening step of all the isolates was the observed genomic similarity between the seemingly non epidemic strains in Group 2 isolates and some strains in Group 3, raising the possibility of an alternative source of sporadic infections with S. Saintpaul diagnosed in people during the epidemic. The low percentage of clinical isolates of S. Saintpaul that contained plasmids limits the scope of plasmid profiling in this epidemiological investigation but the method was a useful complementary tool because as it turned out, all isolates except two, that contained plasmids were the same that had been identified by rep-PCR fingerprinting as different from the epidemic strain. The detection of plasmids in isolate 206917, obtained three years after the epidemic did not constitute a clonal difference between this strain and one that caused the epidemic since the plasmids could well have been acquired within that span of time. Thus the information gained from plasmid profiles does not conflict with the conclusions made from rep-PCR fingerprinting. Considering the genomic information yielded by AP-PCR fingerprinting with the two arbitrary primers the strain discrimination level was quite comparable to what was achieved by rep-PCR fingerprinting.

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Feedborne Salmonella Infantis outbreak in cattle: Identification of affected farms by genotyping

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Summary

Salmonella enterica serovar Infantis (S. Infantis) has been the most common Salmonella serovar among Finnish cattle in the 1990s. In May 1995 S. Infantis was spread to cattle via contaminated feed. In this study we analysed cattle and feed S. Infantis isolates with our molecular typing scheme, based on ribotyping, IS200 -typing, plasmid profiles and macrorestriction profiles. We analysed bacterial isolates from 23 feed samples related to the outbreak. Isolates from 240 out of the 242 cattle farms with S. Infantis -infection in 1995 were also included, among these both isolates related to the outbreak as well as other cattle isolates. To get background information on the Finnish S. Infantis -infection in cattle we analysed cattle isolates from the years 1992 - 1994 (70 isolates).

Based on the chromosomal DNA, the S. Infantis -infection among Finnish cattle is very homogenous. Ribotypes and IS200 -types were determined only in the beginning of the analysis of the data. The analysed strains all had the ribo/IS200 -type 1A, which at present is the predominant ribo/IS200 -type among endemic S. Infantis -isolates. Among the ribo/IS200 -type 1A we found several different macrorestriction profiles, ie. pulsed field (pf) types, which are genetically closely related to each other (F values above 0.7). Pulsed-field type pf1 seems to be predominant in infections related to cattle.

The outbreak strain resembled the genotype typical of Finnish cattle isolates, but its plasmid profile (called pf1, plasmid type 39) differed from those of other S. Infantis isolates. All the feed isolates shared the plasmid type 39, and it was not seen in any of the cattle samples from earlier years. Therefore we were able to identify farms that had got their S. Infantis infection from the contaminated feed. Of the 85 farms that claimed that they had been infected by contaminated feed, 58 farms (68 %) had the plasmid type 39. Another four farms had plasmid types bearing plasmids similar to those of type 39, and were regarded as being infected by the contaminated feed as well.

Plasmid profiles of *Salmonella enterica* serovar Enteritidis phage type 21 strains isolated in Austria during 1994-1996

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SUMMARY

528 *Salmonella enterica* subspecies *enterica* serovar Enteritidis strains of page type (PT) 21 isolated from sporadic and outbreak cases in humans and poultry were studied. The Serovar-specific plasmid (36 MDa) was founds in 67 % of the strains. The frequency of this plasmid varied not significantly according to the source of isolation (p>0,05). Additional large plasmids of ~46 and ~53 MDa were also detected in 19 and 12 % of the strains respectively. Two main small plasmid combinations were recognized among those bacteria. They consisted of 4·7 : 4·0 : 2·3 : 2·2 and 5·3 : 3·3 : 3·0 : 2·8 MDa. Overall 60 plasmid profiles (PPs) were identified among S. Enteritidis PT-21 strains. However 82 % of the tested strains presented 8 PPs only. Plasmid profile analysis showed that 13 PPs were commonly found among isolates from humans (both outbreaks and sporadic cases), poultry, eggs and products containing eggs confirming that poultry infected by S.Enteritidis is an important source of this agent presenting an hazard for infecting humans. Plasmid profile analysis concerning case related strains from both humans and poultry showed that profile patterns were identical or similar in most of the cases. The indicated „similarity“ consisted in the presence and the relatively stable number of the small plasmids while the molecular weight variation shown by large plasmids seemed to render the observed PP(s) „diversity“ among the isolates of the same case.

Keywords: Salmonella Enteritidis phage type 21, humans, poultry, serovar-specific plasmid, plasmids plasmid profile

INTRODUCTION

Salmonellae are wide-spread pathogenic bacteria and salmonellosis constitute an increasing problem in all of the world countries (OIE, 1992; RODRIGUE, 1993). In molecular epidemiology, **plasmid profile analysis** (PPA) has been shown to be a useful method for identifying the case-related and their sources. Purposes of the present study were:

1. To determine the frequencies of various plasmids among *S.* Enteritidis phage type (PT) 21 isolates and specially the prevalence of the so-called serotype specific plasmids.
2. To evaluate the usefulness of plasmid profile analysis for discriminating the strains belonging to the same phage type.
3. To introduce the PPA in the epidemiological investigations and to estimate the approaches

MATERIALS AND METHODS

A total of 528 strains of *S.*Enteritidis PT-21 were studied. They were isolated in Austria during 1994-96. This set consisted of epidemic and sporadic (i.e. apparently case related and/or nonrelated) isolates. They were mostly of human and poultry origin. Plasmid DNA was extracted by a modification of an alkaline lysis method (ZYSKIND and BERNSTEIN, 1992). Phage and serovar typing were carried out by scheme recommended by WARD et al. (1987).

RESULTS

1. Identified Plasmids. 99,6 % of 528 *Salm.* Enteritidis PT-21 1-15 plasmids. Bacteria harbouring 4-6 plasmids predominated. The **36 MDa SSP** was detected in 353 (67 %) strains and of these, 326 possessed the SSP together with one up to nine additional plasmids. Besides the 36 MDa SSP, large plasmids of 46 and 53 MDa were observed in 19 % and 12 % of isolates respectively. Small plasmids of 4·7, 4·0, 2·3 and 2·2 MDa predominated among *S.*Enteritidis PT-21 isolates (68-74 %).

2. Plasmid profiles identified among *Salm*. Enteritidis PT-21. At least 60 plasmid profiles were recognized among the strains under investigation and 82 % of them showed 8 PPs only. **13 PPs were found to be common among Enteritidis PT-21 strains isolated from humans, poultry, eggs, products containing eggs.** Of 45 PPs identified among human isolates, 7 were found to be common between the both sick and carrier persons. **39 strains isolated from poultry (organ material) samples showed 17 PPs and** 5 **of them were exclusively found in poultry**. **11 PPs were shown by 37 isolates from eggs and products containing eggs**. 4 PPs were found to be common among isolates from poultry, eggs and egg products. 5 PPs were found among isolates from eggs only. 4 PPs were found to be common among isolates from poultry house samples, poultry (organ material) eggs and egg products.

3. Outbreaks and cases related to *Salm.* Enteritidis PT-21 infection. 93 of 94 Enteritidis PT-21 strains isolated from 37 cases of community outbreaks carried plasmids of different sizes. The SSP was found in 31 of those 37 outbreaks. Plasmid profile analysis

concerning case related strains from both humans and poultry showed that profile patterns were mostly the same or similar. The indicated „similarity“ consisted in the presence and the relatively stable number of the small plasmids while the molecular weight variation shown by large plasmids seemed to render the observed PP(s) „diversity“ among the isolates of the same case.

DISCUSSION

Although SSP prevalence varied according to the source of isolation (51-69 %), differences were found to be not significant ($p>0,05$). Besides the SSP, larger plasmids of ~46, ~53 and ~66 MDa were frequently detected among the isolates including one third of all of them. Maybe these plasmids are molecular variants of the 36 MDa SSP as it was reported previously by NAKAMURA et al. (1989) and later by BROWN et al. (1993) as well as by RANKIN et al., (1995).

Plasmid analysis showed *S.* Enteritidis PT-21 isolates could be divided into two main groups according to the presence and combinations of small plasmids of 4·7, 4·0, 2·3, 2·2 MDa and 5·3, 3·3, 3·0, 2·8 MDa. However, small plasmids of 4·7, 4·0, 2·3 and 2·2 MDa were the most prevalent. They were frequently found among strains isolated from both humans and eggs. This could explained fact as to why several outbreaks in humans „seemed“ to be caused by consuming eggs and/or eggs products contaminated by *S.* Enteritidis PT-21. 60 PPs were identified among 528 Enteritidis PT-21 strains and 84 % of them showed 8 of them only. 13 PPs were commonly found among isolates from humans (outbreaks and sporadic cases), poultry, eggs and egg products. This confirms that poultry infected by *S.* Enteritidis seems to be an important source of this agent presenting an hazard for infecting humans. Plasmid profile analysis demonstrated that *S.* Enteritidis PT-21 strains isolated from small community outbreaks showed the same PPs as those isolated from eggs and feed containing eggs produced by a poultry farm in the same province. This gave support to identify the source of infection. Although, similar (but not always identical) profile patterns were present in most of the cases concerning both outbreaks in humans and investigation(s) carried in the poultry farms. The indicated similarity consisted in the presence and the relatively stable number of the small plasmids while the molecular weight variation shown by large plasmids seemed to render the observed PP(s) „diversity“ among the isolates of the same case. It is possible that both phage types and plasmid profiles may change (HOLMBERG et al., 1985; RANKIN and PLATT, 1995) and the data regarding this collection consisted of strains belonging to *S.* Enteritidis PT-21, may be seen from this point of view. Although several plasmid profiles were identified, those latter were very similar considering the small plasmid combinations. It should be taken into consideration that all of 528 Enteritidis PT-

21 strains were isolated during a period of longer than a year. On the other hand, if it may be assumed that plasmids of ~46, ~53 and ~66 MDa are molecular variants of the 36 MDa SSP, the epidemiological analysis gets simpler.

Investigation of both epidemiologically related and unrelated *Salmonella* isolates indicated that PPA provided a good discrimination among them. This method could be successfully applied for the characterization of Enteritidis PT-21. However, PPA seems to be more useful when case related strains are examined.

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The time required for the elimination of bovine Salmonella infection

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Summary

During one and a half years 243 herds of cattle were found to be infected by Salmonella, 155 in the province of Vaasa and 88 in the province of Oulu. Salmonella Infantis was found on 151 farms in the province of Vaasa, three of which also harboured S. Typhimurium, one S. Bredeney, one S. Agona and one S. Rubislaw. In addition two herds had S. Typhimurium infection, one S. Rubislaw and one S. Agona. 76 farms in the province of Oulu were infected with S. Infantis, in addition to which S. Agona was found on 8 and S. Mbandaka, S. Enteritidis, S. Typhimurium and S. Gatuni on one each. Most of the infections were symptomless.

Salmonella Infantis infection was successfully eliminated on 79 farms in the province of Vassa (52.3%) and on 48 in the province of Oulu (63 2 %) within four months. The slaughter of symptomless carrier animals more often was suggested at the beginning of the elimination procedure in the latter province, as this was seen as a quick means of eliminating the infection. There was no statistical difference in the time required for Salmonella elimination between the two provinces.

The S. Agona epidemic was mainly located in four local government districts along the River Kalajoki and the source of the infection has not yet been found. Elimination of this serotype seemed to take a longer time than that of S. Infantis.

Keyword: bovine salmonellosis, elimination time, epidemics

Introduction

Salmonella infection in cattle causes hygiene problems in slaughterhouses and meat cutting plants, so that the food industry would prefer only Salmonella-free animals to come for slaughter. During 1994-1996 Salmonella surveys were carried out in the three provinces of Finland, Vaasa, Oulu and Lapland, with financial support from dairies and slaughterhouses. The sites examined were slaughterhouses (pooled fecal samples from animal transportation trucks) and cattle farms of different kinds (pooled fecal samples from each farm). The results of these examinations were collected together by the provincial administrations of Vaasa and Oulu under legislation

concerning Salmonella infection in cattle. Only a few isolated cases of bovine Salmonellosis were found in Lapland.

Material and methods

The Salmonella tests were performed by the ISO method no. 6579/93, either at local laboratories for foodstuffs or at the National Veterinary and Food Research Institute. Those discussed here were carried out during a period from September 1, 1994 until April 20, 1996, and the results were collected by the provincial veterinary officers in Vaasa and Oulu.

Results

Altogether 243 farms were found to be infected by Salmonella, 155 of which were located in the province of Vaasa and 88 in the province of Oulu. The numbers of herds infected with Salmonella Infantis and the times required for elimination of the infection are shown in Table 1 and those of Salmonella Agona in Table 2. The Salmonella Agona cases were located in six local government districts, four of which were by River Kalajoki in the province of Oulu, where most of the cases were found, and two in the province of Vaasa, not far away from these geographically, which had only one infected herd each.

The time required for the elimination of Salmonella was counted from the date of first positive test result to the date of the first negative one. Salmonella Infantis was eliminated within four months from 48 herds in the province of Oulu (63.2 %) and 79 herds in the province of Vaasa (52.3 %). This difference was not statistically significant. Herds infected by Salmonella Agona seemed to become free of infection more slowly than the S. Infantis cases.

Most of the infected animals were asymptomatic carriers, but in one case a few animals developed severe symptoms in the middle of elimination procedure and two cows died. Some farmers or members of their family developed clinical or subclinical Salmonellosis.

Discussion

A Salmonella finding in feces may arise for several reasons. Some animals are active carriers and Salmonella multiplies in their organs. In these cases 10^5-10^8 Salmonella bacteria per g may be found in the feces. Passive carriers receive Salmonella orally and the bacteria do not multiply in the body. Salmonella can be isolated from the feces in such cases. Latent carriers are animals which carry the infection in organs such as lymphnodes, gallbladder etc and fecal samples vary from positive to negative in the tests. Our material most probably includes all of these carrier types, which must also have had some influence on the elimination time.

The infective dose of Salmonella bacteria has been considered to be high, 10^5-1011/g feces, whereas Jones (1992) reports that less than three S. Mbandaka bacteria in a gramme of feed could infect a calf. The only S. Mbandaka case found in this material has not yet improved.

The time required for elimination depends on the number of infected animals, the age and condition of the cattle house, the farmer´s physical condition and capacity to do the large amount of extra work needed, and finally his ability to co-operate with the veterinarian (and vice versa).

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Table 1. Total numbers of farms infected with Salmonella Infantis in the provinces of Oulu and Vaasa as of April 20, 1996.

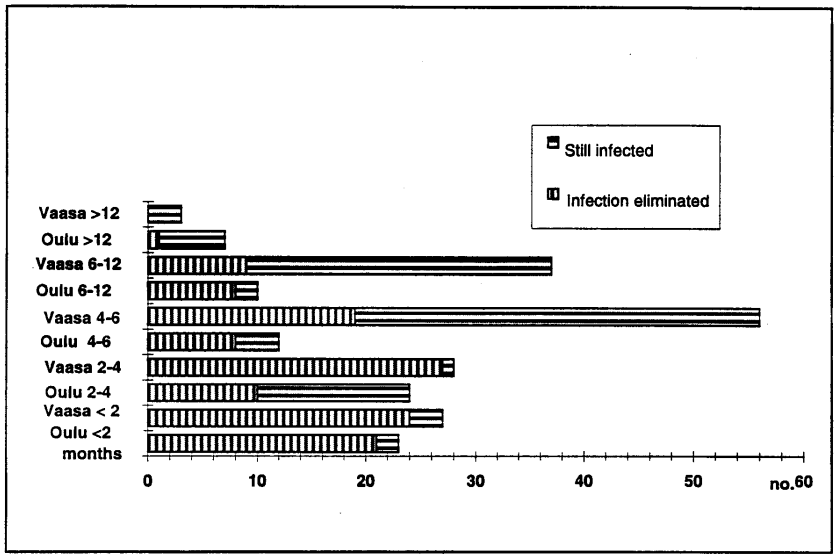

Table 1. Total numbers of farms infected with Salmonella Infantis in the provinces of Oulu and Vaasa as of April 20, 1996.

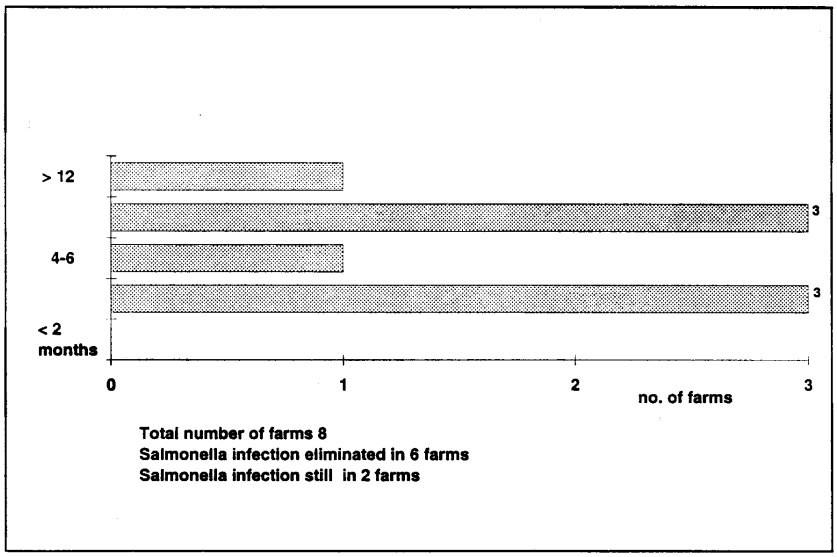

Table 2. Total numbers of farms infected with Salmonella Agona in the province of Oulu as of April 20, 1996.

Table 2. Total numbers of farms infected with Salmonella Agona in the province of Oulu as of April 20, 1996.

Review of equine salmonellae serotypes causing outbreaks of disease: Disinfection and management protocols for use in equine disease outbreaks

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Summary

Salmonella typhimurium is the most frequently isolated serotype from equine cases in central Kentucky and from samples submitted to the National Veterinary Services Laboratory, Ames, Iowa. However, other serotypes have caused outbreaks of serious disease: *anatum*, *infantis*, *krefeld*, *ohio*, and *saint-paul*. Disinfection of typical equine facilities includes thorough cleaning with a detergent followed by application of a disinfectant which is germicidal in the presence of organic matter. Phenolic compounds have many beneficial properties which make them most useful in the disinfection of equine facilities.

Key words: salmonella, serotype, disinfection, equine, outbreak, phenol

Salmonellosis has been a constant problem to equine practitioners not only in individual animal cases, but also in farm outbreaks of disease and nosocomial infections. Studying documented outbreaks and disinfection practices used are helpful in understanding the control measures needed to contain this disease.

Equine salmonellae serotypes identified in central Kentucky and the US

Equine salmonellae serotypes have been monitored at the Livestock Disease Diagnostic Center, University of Kentucky since 1981. Positive cultures are sent to the National Veterinary Services Laboratory (NVSL), Ames, Iowa for serotyping. *Salmonella typhimurium* has consistently been the predominant serotype isolated from necropsy and clinical cases submitted. From January 1990- June 1996, 301 *Salmonella* isolates representing 32 different serotypes were cultured from equine samples: 207 necropsy cases and 94 clinical cases. A positive *Salmonella* culture from necropsy cases was not necessarily indicative that salmonellosis was the cause of death. Of the clinical cases, the majority of isolates were *typhimurium* (34), *newport* (21) or *thompson* (14). Of 14 other serotypes detected, there were five or fewer isolations. The sources of these isolations were feces (89), joints (3) and abscesses (2) (Dwyer and Donahue 1996).

Bacterial cultures from multiple species are received by NVSL from throughout the United States for serotyping. The most common equine salmonellae serotypes from 10/94-9/95, included *typhimurium* (149), *typhimurium* var. *copenhagen* (24); *newport* (37); *anatum* (28); *oranienburg* (21); *dublin* (11) and *krefeld* (10). Serotypes of 45 other *Salmonella* were also isolated from equine samples (Ferris 1996).

Hospital/Farm Outbreaks

Equine hospitals have encountered infections with *S. saint-paul* (Hird 1984) *anatum* (Hartmann 1996), *krefeld* (Pare, 1996), *infantis* (Traub-Dargatz 1997) and *typhimurium* (personal communication, April 1997, Susan Ewart, East Lansing, MI). One California Thoroughbred farm had a highly contagious outbreak of *S. ohio*, possibly due to contaminated feed (Madigan 1990). Multiple cases of horses with salmonellosis have been documented in central Kentucky during 1985-1986 due to *S. saint-paul* (Powell 1988), and during 1980-1984 with an antibiotic resistant strain of *S. agona* (Donahue 1986). Serotypes *newport* and *thompson* have also caused morbidity and mortality on farms in central Kentucky (Dwyer and Donahue 1994).

Disinfection of equipment, stalls, islways, drains and feeding equipment have been recognized as an essential procedure during outbreaks, although methods and disinfectant details have not always been clear in published papers. In a 1991 hospital outbreak involving *S. anatum*, a phenolic disinfectant was used in the cleaning of stalls, isleways and drains. The debris was rinsed with hot water sprayed by a power washer, followed by application of a freshly made 10% hypochlorite solution. After 15 minutes surfaces were rinsed with water. In follow up studies of this outbreak, the use of a power washer was discontinued because of possible particle aerosolization (Pare 1996). Other hospitals routinely use only chlorine bleach for stall disinfection (Murray 1996). Phenolic compounds have been successfully used on multiple farm outbreaks (Dwyer 1995, Madigan 1990) and large animal hospital outbreaks (personal communication, April 1997, Susan Ewart).

Choice of disinfectant in equine facilities

The surfaces found in equine facilities can be vastly different from other animal and human facilities. Raw wood, dirt flooring and porous surfaces are commonly found, although high level equine farms and hospitals often have painted concrete block walls, sealed rubber matted floors and asphalt isleways with drains which are most conducive to successful disinfection.

Salmonellae can survive in bovine feces for six years on a variety of stall surfaces (Plym-Forshell 1996). An effective disinfectant must be germicidal in the presence of organic matter in order to be effective.

Organic mater in chlorine solutions reduces germicidal activity (Dychdala 1991) and also affects quaternary ammonium compounds and chlorhexidine (Russell 1987). Phenolic compounds have several advantages of having broad spectrum antimicrobial and virucidal activities, tolerance for organic mater and hard water, residual activity and biodegradability. Compounds containing o-phenylphenol and o-benzyl-p-chlorophenol show a wide range of activity against gram negative and gram positive bacteria, including four different serotypes of *Salmonella* tested (O'Connor 1991). Both organic phenols and sodium phenate disinfectants are commercially available. Sodium or potassium salts of phenolic compounds have less bacteriocidal power, but are more soluble in water (O'Connor 1991).

Disinfection of equine facilities

Effective disinfection of equine facilities begins with thorough cleaning of surfaces with a detergent or disinfectant/detergent compound. After completely rinsing, a phenolic disinfectant, diluted to manufacturer's recommendations, should be applied and left on the surfaces since the contact time of the disinfectant is important to germicidal action. No rinsing is needed, except for feeding or drinking equipment such as buckets, hay nets and feed troughs which should be thoroughly flushed with water prior to use (Dwyer 1995).

In containing a salmonellosis outbreak, attention to detail with disinfection procedures is particularly important. Bacteria can be found in corners of stalls, under rubber mats, in drains and at the ends of water hoses which have been left on the floor. People are also effective cross-contaminators of bacteria within a barn or hospital, so developing a one-way traffic pattern for people from clean areas (healthy horses) to dirty areas (sick or convalescing horses) is especially important. Disinfection of equipment used on sick animals, including pitchforks, brushes, halters and nasogastric tubes is essential, as is the use of protective clothing and handwashing.

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*The author wishes to acknowledge Bio-Tek Industries, Inc., Atlanta, Georgia for the travel grant which allowed presentation of this paper.

Situation of *Salmonella* contamination in Styrian broiler flocks

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Summary

According to the Austrian „Poultry Hygiene Regulation“, every poultry flock scheduled for slaughter must be subjected to a *Salmonella* test, which is to be performed by taking 9 cloacal swabs. In 1994, the Styrian Poultry Health Service started collecting the results from these tests. The test records are supplemented by the collection of epidemiological data of the flock to be slaughtered. The results of the data acquired during the years 1994, 1995 and 1996 are shown and compared. An attempt is made to discuss the possible influence of the origin of the chicks, the hatcheries, the feed and the environment of the flock on the degree of contamination with *Salmonella* of the broiler flocks.

Key words: broiler, cloacal swabs, *Salmonella* situation

Introduction

Epidemiological researches are required in order to estimate the extend of *Salmonella* infection in poultry flocks. Beside *S. enteritidis* and *S. typhimurium* other epidemiological relevant serovares are to be taken in consideration (Schlüter et al., 1994). According to the Austrian „Poultry Hygiene Regulation“ (BGBl. Nr.274/1991), every flock of poultry scheduled for slaughter must be subjected to a *Salmonella* test. The examination is to be performed within 3 weeks of the intended slaughter date by taking 9 cloacal swabs. The number 9 applies irrespective of the size of the flock. However, this monitoring system is not regarded as a suitable tool to ensure that the flock is free of *Salmonella*. Taking 9 cloacal swabs per flock means, statistically, that a negative result allows upto 30% contaminated birds with a safety factor of 95%. In addition also no pre-enrichment for the analysis of the samples in the laboratory is required. Nevertheless, this examination does enable us to collect facts and data about the situation of *Salmonella* contamination in Styrian broiler flocks. In this report, we will be presenting the results of 3 years of data acquisition associated with the legally required cloacal swab test for *Salmonella* (Fuchs, 1996) and we compare these results with the results of *Salmonella* tests in man during the same period.

Material and methods

The cloacal swabs are taken by the veterinarians, and they are then delivered to the investigation centre. The swabs are of the usual, readily available cotton head type. The examination is to be performed no more than 3 weeks prior to the scheduled slaughtering date. A standard form is used by the veterinarian to advise the Poultry Health Service of the results of the examination. In addition to the test results, the form also includes a code number for the flock owner, the date when the sample was taken, the size of the flock, the age of the flock, the hatchery, the origin of the feed and, if other test material is submitted as well, the type of sample.

Results

Table 1 shows the results of the cloacal swab examinations for the years 1994 to 1996.

For positive samples, the serovar and the phage type was determined. **Figure 1** shows the distribution of the different *Salmonella* serovars.

Figure 2 shows the results of *Salmonella* tests performed in man during the same period, by serovar.

Table 1: Comparison of the results of the total number of positive samples taken between 1994 and 1996.

Samples	1994	1995	1996	Total
Positive	384 (15.5%)	141 (7.6%)	169 (10.6%)	694
Negative	2087 (84.5%)	1701 (92.4%)	1427 (89.4%)	5215
Total	2471 (100%)	1842 (100%)	1596 (100%)	5909

Conclusions

The collection of data concerning origin, hatchery and feed does not permit any epidemiological conclusions. The monthly distribution of positive findings shows a conspicuous accumulation in the winter months December to February. A striking similarity of the

distribution pattern is noted when the serovars and the phage type in the cloacal swab examinations is compared with the distribution in the human *Salmonella* test results. This shows the close dependence of human *Salmonella* illnesses on the *Salmonella* situation in poultry production.

Figure 1: Distribution of the different *Salmonella* serovars.

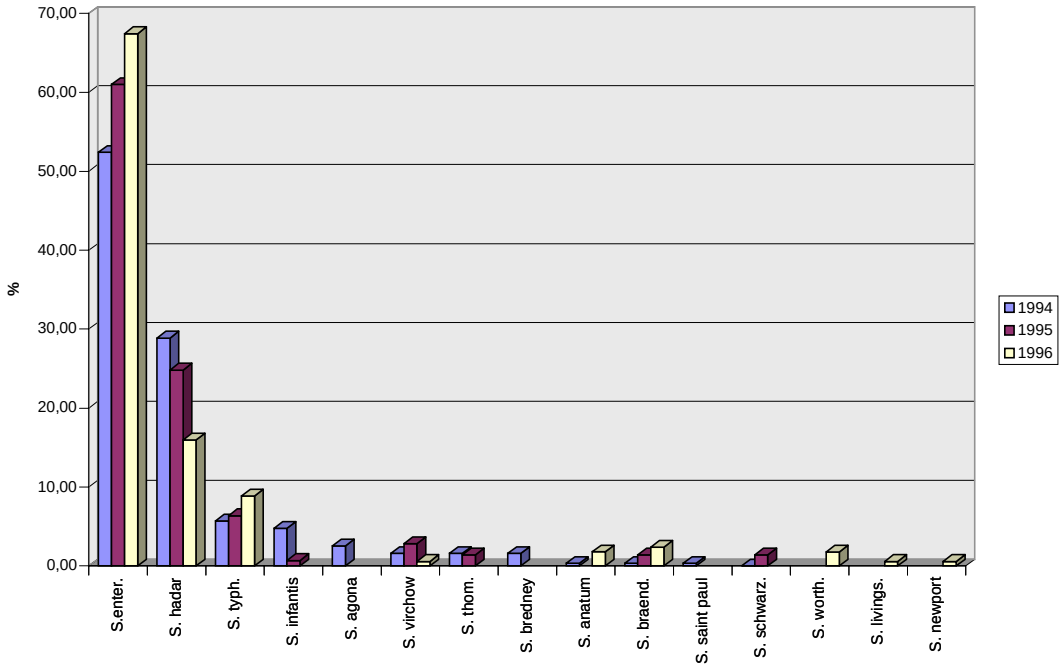
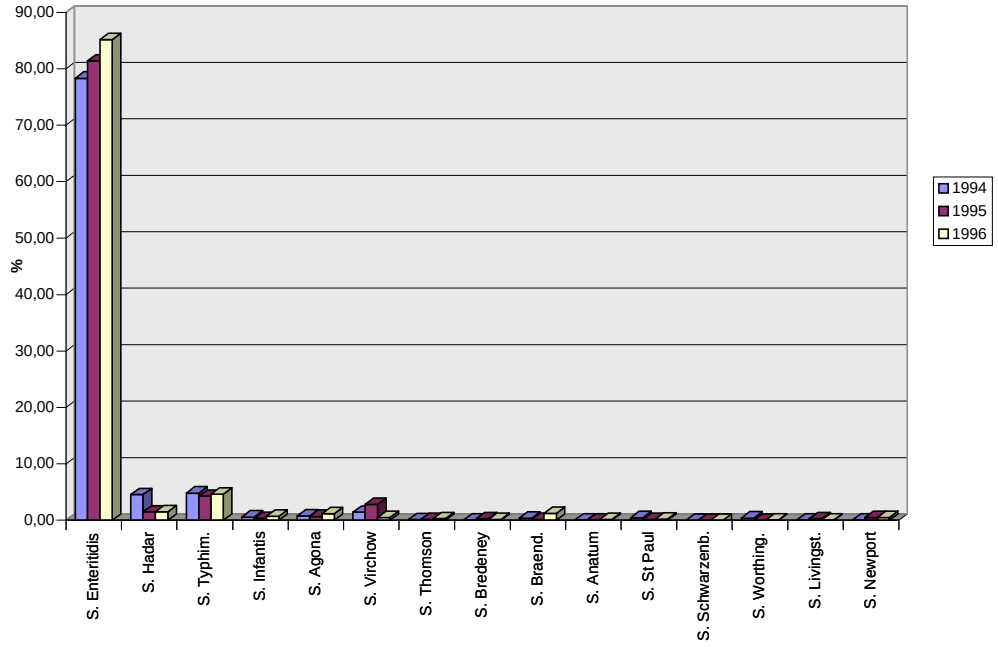


Figure 2: Distribution of serovars in human *Salmonella* test results.



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SALMONELLA ISOLATION AND EVALUATION OF ANIMAL FOOD HYGIENE IN TlIE PROVINCE OF CASERTA (ITALY).

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Summary

Salmonella spp. bacteria are still nowadays one of the commonest causes of food toxinfection in man. Anyhow, while Salmonella typhi is a typical human parasite meaning that man is the only source of infection, other species of Salmonella, responsible for food poisoning, are passed on to him by animals. The poisoning takes place by consuming raw meat or sausages from either infected animals or healthy carriers. During our investigation, Salmonella bacteria were detected both in processed meat (minced meat, sausage, hamburger, etc.) and chopped meat from different species of animals. Besides this, the samples were analysed in order to check other hygienic parameters such as total bacterial count, E.coli and coagulase-positive Staphylococci count. Finally, Salmonella strains were typed.

Key words: processed meat, non-processed meat, microbiology, Salmonella, food poisoning.

Introduction

Minor Salmonella infections are nowadays considered one of the commonest causes of toxinfections in man. Several epidemic outbreaks of S. enteritidis, S. derby, S. typhimurium and S. infantis are yearly reported in Italy and throughout the world (Legnani et al., 1986, Orefice et al., 1989, Tommasi et al., 1994). From the epidemiological standpoint, animals and animal foods in particular are thought to be the main sources o f infection for man . Some investigations have also pointed out that chicken and pork meats are the most contaminated of all meats, followed by the bovine and horse ones (Cantoni et al., 1991). In Italy, the National Sanitary Council (1990-1991) reports 19,957 cases of human salmonelloses in 1990 and 17,996 in 1991 (Fabio et al., 1996). As for the province of Caserta (South of Italy), according to the data recorded by the local Hospital Services (Department of Infective Diseases), 327 cases of clinically manifested salmonelloses were detected in the period from January 1991 to October 1996. 3.7% of the total amount of cases was due to S. typhi, 11.9% to S. typhimurium, 28.7% to S. enteritidis, 20.1 % to group B Salmonellae, 10.3% to group C Salmonellae and, finally, 25% to group D Salmonellae. Data reported here show that, during the last five years, in the province of Caserta, food salmonelloses were by far the most numerous of all. Most epidemic outbreaks have been related to egg-based foods and meat products, while 12 cases caused by S. enteritidis have been related to the consumption of roasted food prepared with lyophilized potato flakes contaminated by S. enteritidis. Anyhow, since a number of cases is not noticeable owing to its benign course, an exact view of the problem as regards its dimensions is not possible. In the light of this, our purpose in the present investigation was to highlight the relation between human salmonellosis and the presence of salmonellae in animal food. We report data showing the frequency of salmonella isolation in meat samples analysed routinely in our laboratory and, finally, we identify the serotypes isolated in the samples. In addition, in compliance with EEC Directive 88/657, some meat samples (minced meat, sausages and hamburgers) were also microbiologically checked as regards their total bacterial count, E.coli and coagulase-positive Staphylococci.

Materials and methods

Our investigation covers the period from January 1995 to March 1996. During these months we analysed a total amount of 567 meat samples consisting of fresh sausages, chopped meat, minced meat and hamburgers (**Fig. 1**). The samples were taken by the local Sanitary Services (ASL CE/1 and ASL CE/2) during their routine survey of the territory; all the analysed

samples came from commercial firms carrying out both the preparation and the sale of their products.

In order to isolate Salmonella spp., 25 g of each sample were homogenised in Peptone Water (Buffered) and incubated at 37° C for 24 hours; secondly, 0.1 ml of pre-enrichment medium was inoculated into 10 ml of Rappaport Vassiliadis and then incubated at 43° C for 24-40 hours. Finally, in order to isolate Salmonella, we used Brilliant Green Agar, SS Agar and Rambak Agar. Salmonella strains were typed using either Enterotube II Roche or Api System 20 E. The serological identification of the isolated strains was carried out by the Pathogenic Enterobacteria Centre in Palermo (Italy). In addition to this, 169 samples were also checked as regards their total bacterial count and Staphylococci by means of plate-counting; dilutions prepared for the samples (10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}) were inoculated into Plate Count Agar and Parker plates. E.coli were detected by means of MPM (Most Probable Number) methodology.

Results

During the work, 39 Salmonellae spp. were isolated from 567 meat samples, the percentage of isolation being the following: 7.6% in pork sausages, 20% in bovine-pork sausages, 16.6% in sheep meat, 5.1% in bovine minced meat and, finally, 4% in chicken meat. 24 Salmonella strains, out of 39 isolated, were typed and the following serotypes were identified: S. derby (2 isolations), S. livingstone (6), S. bredeney (1), S.panama (1), S. london (3), S. typhimurium (5), S. infantis (1), S. lexingtone (1), S. de moy (1), S. virchow (1), S. bochum (1), S. eimsbuettel (1). Among the serotypes reported, S. livingstone and S. typhimurium were those most frequently detected. The data obtained from total bacterial count, coagulase-positive Staphylococci and E.coli were the following: 2.9% of the samples showed a bacterial titre < 5.000.000 ufc/g, 12.4% of them ranged between 500.000 and 5.000.000 ufc/g and, finally, 84.6% was > 5.000.000 ufc/g. Moreover, Staphylococci were detected only in 2.3% of the samples checked while E.coli were found to be absent in 86.9% of the samples and present in 20.7% of them with a titre ranging from 50 to 500 ufc/g (**Figg. 2 - 3**).

Conclusions

Our data confirm what others (Cantoni et al., 1991) have previously reported in Italy. The detection of highly pathogenic strains such as S. typhimurium, derby and infantis in meats with microbiological requisites different from those established in EEC Directive 88/657, must not be undervalued; in fact, serotypes such as these might cause epidemic outbreaks if food is not cooked or preserved properly or if it is eaten after slow cooling without previous heating. In the light of this, then, we think it is necessary, in order to protect the consumers from the risks linked to this kind of infections, that the competent authorities carry out a lasting and systematic survey of all the phases of production. The program must check not only those commercial firms where the meats are prepared and put on sale, but also the phases of slaughter, processing and meat transportation when it is more probable that a secondary contamination may take place. Finally, people involved in meat processing should be well informed about the risks for human health caused by the contamination of food due to salmonella bacteria and other important pathogens in order to attain a more hygienic production of meat.

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Fig. 1: Animal foods checked for Salmonella

	Sausages			Chopped meat				Minced meat	Hamburger	
	Pork	Pork-bovine	Chicken	Bonive	Pork	Sheep	Chicken	Bovine	Bovine	Chicken
No. samples	288	50	5	84	19	6	24	78	6	7
Pos. % (no.)	7,6 (22)	20 (10)	-	-	5,2 (1)	16,6 (1)	4,1 (1)	5,1 (4)	-	-
ASL CE/1	256	23	4	73	19	6	22	43	5	7
Pos. % (no.)	5,8 (15)	34,7 (8)	-	-	5,2 (1)	16,6 (6)	4,5 (1)	2,3 (1)	-	-
ASL CE/2	47	12	1	11	-	-	2	35	1	-
Pos. % (no.)	14,8 (7)	16,6 (3)	-	-	-	-	-	8,5 (3)	-	-

Fig. 2: % T.B.C. (x1000)

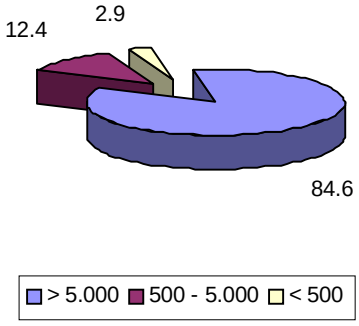
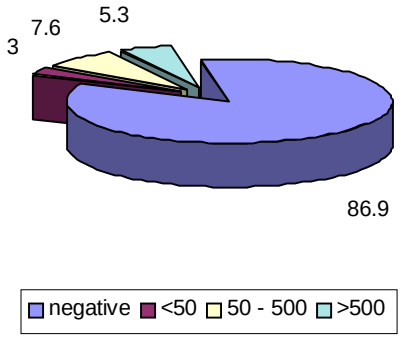


Fig. 3: % E. coli count



A survey of bovine salmonellosis in Northern Finland

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Summary

Salmonella examination was carried out among the cattle farms in the two northernmost provinces of Finland, Oulu and Lapland, served by the slaughterhouse of Atria Ltd in Ylivieska, as the company wanted to ensure that its meat products were free of the Salmonella.

Fecal samples were collected from the floors of cattle transportation trucks just after the animals had been taken into the slaughterhouse, and the samples from each truck were pooled and examined for the presence of Salmonella in the slaughterhouse´s own laboratory using method no. 71/1991 of the Nordic Committee on Food Analysis. Unlike the method used in the Salmonella Control Programme this includes preincubation in peptone water, which was considered desirable for such a survey. All the Salmonella strains were sent to the National Veterinary and Food Research Institute (EELA) for serotyping. When a Salmonella-positive truck was found, each farm which had sent animals in this particular truck was examined by the same method. Individual samples were collected from all the cattle, pooled and examined for Salmonella. The method was successful even in a case in which fecal samples from 66 animals were pooled and only one of those animals was Salmonella carrier.

This survey indicated that the occurence of the Salmonella carrier state is very low among the farms concerned, 0.6 and 1.2 % respectively in two sampling periods. The survey covered 95 % of all the farms served by this slaughterhouse, a total of 3856 farms. Our practical experience is that fecal samples from 30 animals can be successfully pooled. This method is considered to have good cost-benefit value and it is reliable enough for this kind of survey. To our knowledge this is the first time a Salmonella survey has been carried out using these sampling and culture methods.

Key words: bovine salmonellosis, pooled fecal samples

Survey on cattle transportation trucks

When Atria Ltd in Ylivieska decided to undertake a survey of its cattle transportation trucks, instructions for the taking of samples were sent to every agent and truck driver on the 14th of September in 1994, and the first survey of every consignment of cattle began on Sept. 16 and ended on December 22, 1994. After unloading the truck, the driver collected as representative a sample of the faeces as possible from the floor of the truck using a plastic glove. About a tablespoon of farces was to be taken from the place occupied by each animal. After that the sampler turned the glove inside out and wrote on it the number of the delivery note and the date. The samples were delivered to the slaughterhouse refrigerator. Each sample was thoroughly blended the next day and cultured by the method of the Nordic Committee on Food Analysis (NMKL). This method consists of preenrichment in buffered peptone water, enrichment in Rappaport-Vassiliadis broth and plate culturing on brilliant green-phenol-red agar and XLD agar. If Salmonellae occur in this kind of specimen, they often do so only in small quantities, sometimes sublethally injured and often covered with other Enter-obacteriae. Consequently this method, and especially its pre-enrichment step, was estimated to have the effect of promoting the formation of Salmonellae colonies. The Salmonella-positive cultures were sent to EELA for confirmation.

The agents collected pooled samples from the individual farms that had contributed to the truckloads that proved positive, and these were also examined in the slaughter-house laboratory, the positive samples being sent on to EELA for confirmation. A second similar Salmonella survey was carried out from 21 March to 28 April, 1995.

The occurence of Salmonella sp. in pooled samples taken from the cattle transportation trucks and infected farms in shown in Table 1. The results indicate that even one infected case among 19-66 animals (5.3 and 1.5 % of animals respectively), could be found in the pooled sample with the isolation procedure used.

After beginning with systematic sampling and pre-enrichment according to the new method at Atria Ltd in Ylivieska, more Salmonella-positive herds were found in the provinces of Oulu and Lapland than had been identified in the whole of Finland before this. The most important reasons for this may have been both the new sampling system and good coverage, together with the efficient method.

The incidence of bovine Salmonellosis in the area is evidently is very low, as only 0.6 % positive herds were found in the first survey and 1.2 % in the second. About 95% of the herds belonging to farms supplying animals to the Lihakunta meat processors were examined and several farms participated in the survey 2-3 times. At most 87.5 % of the animals on a farm were infected (28/32). After the first positive Salmonella finding in a pooled sample, each animal on the farm in question was examined separately. On 13 of these farms there was only one infected animal and on 12 there were none. The following reasons can be given for the latter situation: either the positive animals on the farm had already been slaugtered, the farm had eliminated the infection between the samplings, or the new sampling was not representative.

Table 1. Occurrence of Salmonella sp. in pooled fecal samples originating from cattle transportation trucks and infected farms. Samples were collected from 16th Sept. to 22nd Dec. 1994 and from 21st March to 28th April 1995.

Transporta-	Salmonella-		Farms	Farms	Farms	
tions	positive		total	suspected	infected	
No.	No.	%	No.	No.	No.	%
1001	38	3,8	2305	176	28	1,2
189	9	4,8	1551	120	9	0,6

References

Anom.: Nordic Committee on Food Analysis, Nr 71, 4th edition 1991, Espoo, Finland

Finland’s low Salmonella infection rate is based on a high risk control level in the feed industry

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Summary

The Salmonella infection rate on Finnish farms is very low by international standards. One of the most important reasons for this is the high hygienic level of the animal feed. Salmonella control programme have a long tradition in this industry, as a result of earlier legislation and risk management is being continued voluntarily even though Finland has become a member of the EU. The Association for Animal Disease Control (ETT) lists the feed factories and suppliers, which employ adequate risk management. This list is published every week in the farmers´ newspaper in order to help farmers to choose safe suppliers.

Keywords: voluntary risk control, feed industry, internal inspection, preventive measures, controlling emergency situations, positive list

”A positive list” to guide the voluntary control of imported complete mixed feeds and raw materials

The aim of the ”positive list” is to coordinate voluntary risk control in the animal feed industry aimed at eliminating the occurrence of Salmonella in feed and thus at minimizing infections in farm animals. The ETT in co-operation with the animal feed industry and the government authorities has tried to find measures to control critical points in the feed industry. These are the examination of each batch of imported feed for salmonella, a hygiene program for the transport and storing of feeds and successful internal inspection.

Since Finland became a member of the EU, the Finnish authorities have had the opportunityto carry out only occassional tests of imported complete mixed feed or raw materials, instead of the compulsory testing of each batch that was in force earlier. As this was not considered sufficient, the companies listed in the ”positive list” undertake to carry on with the previous Salmonella testing programme for each imported batch. The Plant Production Inspection Center (KTTK) organizes the official sampling and carries out the laboratory tests in its own laboratory. The number of importers has decreased recently, although the amount of imported feed has remained the same. Importation is now done in the hands of certain companies, which have found reliable

partners abroad, mainly in the Scandinavian countries (Denmark, Sweden and Norway). These importers supply tested materials to Finnish animal feed producers.

Risk control in feed factors - internal inspection is the keynote

The animal feed industry is in general better prepared nowadays to control the risks than it was before. It stresses preventive measures, which are also required before a company can qualify for the ”positive list”. Imported raw materials are bought only from foreign companies, which have agreed sales in advance and if Salmonella is found in the imported batch on arrival in Finland, the buyer has the right either to send it back or to subject it to heat treatment at the supplier’s expense.

The Finnish feed factories make contracts for transport and storage that incorporate hygiene demands; chiefly that trucks and storehouses, both of which must have quality certificates, must be cleaned and desinfected regularly. The industry carries out its own hygiene programme and ensures the maintenance of its equipments. Quality control at all levels includes training of the employees and the issuing of written instructions for each action.

Another part of risk control is supervision by the goverment authorities, i.e. KTTK and Department of Veterinary Medicine and Food Hygiene at the Ministry of Agriculture (MMMEO), which organize market controls to a certain extent by testing mixed feed leaving the factory and by checking the internal inspection routines used in the industry. The industry itself is therefore mostly responsible for risk control. Raw material suppliers keep all the products in their own quarantine stores until the KTTK Salmonella tests have been completed with negative results. The mixed feed manufactures do not keep the products in quarantine, but official tests are performed on them all the time. Officials from KTTK visit the animal feed factories regularly, and those which use animal waste are inspected by the veterinary authorities mentioned above.

The internal inspection programmes are accepted by the authorities. These include information on site, date and methods of sampling and the laboratory, where the tests are carried out. Samples are taken from raw materials, the process and the surroundings (dust filters, floor, fodder silos). Everything is documented, including the temperature and pressure during the process. All this is done to ensure that abnormalities are noticed immediately and emergencies can be avoided. The results of this internal inspection are reported regularly to KTTK and MMMEO, except in abnormal situations, when reports are made at once.

Exceptional situations must be handled with great care,taking measures, which have been planned in advance. Salmonella contamination in the surroundings, the product or the process always causes a risk situation, especially when there is an interruption or failure in process.

Problems must be rectified as soon as possible irrespective of how they were found out (official or internal inspection). Instructions for remedying the situation are given by KTTK or MMMEO depending on the finding. When the risk has been evaluated measures may vary between informing customers and requesting return of the products and closing the factory down for a certain time. The necessary cleaning and disinfection, sampling and investigation of the cause of the score are carried out. As sales ban is enforced until the situation has been resolved and the risks are under control. When the factory has completed all the measures needed, it is permitted to start the process on certain conditions, eg. more frequent sampling together with quarantine before the product is released for sale. If contaminated feed has been delivered to farms, it must be returned to the supplier and the silos must be disinfected etc. When the situation has returned to normal the special conditions are lifted. The fact that the factory is found to harbour Salmonella, does not always mean that it is dropped off the positive list, but that will always be consequence if the rules are not followed.

Some suppliers/factories have found Salmonella in their process, since this voluntary internal inspection system was introduced and in most of these cases they have successfully eliminated the risk on their own.

Responsibility of the feed factories

Animal feed plays a very important role in the spreading of Salmonella epidemics to farm animals, and therefore the feed factories´ programme of risk control is extremely important for the whole food chain from farms to market. A lot of work has been done to eliminate risks, but measures can be still improved in the spirit of close co-operation.

The Styrian *Salmonella* prevention programme in poultry

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Summary

The *Salmonella* prevention programme of the Styrian Poultry Health Service introduces measures at all levels of poultry production. The programme is based on a complete *Salmonella* monitoring of parent flocks and hatcheries by a regular bacteriological examination of wipe and drag swabs. The main activities of the programme are the evaluation of the results of the investigation of cloacal swabs from the broiler flocks as required by the „Austrian Poultry Hygiene Regulation“, the efforts to slaughter *Salmonella* positive and Salmonella negative flocks separately and, the introduction of a feedback system for slaughter findings. The programme further includes projects at different levels of the Styrian poultry industry such as serological analyses of parent flocks, the determination of the contamination of rodents with *Salmonella* in poultry farms and the monitoring of residues in broilers.

Key words: *Salmonella* monitoring, consumer protection

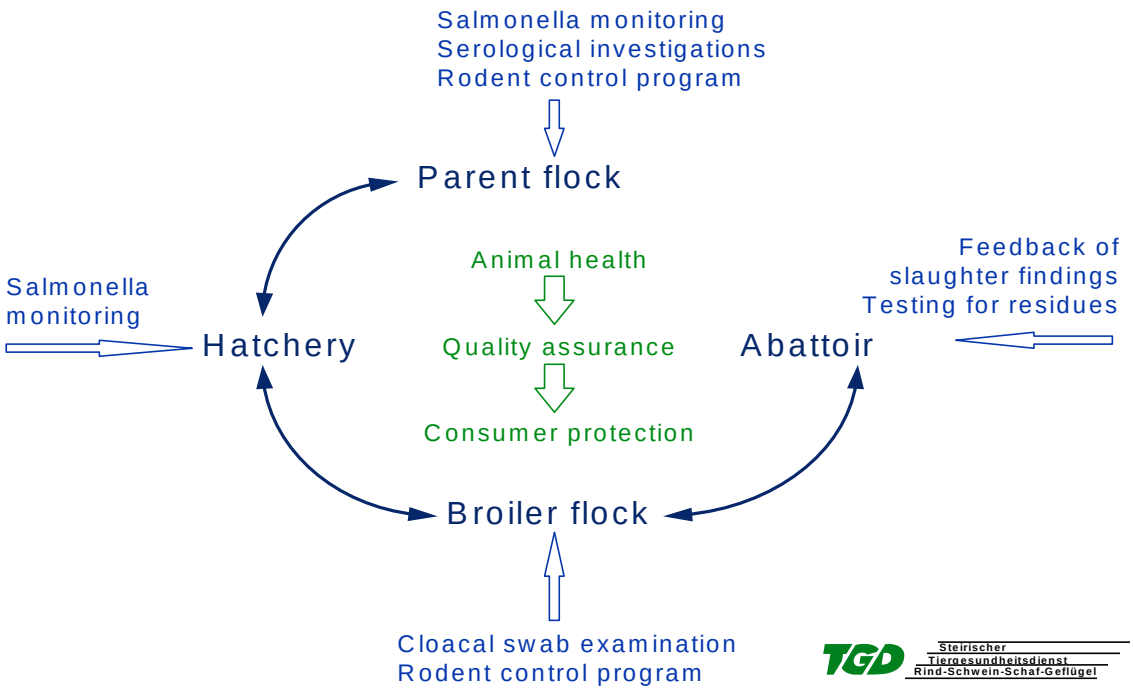
Introduction

Salmonellosis is still one of the most important zoonosis. Like other industrialized countries, Austria suffered a significant increase of salmonellosis occurrences from 1985 onwards, mostly due to *S. enteritidis*. In Austria, the incidence of foodborn salmonellosis increased from 19 cases per 100.000 inhabitants in 1985 to 144 cases in 1991. Almost every outbreak of salmonellosis in man is associated with the consumption of poultry products. Responding to this situation, the Federal Government of Austria passed the „Poultry Hygiene Regulation“ (BGBl. Nr.274/1991). In addition to general provisions, the „Poultry Hygiene Regulation“ also introduced concrete hygiene measures in parent flocks, hatcheries, broiler flocks and poultry abattoirs.

For parent flocks, the examination for *S. pullorum gallinarum* and *S. enteritidis* of the chick delivery box inserts or bedding and of a max. of 10 deceased chicks is compulsory within the first 48 hours after birth. When the chickens reach 10% of the laying performance, the blood of 600 animals must be tested for *S. pullorum gallinarum* and *S. enteritidis*. Of each parent flock, the hatchery has to examine 20 to 30 chicks deceased in the shell each year. According to the

provisions of the „Poultry Hygiene Regulation“, broiler farms are required to take 9 cloacal swabs for a *Salmonella* test, which is to be performed within 3 weeks of the scheduled slaughter date. In our opinion these measures are not sufficient to effectively reduce the incidence of *Salmonella* in poultry and poultry products. Some countries such as Switzerland (Hoop,1994) and Sweden (Wierup,1994) for example, have introduced promising *Salmonella* monitoring and prevention measures. These programmes are based on tighter controls of the parent animal farms and of the hatcheries. The recognition of a *Salmonella* infection in the early stages of production is of decisive importance to prevent the infection from spreading. Nevertheless, effective measures are required at all stages of the production chain. The Styrian *Salmonella* prevention programme generally follows the recommendations of the WHO Guidelines (1994). All activities aim at reducing the occurrence of *Salmonella* in poultry products in the interest of consumer protection.

Figure 1



Material and methods

23 parent flocks with approx. 100.000 animals participate in the Styrian *Salmonella* prevention programme. The first measure is to take stock of the buildings and the hygienic conditions and fill in a check list, as well as a bacteriological control of the unoccupied stable. On the day the chicks are delivered, 10% of the chicken delivery box inserts and some dead animals from the batch are examined for the presence of *Salmonella*. The checks during the raising period include the bacteriological analysis of dying or ill animals on the 7th day of life and the examination by wipe and drag swabs during weeks 7 and 16. Thereafter, at monthly

intervals, the owner of the animals and the veterinarian send in wipe swabs and drag swabs for examination. The hatcheries have 50 chicks deceased in the shell or one meconium mixing sample of 250 chicks of each domestic parent flock analysed on a monthly basis.

In 1996, the *Salmonella* vaccination of parent flocks was initiated. The parent animals are vaccinated with *Salmonella* vac T® and Talovac 109 SE® of co. TAD. The peroral administration with drinking water of Salmonella vac T® is performed on the 1st day and during the 7th week, respectively. Talovac 109 SE® is administered by subcutaneous injection into the neck during the 16th week after birth. As part of the serological examination of parent flocks, 60 blood samples were taken per flock and investigated for the following parameters: NCD, IB, ILT, TRT, CAA, Gumboro, Celo, Reo, M. gallisepticum, M. synoviae, S. enteritidis.

Operators of farms where positive *Salmonella* tests were obtained, are motivated to introduce a tighter rodent control. The programme workers offer to establish the initial contact with professionals in this area. Caught animals are examined for the presence of Salmonella.

The results of the cloacal swab examinations (Gruber et al.,1997) and of the separate slaughtering of *Salmonella* positive and negative flocks (Pless and Köfer, 1997) will also be presented at this congress.

Results

The results of the *Salmonella* examinations in the parent flocks are shown in **table 1**. All samples from flocks subjected to a vaccination with *Salmonella* vaccines were negative in the bacteriological test.

The results of the *Salmonella* tests in the hatcheries are shown in **table 2.**

Table 1: Comparison of the results of the total number of positive samples 1995 to 1996.

Samples	1995		1996		Total
Positive	71	(7.5%)	19	(3.9%)	90
Negative	879	(92.5%)	466	(96.1%)	1345
Total	950	(100%)	485	(100%)	1435

Table 2: Comparison of the results of the total number of positive samples 1995 to 1996.

Samples	1995		1996		Total
Positive	59	(9.9%)	58	(6.8%)	117
Negative	536	(90.1%)	792	(93.2%)	1328
Total	595	(100%)	850	(100%)	1445

In the serological examination for *S. enteritidis*, no further flocks were diagnosed as *Salmonella* carriers. All serologically positive flocks were also positive in the bacteriological test.

Conclusions

Both the results of the Salmonella tests in parent flocks and in the hatcheries show a significant decrease of positive findings. The effect of the Salmonella vaccination is certainly felt. The positive effect of regular controls and the associated measures also contribute to the reduction. However, the measures to prevent salmonellosis cannot improve the situation in isolation. Any significant progress must rely on a combination of all measures.

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Antimicrobial Efficacy of Different Drugs Against Experimentally Induced *Salmonella Pullorum* Infection in Broilers and Estimation of some Blood Parameters

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Summary

Groups of 4 weeks old broiler chicks were given four antimicrobial agents in their drinking water for 7 days, after experimental oral infection with *Salmonella pullorum.* Growth rate and mortality studies showed that the best therapeutic effects were produced by *Trimodin Forte (Trimethoprim Plus Suiphadiazine).* All the four antimicrobial agents reduced mortality associated with experimental pullorum disease. Post Mortem findings were also described. The total Erythrocytic Count (TEC) and Haemoglobin content (Hb), significantly decreased and Total Leukocytic Count (TLC), significantly increased, 3 days post challenge.

Key words: Salmonella pullorum, Trimethoprim plus Sulphadiazine, Post mortem, TEC, Hb, TLC.

Introduction

The pullorum disease has long been recognized as a serious economic menace to the poultry industry throughout the world. Without effective control measures against this insidious problem, real development of our poultry would be impossible.

The present project was designed to evaluate the efficacy of Ampicillin, Oxytetracycline plus Neomycin, Chloramphenicol &Trimethoprim plus Sulphadiazine as medicinal therapy, against the experimentally induced *Salmonella pullorum* infection & estimation of some blood parameters in broiler chicks.

Materials and Methods

Salmonella pullorum inoclum

A virulent smooth field strain of *Salmonella pullorum,* preserved by freezedrying was used.

Design of Expenment

At 4 weeks of age, 120 chicks were randomly selected and divided into six groups having 20 birds each. Five groups A to E were inoculated with 1 ml of 24 hours broth culture of *Salmonella pullorum* per birds, orally. The control group E was infected but nonmedicated, while control group F was non-infected and non-medicated.

Protocol for Group Treatment

At the onset of clinical sings, group A to D were treated with allotted drugs, as mentioned below.

1. **Group A**: Infected and medicated with Ampicillin 20% (Ampicillin Trihydrate) at a dose rate of 0.5 grams/liter of water.

2. **Group B**: Infected and medicate with Oxy-N-50 (Oxytetracycline plus Neomycin) at dose rate of 2.5 grams/liter of water.
3. **Group C**: Infected and medicated with Chloricol-10 (Chloramphenicol) at a dose rate of 2.5 grams/liter of water.
4. **Group D:** Infected and medicated with Trimodin forte (Trimethoprim plus Sulphadiazine) at a dose rate of 1 ml/2.5 ml of water.
5. **Group E**: Infected and non medicated control group.
6. **Group F**: Non-infected and non-medicated control group. The antemortem symptoms, morbidity, mortality, necropsy findings and weight gain were noted. Mortality after medicated and compared for the efficacy of four experimental drugs.

Hematological Studies

The following blood parameters were recorded 3 days before and after challenge.

1. Total Erythrocytic Count (TEC) (Natt & Herrick, 1952).
2. Total Leukocytic Count (TLC) (Natt and Herrick, 1952).
3. Haemoglobin Content (Hb) (Coles, 1986).

Results and Discussion

Comparison of Treatment Effect

Observation were recorded daily relative to mortality. Deaths were first commenced on 4th day of experimental infection in groups A to E. No mortality was recorded in control group F. throughout the study period. In this study, mortality varied from 10-70 percent i.e. lowest in group D and highest in group E. The overall mortality in all groups A to F was 25.83 percent and the recovered percentage was 74.17 percent.

Growth rate and mortality studies showed that the best therapeutic effects were produced by Trimodin Forte (Trimethoprim plus Sulphadizine) followed in effectiveness by Chloricol-10 (Chloramphenicol), Ampicillin-20% (Ampicillin) and OXY-N-50 (Oxytetracycline plus Neomycin). The present results favorably confirm the findings of Smith & Tucker (1975), Reddy (1985) and Reddy et al. (1987) who described the superiority of Trimethoprin plus Sulphadiazine combination against salmonellosis. Majid et al. (1991) also mentioned the same results.

Postmortem Examination

Postmortem changes observed were, enlarged and congested liver streamed with hemorrhages; spleenomegaly; enlarged heart, pericarditis and thickened and inflamed intestinal walls. These necropsy findings were in agreement with the findings of Nafees (1984), Calnek et al. (1991) and Younis (1964).

Hematological Study

Hematological alterations were studied in all experimental groups at the age of 25 days (3 days pre-infection) and 31 days t3 days post infection). The data of Total Erythrocytic Count, Total Leukocytic Count and Haemoglobin content were obtained which were analysed statistically. A significant decrease ($P < 0.05$) of Total Erythrocytic Count and Haemoglobin content was found post challenge, in all the infected groups (A to E), except control group F. whereas a significant increase ($P < 0.05$) of Total Leukocytic Count (TLC) was observed in the infected groups A, to E except control group F. post infection.

These Hematological findings in the present study are in line with the observations of Kokosharov and Todorova (1987), Calnek et al. (1991) and Younis (1994).

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A Campaign against bovine Salmonellosis in the provinces of Vaasa, Oulu and Lapland in Finland

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Summary

The area of Ostrobothnia has been considered to be the most common area for salmonellosis in animals in Finland, on the basis of practical experience. Environmental specimens taken at a slaughterhouse in this area in 1993 were unusually often found to be positive, especially for Salmonella infantis. This finding was the starting point for the campaign against Salmonellosis in cattle in the provinces of Vaasa, Oulu and Lapland. The resulting surveys, which were supported financially by the local dairies and slaughterhouses, showed that over two hundred farms were infected. Most of these farms are now free of the infection. New instructions for eradicating salmonellosis from cattle and from environment were provided for veterinarians, farmers and other groups associated with cattle in the course of the campaign.

Key words: Salmonella infantis, epidemic, cattle

Introduction

Ostrobothnia, an extensive region on the west coast of Finland, is considered to be the most common area for salmonellosis in cattle. It is also the most important area for cattle-rearing, with cattle houses located close to each other on the both sides of the numerous rivers. Calves and heifers have traditionally been sold from one farm to another, and this is nowadays a form of organized business. Both the densitity of cattle farms and the sale of animals pose a risk of the spread of epidemics, which is thought to be further compounded by the prevalence of fur farming in the same region.

In the early 1990´s salmonellosis was found only individual cases in the province of Vaasa, with around ten farms annually being found to be infected and being issued with restrictive orders under the Animal Diseases Act. The first signs of a change in the Salmonella situation in this province appeared in autumn 1993, when Salmonella was frequently isolated from the specimens taken from the sewer in the intestine department of one of the slaughterhouses, although clinical salmonellosis had not increased in the area. This led to the conclusion that subclinical salmonellosis was becoming a problem in Ostrobothnia.

Another significant finding was that the serotype ”infantis” was increasing among those identified by the National Veterinary and Food Research Institute. This serotype had first been found in soy fodder and farm animals in 1971, and then in slaughter waste in 1976. Since the late 1970´s it was found annually both in cattle and in broiler chickens. It has also been found on fox and mink farms in the province of Vaasa since the early 1980´s.

Effect of Salmonella infection on animal husbandry and the food industry

Salmonella infection, clinical or subclinical, causes farmers a huge amount of extra work and great expense. It also leads to increased production costs in the food processing industry, on account of the need for separate milk collections, sanitary slaughter of carrier animals, special treatment of milk and meat etc. Eradication of Salmonella infection on farms is the most important step towards insuring the purity of foodstuffs and safe food for the consumer. This was the background to the campaign against Salmonellosis.

The campaign against Salmonellosis in the provinces of Vaasa, Oulu and Lapland

A working group which later came to be called the ”Salmonella project for the provinces of Vaasa, Oulu and Lapland” was established in February 1994. This involved contributors from two directions, the veterinary advisors (provincial and local authorities, health service, meat inspectorate and laboratory veterinarians) and industrial representatives, including those from the local dairies and slaughterhouses.

The aims of the project were:

- to eliminate and prevent Salmonella infection in farm animals,
- to introduce new instructions for preventing salmonellosis and eradicating Salmonella on the infected farm where it is identified,
- to train veterinarians and others involved with animal husbandry in the prevention of infectious diseases,
- to introduce new measures to reduce the risk of Salmonella infection via animal sales,
- to arrange a Salmonella insurance for farmers to guarantee the farm´s survival after the economic losses caused by Salmonella, and
- to find out the real occurrence of Salmonella on Finnish farms

Practical actions

In 1994

- First instructions issued to farms, veterinarians and dairies for the prevention of salmonellosis.
- Training meetings for veterinarians.
- Collection of information of Salmonella-positive farms raising cattle, broiler chickens or mink/foxes from official files for 1988-1994. This revealed two highly infected areas, the districts of Teuva, Karijoki and Jurva and those of Himanka, Ullava and Lohtaja.
- Introduction of a new pooled fecal sampling method, which was cheaper and enabled the industry to carry out larger surveys.
- Initial surveys at two slaughterhouses, one in the province of Vaasa and the other in Oulu. The aim was to discover in which areas infected herds were to be found.

In 1995

- A second survey was carried out at seven slaughterhouses. 1274 animal transportation trucks were examined, out of which 68 (0.05 %) were positive for Salmonella. 37 new infected herds were found. Some of these had contracted the infection via contaminated feed delivered by a feed factory.
- Organized calf sales demanded that farms which sell calves to other farms should undergo an annual Salmonella examination of their entire herd by the pooled fecal sampling method. Over 80 % of the dairy herds in the area were examined in autumn 1995.
- Instructional meetings were arranged for farmers and other groups involved. Farmers whose herds were infected were invited for open discussions.

In 1996

- Instructions were given on how to treat Salmonella contaminated feces and pasture lands.
- Herds from which calves will be sold to other farms through organized channels were examined again for the presence of Salmonella.
- Further advisory meetings were held. Farmers who had Salmonella problems in their herds received counselling from a psychologist on stress control.
- Salmonella insurance was established on the farm level.

Conclusions

The main goal and strength of this project has been the good co-operation achieved between the different parties. The farmers have shown a good motivation to win the fight against Salmonella, which has been the basic requirement for success. The farmers and others involved are now much more prepared to resist any other infectious disease which might appear in the country. Veterinarians working in these provinces have done a great deal of work, and the other groups involved have also made an indispensable contribution to the project.

The number of herds infected with Salmonella has decreased quickly since 1995, so that where there were 146 such herds in the province of Vaasa at the end of 1995, there were only about 30 in February 1997, just 13 months later. Another proof of the success of the campaign is that the environmental samples taken in the slaughterhouses are now consistently negative.

The Salmonella project group has achieved most of its aims, but it still is active with an emphasis on the preventive measures. The future goal will be to go on producing Salmonella-free foodstuffs in the main cattle rearing region in Finland.

Bovine Salmonellosis in a restricted area in Finland

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Summary

The Salmonella project in the provinces of Vaasa, Oulu and Lapland showed that farms in Karijoki and Teuva, two adjacent local government districts in Southern Ostrobothnia, had an abnormally high rate of infection with Salmonella. All the 245 cattle herds in this area were therefore examined, comprising 195 herds of dairy cattle and 50 of beef cattle. 32 of these were infected with Salmonella infantis. Four village epidemics were found, each involving 3 to 7 infected herds.

An eradication programme was drawn up for each farm and the farmers and other people involved with the farms were instructed on how to eliminate Salmonella infection and prevent it from spreading. All the farms were examined again one year later and only one new infected herd was found.

Keywords: bovine salmonellosis, Salmonella infantis, pooled fecal sample, epidemics, Salmonella eradication program

Background to the Salmonella project

A project was organized in the provinces of Vaasa, Oulu and Lapland, in which pooled fecal samples for Salmonella tests were collected from the floor of each cattle transportation truck after the animals had been taken to the slaughterhouse (Nauholz 1996). The survey revealed that two adjacent local government districts in Southern Ostrobothnia, Teuva and Karijoki, formed a high risk area for bovine salmonellosis, with 12 infected herds (7.3 % of all herds) in Teuva and 3 in Karijoki (3.8 %). The Veterinary and Food Department of the Ministry of Agriculture and Forestry in co-operation with the local authorities decided that all the herds in this area should be examined for the presence of Salmonella in autumn 1995, an operation supported by the local dairy and slaughterhouse and the Association for Animal Disease Control.

Salmonella examination

A veterinarian was hired for three months to take samples, plan an eradication programme for the farms and instruct the farmers and others involved. Even the practical cleaning and disinfection on some of the infected farms was supervised by the veterinarian. For practical reasons the instruction was organized in co-operation with the local dairy and slaughterhouse.

The survey covered 195 herds of dairy cattle and 50 of beef cattle. Samples were taken from each animal or in some occassions from groups kept in the same pen. The method of using pooled fecal samples was also tested for its reliability (Kivelä 1996). The samples were examined either at the National Veterinary and Food Research Institute in Helsinki or at the Foodstuff and Environmental laboratory in Kauhajoki.

Salmonella findings and village epidemics

The survey revealed 17 new herds infected with Salmonella in addition to the 15 found before so that at the end of 1995 there were 23 infected herds in Teuva (13.9 %) and 9 in Karijoki (11.3 %).

Four village epidemics were found, in which 3 to 7 adjacent farms were Salmonella-positive. Some farms in other locations had gained their infection from these villages via purchases of animals or animal feed, which are the most common ways in which infection spreads. Other contacts such as a short distance between farms, a high density of animals, the use of feces as fertilizer, the loan of agricultural machines from one farm to another and other forms of co-operation increase the possibility of infections to spreading. Other animals such as pets, pests and especially wild birds may act as vectors if they have free passage to cattle houses and fodder stores.

Eradication of Salmonella in infected herds

Written instructions were drawn up for each farm individually containing detailed advice on cleaning and disinfection of the cattle house, isolation of carrier animals, slaughter of permanent carriers, prevention of the spread of Salmonella and destruction of contaminated animal feed, urine and feces. The main goal was to cut the infection chain from feces to feed and back to the intestines. A great number of animals were free of infection in about four months, once the infection chain had been successfully broken. Farmers were advised to send chronic carriers for sanitary slaughter to reduce the infection pressure.

Some farms failed to eliminate Salmonella caused by contamination in feed as demonstrated by bacteriological examinations. There were also motivation problems for the farmers, and contributory factors such as too many animals in the cattle house or old cattle houses usually lay behind the cases of failure.

Salmonella follow-up in the area

All herds in the area were examined again in autumn 1996, and only one new case was found. There were only five infected herds remaining in the area in April 1997.

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Fig.1. The Salmonella findings and village epidemics in Teuva-Karijoki area.

Fig.1. The Salmonella findings and village epidemics in Teuva-Karijoki area.

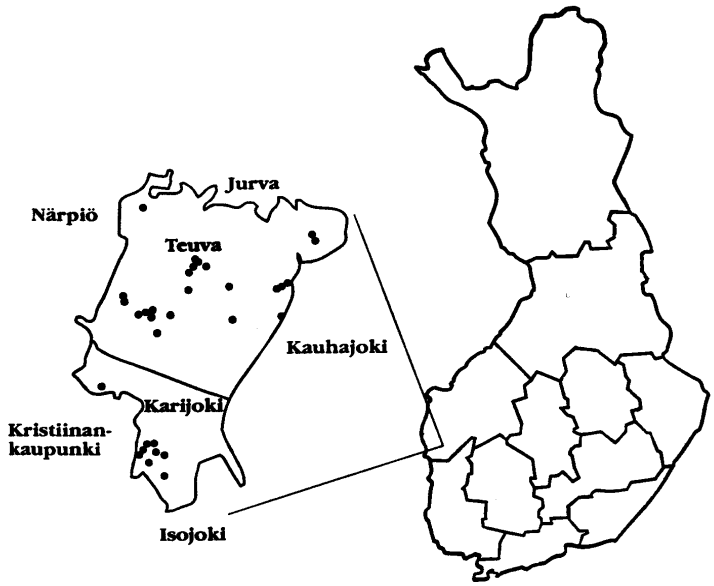

Fig.1. The Salmonella findings and village epidemics in Teuva-Karijoki area.

Viability of Salmonella typhimurium *in the solid fraction of slurry from agricultural wastewater treatment plant stored at two different temperatures*

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Summary

The solid fraction of slurry obtained by mechanical separation was stored at two different temperatures - in a refrigerator at 4°C and a thermostat at 20°C. The Salmonella typhimurium bacteria tested remained viable for 111 days at 4°C and for 63 days at 20°C. During the experiment, at both temperatures tested, we observed a decrease in the values of pH from the initial value of 8.8 to the final values of 6.63 at 4°C and 7.45 at 20°C. The concentrations of ammonia nitrogen in the solid portion of slurry stored at 4°C amounted to about half of the initial value while the storage at 20°C resulted in a 20-fold decrease. The values of total nitrogen increased at both temperatures. No significant changes in the initial and final concentrations of total phosphorus were observed.

Key words: wastewater treatment plant, pig farm, solid fraction, Salmonella typhimurium, indicatory microorganisms, survival

Introduction

Recently, salmonellosis is a widespread alimentary disease. Salmonella organisms are capable of surviving for a long period of time in the environment before they are totally eliminated and it is this period which forms an important part of the infectious process not only with regard to salmonellae but also to other enterobacteria.

With the increasing concentration of animals, particularly in rearing of calves, pigs and poultry (Ivanová et al. 1995), the risk of occurence of undetected diseases or their carriers on agricultural farms increases. In order to decrease the negative effect on the environment, agricultural wastewater treatment plants (WTP) have been built on agricultural farms. Wastewaters from animal housings fed to these WTP are treated by technologies which in the first stage separate the liquid and solid fractions of slurry by means of vibrating screens or belt presses. The solid fraction is transported to field manure heaps or dumps and is subsequently used as an agricultural fertilizer. For this reason we aimed our studies at investigation of survival of Salmonella typhimurium microorganisms introduced indirectly into the solid fraction of pig slurry under laboratory conditions and stored at two different temperatures imitating the winter and summer periods.

Material and methods

The solid fraction from mechanical separation of slurry was obtained from the WTP operating on a pig farm in Košická Polianka and was stored at two different temperatures, at 4°C in a refrigerator and at 20°C in a thermostat. A lyophilised strain of Salmonella typhimurium SK 14/39 (obtained from State Veterinary Institute in Prague) was used as a tested strain. The revitalized culture was inoculated to leather squares (4 x 4 cm). The carriers obtained were examined in time intervals specified in Table 2 according to the method of Müller (1973).

Chemical examinations were carried out using 50g average samples taken in time intervals shown in **Table 1.**

Determinations of physico-chemical parameters, pH, dry matter, ammonia nitrogen and total nitrogen were carried out according to the national standard ČSN 83 0540 and the total

phosphorus was determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 1985).

Results

The Salmonella typhimurium bacteria tested remained viable for 111 days at 4°C and for 63 days at 20°C (**Table 2**). The carriers stored at 4°C allowed us to carry out quantitative salmonella determinations up to day 111 and that one stored at 20°C up to day 37. Qualitative determinations on the carriers stored at 20°C allowed us to detect Salmonella up to day 63. Cultivation of the solid portion of slurry showed no presence of Salmonella spp. at the beginning of the experiment.

Results of physico-chemical examinations are summarized in **Table 1.**

Discussion

Results obtained indicate that the Salmonella typhimurium strain tested survived longer at lower temperatures which is in agreement with the statement of Strauch et al. (1991) who observed in their studies better viability of salmonellae in slurry with 5% content of DM stored at temperatures below 10°C. Our investigations confirmed the findings of Strauch (1987) that salmonellae survive in most cases for 150 days and their reduction by 90% occurs during the first two to four weeks along with the decrease in pH. The decrease in pH during the storage of slurry is related to the production of fatty acids by natural bacterial flora. The toxic influence on salmonellae, combined with the observation that salmonellae in comparison with natural bacterial flora are unable to secure nutrients for themselves, are the probable causes of their death.

Strauch et al. (1989) stated that the viability of various types of salmonella in liquid slurry varies from 4 to 97 days in summer and up to 87 days in winter. Our results indicate that Salmonella typhimurium organisms can survive even longer in the solid fraction of slurry during the winter period. For this reason it is necessary to pay appropriate attention to the hygienic disposal of the solid fraction which can serve as a source of spreading of infectious disease agents in the environment.

The high content of water in the solid fraction of slurry prevents the onset and development of thermic processes which take place during the storage of solid animal manure (ensuring devitalization of pathogens) therefore this fraction must be subjected to further processing, preferably by composting. Besides devitalization of pathogenic microorganisms, the composting process provides a high quality product with excellent fertilization properties (Novák 1994; Krupicer et al. 1996).

In the case, when for various reasons the solid fraction cannot be subjected to composting or some other appropriate hygienic treatment, it is necessary to pay maximum attention to its storage. For that reason, in the interest of minimization of the risk of transfer of pathogens by means of the solid fraction of slurry, it is necessary to carry out systematic epidemiological monitoring of this material.

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Table 1. Physico — chemical parameters

Date of collection	**Temperature of storage**	**pH**	**dry matter** (%)	**organic matter** (%)	**inorganic matter** (%)	**N-NH_4** ($mg.kg^{-1}$)	**N-total** ($g.kg^{-1}$)	**P-total** ($g.kg^{-1}$)
10. 1	original sample	8,80	15,62	90,49	9,51	420,21	10,78	19, 36
25. 10	4°C	7,92	13,79	91,77	8,23	336,17	13, 46	6, 96
	20°C	6,86	23,24	80,97	19,03	112,06	22, 21	11, 15
16. 11	4°C	7,07	13,50	89,50	10,50	395,00	15, 92	9, 43
	20°C	6,81	18,20	78, 9	31,10	67,20	24, 38	21, 14
11.12	4°C	6,70	26,30	93,10	6,90	357,20	14, 62	6, 24
	20°C	7,20	26,90	84,10	15,90	47,60	26, 54	21, 73
8 . 1	4°C	7,30	13,30	89,40	10,58	84, 04	5 9,94	3 5,00
	20°C	7,46	14,30	73,00	26,96	21,01	12 0,22	14 9,00
30. 1	4°C	7,08	24,40	89,92	10,08	42,02	190,22	9 5,10
	20°C	6,98	16,20	77,49	22,51	7,00	222,55	18 9,60
12 . 3	4°C	6,63	14,16	89,13	10,87	190,50	26,08	11,86
	20°C	7,45	14,81	72,00	28,00	22,40	3 0,32	1 7,99

Table 2. Survival time of Salmonella typhimurium in solid fraction

Survival time (days)	**4°C — CFU.ml^{-1}**	**20°C — CFU.ml^{-1}**
0	$8,71 \times 10^4$	
13	$1,7 \times 10^5$	1×10^3
37	$8,75 \times 10^2$	$3,9 \times 10^3$
63	$2,1 \times 10^3$	+
92	$1,2 \times 10^4$	—
111	$6,15 \times 10^3$	—
156	—	—
190	—	—

Free papers

Animal hygiene in the field of small and companion animals

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Summary

There is an obvious discrepancy between the topics presented and discussed during the last meetings of the International Society for Animal Hygiene and the everyday work at a Veterinary Faculty, at least within Germany. This is mainly influenced by the latest developments in the European countries regarding the veterinary profession, represented by the veterinary graduates desire to go into small- and companion animal practice. Since Animal Hygiene is mainly a discipline focusing on prevention, it could definitely gain importance in the field of small- and companion animal medicine, but also in the area of zoo animal care if the present requirements are recognised and methodological measures taken by the institutions in charge accordingly.

Key words: animal hygiene, small and companion animals, trends in veterinary medicine

In Germany, approximately one half of the veterinarians is currently working in mixed practice. One third is working exclusively with small animals and only 18% work in large animal practice. One the one hand, this development is caused by a structural change in agriculture, on the other hand, it is related to the increasing relevance companion animals have in society. This development is well represented by the constant growth seen pet product and food industry.

Yet another important factor is the constantly increasing percentage of female veterinarians within this profession. 1995, 32,7% of the veterinary practitioners were female. In the same year, the percentage of female graduates from veterinary schools came up to 61,2 and at the same time 80,4% of the students starting their studies were female. This means that the veterinary comunity - at least in Germany - will consist mainly from women with in few years, who are generally known to be more interested in small- and companion animal medicine and equine practice.

It is evident that we’re not dealing with a purely German problem. This observation is supported by the statistics published by EUROVET regarding veterinary specialisation in large-, small-, mixed-, and equine practice within the European Union. Numbers are available for 12 out of 15 countries; only Finland, Italy, and the Netherlands don’t provide detailed statistics differentiating the groups in this manner. In only one out of 12 countries, namely Ireland, more than half of the practising veterinarians work in large animal practice. Countries with predominately small animal practices are Belgium, France, Greece, Luxembourg, Portugal, Sweden, and Spain. Mixed practices are mostly found in Denmark, France, Germany, and the United Kingdom. German statistics show that veterinarians working in mixed practice used to be specialised in large animal medicine and changed their focus to companion animal medicine with in the last 30 years (figure: Specialization of veterinarians within Europe (1996)).

This leads to the conclusion that small- and companion animal medicine is playing an important if not the most important role in the majority of the European countries.

We should ask ourselves if this field is recognised accordingly by the International Society for Animal Hygiene, or if this area needs to receive more attention in the interest of the field itself and for the benefit of the veterinary community in general.

Looking at the last four meetings of the International Society for Animal Hygiene, we can see that some species have been clearly preferred. There is no question that certain topics which are not related to a specific species, dealing with basic scientific questions, play an important role and there's no intention to change this. But we have to ask ourselves if we should continue to focus almost exclusively on farm animals in general and specifically on cattle, pigs, and poultry if this is reducing the attention we invest in species playing an increasing role for the practising veterinarian. It is striking that meetings of the International Society for Animal Hygiene included the same number of presentations on fur animals, buffaloes, and camels, or even insects as on dogs, cats, small companion animals, horses and zoo animals. For this reason, one should think about the possibility to support this field to at least a minimum extend by offering a special session at next years meeting.

SPECIES-RELATED TOPICS OF THE ISAH-MEETINGS 1985-1994

Species	1985	1988	1991	1994
Non-species-specific topics	40	42	68	32
Farm animals in general	11	11	7	14
Cattle	29	39	38	29
Pigs	32	50	51	32
Sheeps	1	12	4	6
Goats	1	3	-	1
Poultry	16	25	30	33
Meat rabbits	-	1	1	1
Fur animals	-	1	2	2
Buffaloes, camels	-	2	-	2
Game, wildlife	-	1	-	1
Rats, mouses	-	-	1	-
Fish and other aquatic animals	-	-	1	2
Insects	-	-	-	2
Horses	3	1	1	3
Small animals in general	1	-	-	-
Dogs	-	-	-	2
Cats	-	1	1	-
Zoo animals	-	-	-	1
TOTAL	**133**	**189**	**205**	**162**

MAP

A different focus regarding the spectrum of species would subsequently have impact on other important aspects of animal hygiene, which have obviously received limited attention so far, including a reduction of the risk of infections for small- and companion animals using systematic hygienic measures. This includes the prevention of anthroprozoonosis, meaning infectious disease transferred from humans to animals, as well as zooanthroprozoonosis, meaning infectios disease transferred from animals onto humans. Anthroprozoonosis includes the risk of

infection from humans to specifically dogs and cats, but also the transmission of germs by feeding raw meat and organs, uncooked milk products, kitchen scraps, and contaminated food products. Zooanthropronosis include numerous germs which may be transmitted from dogs, cats, rodents, pet birds, and fish to humans. The risk is mostly overestimated. However, it is definitely important to take care of this topic and to inform animal owners regarding the potential danger and prophylactic measures. Danger for humans is represented by rabies, leptospirosis, salmonellosis, cat scratch disease, cat pocks, echinococcosis, toxoplasmosis, toxocara infection, mange mites, mycosis, allergies and many others. Rodents can transmit choriomeningitis, mainly known in hamsters. Other rare dangers include diplococcus infections transferred from guinea pigs and rabbits, leptospirosis seen in chinchillas and ferrets, listeriosis and yersiniosis in small animals, predominately mycotic infections and toxoplasmosis.

Pet birds have a high risk for their owners, mainly represented by allergies, psittacosis, avian mycobacteria and last but not least newcastle disease, causing to sever cases of conjunctivitis. Even keeping fish is not free of hygienic risk since it may cause tuberculosis granuloma in humans.

Aside from listing potential infectious risks of small- and companion animals we have to pay more attention to areas which are prone to cause hygienic problems regarding infections. Therefore, we should investigate the problems seen in veterinary practices and clinics, animal shelters, but also play grounds polluted by dogs and cats as well as streets, side walks and parks. It would be important to make suggestions how environmental pollution caused by dogs and cats could be prevented. In this regard, it is very important to emphasise hygienic-epidemologic investigations to increase transparency for realistic figures regarding the health risks for humans.

In the interest of our speciality, we need to clarify that epidemiology is a preventative discipline, belonging into the field of animal hygiene. There is a distinct lack of epidemiological studies in the area of small- and companion animals regarding health risks for the veterinarian’s patients in small animal practices and animal clinics. From epidemiological studies carried out in German hospitals we learned that approximately 40.000 people are dying every year from illnesses they acquired within hospitals. This lead subsequently to the fact that research is paying more attention to the area of hygiene in human medicine than before. Analogue epidemiological studies are not available in veterinary medicine, but they ought to be an important field of research in animal hygiene.

Another substantial hygienic aspect is represented by the question if we can use animal - in this case companion animals - as an indicator for environmental pollution affecting humans. This would also be considered an hygienic-epidemiological study which would help to evaluate regional differences regarding the environmental pollution affecting companion animals. These results could easily be generalised to humans - at least as a work hypothesis.

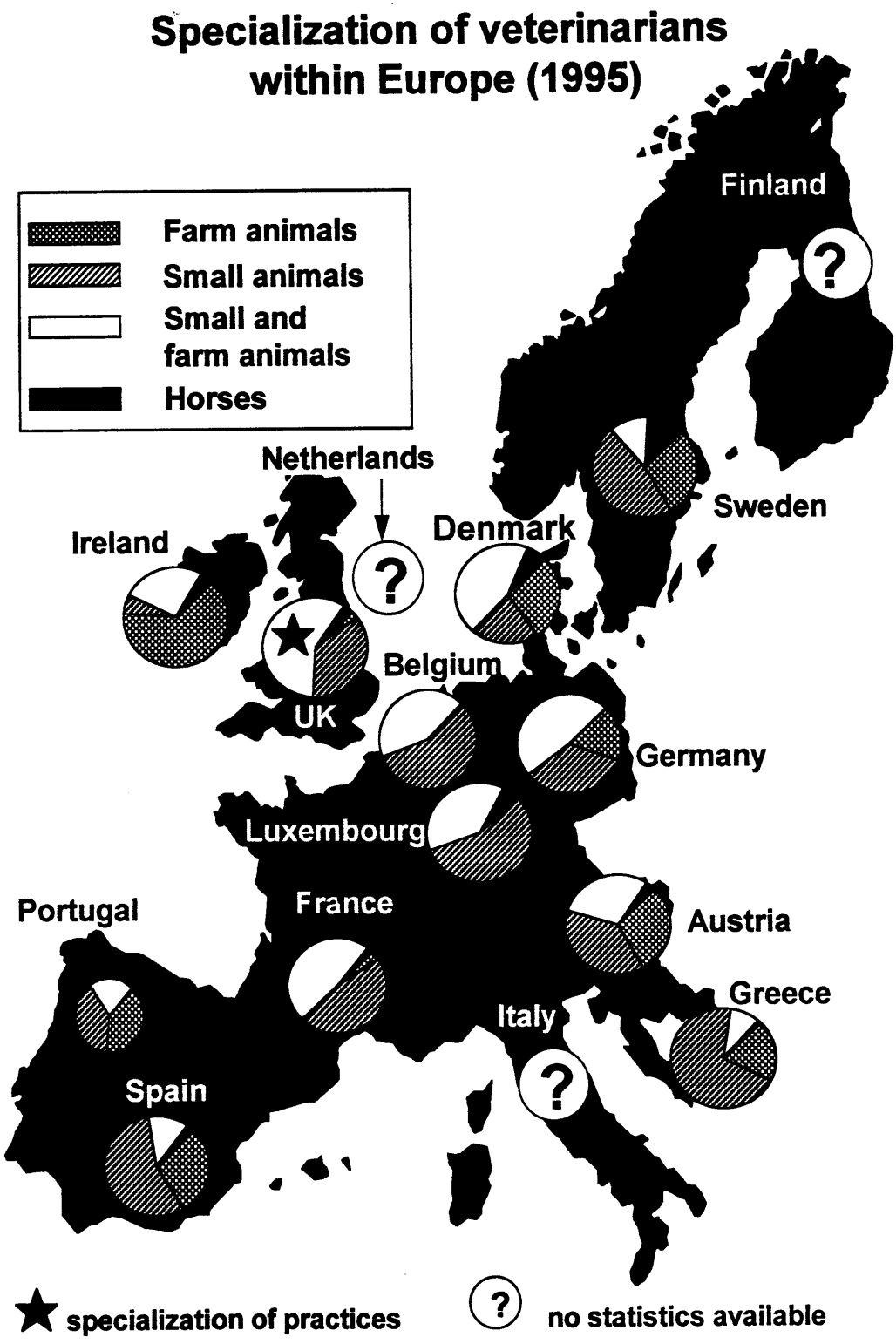
Another question which hasn’t received enough attention are questions related to the human-animal bond. In housing animals - especially farm animals - the major focus is too much based on technical questions, climate, risk of infection and other very important hygienic parameters. We tend to ignore the fact that a good stock person can compensate sub-optimal

hygienic conditions and other environmental influences. The positive effects of ideal hygienic conditions are worthless if the care taker treats the animals in a negative manner.

Most countries are not suffering from a lack of veterinarians. Some countries, like e.g. Germany, train significantly more veterinarians than needed. Therefore, we should not allow the veterinary profession o give up certain fields of interest and leave them to others. In contrast, we need to accomplish new fields in the interest for our profession. Taking the field of animal hygiene into consideration, there are numerous areas regarding small- and companion animals that are basically not taken care of and which veterinarians feel not responsible for. However, animal owners signal that there is an enormous lack of information and counselling. Therefore, another important field of interest in animal hygiene could be seen in taking care of instructing owners before they acquire a new pet, since this is representing a prophylactic and therefore hygienic aspect. Further, many practitioners in veterinary clinics ask for information from affiliated hygienic institutions how animals could be protected from infections and housed properly in clinics, shelters and practices. The field of veterinary hygiene must emphasise the fact that one of the most important future aspects in animal housing will be related to prevention, for which our speciality is predisposed. We must take care of these fields of interest to support young scientists and to guarantee a better future for both, the animals and their owners.

It would be much appreciated if these briefly summarised ideas would help to extend the professional spectrum of animal hygiene in the way it was explained.

Literature can be requested from the author


Specialization of veterinarians within Europe (1995)
Farm animals
Small animals
Small and farm animals
Horses
Finland
Sweden
Netherlands
Denmark
Ireland
Belgium
UK
Germany
Luxembourg
France
Portugal
Austria
Greece
Italy
Spain
specialization of practices
no statistics available

Problems of cat-housing in animal shelters
- results of a survey at animal shelters in eastern Germany -

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Summary

The increasing number of free-living or wild and stray unneutered cats in eastern Germany represents a problem of animal hygiene and animal welfare particularly for the fairly new animal shelters in eastern Germany.

A survey including 44 animal shelters in eastern Germany using a questionnaire (return rate: 55 %) led to the following results: according to the overpopulation of free-living, wild and stray cats (approximately 70% of the cats housed in animal shelters) the animal shelters are over-crowded and experience financial problems managing the large number of cats. Since not all shelters neuter every cat (1995: 39% of all adopted cats have been neutered in the shelter) and the release of unmarked cats (27% of the questioned animal shelters mark the neutered cats) it is impossible to control the free-living feline population. Due to the overpopulation, there is a distinct hygienic risk for the cat owners, other people, conspecifics and other animals (e.g. birds).

For reason of limited space, the shelters are unable to fulfil basic requirements for cat-housing which has impact on the individual's welfare. Because of the close contact of animals, insufficient quarantine wards (55% of the questioned shelters have a quarantine ward) varying duration of quarantine and altering reasons why to stay in quarantine, there are frequent health problems within the shelter cat-population, especially in young animals, subsequently increasing costs for veterinary bills.

It is therefore suggested that the veterinarians in charge should support neutering and marking of cats in the shelter for control the feline population and to manage the further training of the shelter-staff about animal hygiene, prevention of infectious diseases and animal and individuals welfare.

Keywords: Cats - animal shelters - cat-housing - hygienic risks - straying cats - feral cats - neutering

Introduction

About 6 million cats are kept in German households (DPA, 1997) but there is no exact information regarding the number of free-living, wild, and straying unneutered cats. Since animal shelters are particularly affected by an increasing number of cats, one can presume that the number of cats brought to the shelters reflects the increasing numbers of free-living or wild cats in general. Cats seems to be a special problem in eastern Germany and some of the new local animal shelters are over-crowded (Wormuth, 1993). The problems resulting from the increasing number of cats housed in or brought to shelters are to be regarded under hygienic, welfare,

financial and organisational aspects. For evaluation of this range of problems, animal shelters were interrogated using a questionnaire which was sent to the shelter.

Material and Method

On the basis of a standardised questionnaire 44 animal shelters in eastern Germany were questioned in June 1996. The rate of return was 55%. The following subjects were part of the survey: dynamic of the cat-population, hygienic aspects and animal welfare in the animal shelters, including the shelter statistics from 1993 until June 1996.

Results

Dynamic of the cat-population in animal shelters

The total cat-population can be subdivided into unneutered and neutered domestic and stray pets living with or close to humans, and a group of unneutered feral cats. The domestic cat-population is subdivided into cats allowed outside and those that are exclusively kept inside the house. This subdivision of the feline population was also found in the questioned animal shelters of eastern Germany whereas the majority of the shelter cats are represented by free-living, wild and straying cats (70%). Unwanted cats being brought to the shelters by their owners make up 10-15% of the shelter population, approximately 10% of the cats are brought to the shelter during the owner's vacation or due to confiscation by officials (2-3%). Due to the uncontrolled reproduction of the free-living or wild cats, the number of cats admissioned to the animal shelters is increasing constantly and distinctly. In average 163 cats was housed per shelter in 1993. Numbers rose to 169 cats in 1994, 203 cats in 1995, and 232 cats in 1996 (projected from July to December 1996). Since the cats brought to animal shelters are mostly ferals, placement of these cats with new owners is fairly difficult. In 1993 only 40% (rate of return 2 %) of all shelter cats was placed with a new owner, 1994 approximately 58% (rate of return 6%), 1995 60% (rate of return 2%) and 1996 (projected from July to December 1996) 46% (rate of return 2%). For control of reproduction, all questioned animal shelters try to neuter cats upon their arrival in the shelter. In addition, 15 of 22 questioned shelters neuter pregnant queens and 5 of 22 shelters euthanasise new born kittens. 1993 only 15% and from 1994 to 1996 in average 33% (1994 approx. 33%, 1995 approx. 32% and 1996 approx. 35%) of the cats were neutered in the shelter. This relatively low percentage is probably due to financial problems and the fact, that it is not detectable if a female cat has been neutered previously. This problem could be solved if ultrasound was available or if shelters and local veterinarians would routinously tattoo cats or implant microchips at the time of castration. Only 4 of the 22 shelters mark their cats with a tattoo when neutering the animal. In one shelter cats are marked with a microchip and one shelter notches the ears of the neutered cats.

Animal hygiene and cat-housing in animal shelters

Hygienic measures includes veterinary care, the existence of quarantine wards, duration and organisation of cat-housing in quarantine, cleaning and disinfektion, and human diseases causally connected with cat-housing.

Most animal shelters vaccinate cats (89%) upon their arrival for prevention of infectious diseases. Approximately 95% of the shelters vaccinate the cats against snuffles and feline panleukopenia, but only 32% of the shelters vaccinate cats against rabies. All animal shelters deworm the arriving cats and treat them with a ectoparasitizide.

Only half of the questioned animal shelters (55%) have quarantine wards. In some cases (10%) the sick and quarantined animals are kept in the same room. Only 4 animal shelters with a quarantine ward put all arriving cats through quarantine. It seems to be unclear for how long cats should be quarantined since quarantine periods differ from 5 days up to 4 weeks. Also the organisation of cat-housing in the quarantine wards varies: 60% of the questioned shelters with a quarantine ward keep the cats in individual cages and 30% house simultaneously arriving cats in groups. 5% of the shelters put all new arrivals into groups of different size, and 5 % keep queens and their litter together in quarantine.

Nearly 53 % of the animal shelters are cleaning the quarantine ward every day and only 23% are disinfecting these wards on a daily basis. The remaining shelters are cleaning and disinfecting the quarantine ward weekly or only when new animals are placed in the cages. Other methods reducing the risk of infections include the use of special clothing for the quarantine ward (2 shelters), provision of separate food and water dishes (1 shelter), and washing and disinfecting the employees hands before and after entering the quarantine section (8 shelters). Frequently used equipment in the quarantine wards includes blankets (100%), baskets (85%), toys (77%), wooden boards (54%), furniture (39%), scratching posts (31%), and pillows (23%). The occurrence of specific diseases, causally connected to hygienic conditions, reflects these deficiencies. Nearly all animal shelters point out that they frequently experience health problems and infections with snuffles (95%), followed by parvovirosis (61%), feline infectious peritonitis (39%), leucosis (22%), and diarrhoea (17%). Feline pasteurellosis, mange, ear mites, and ocular diseases (each occurring in 6% of the questioned shelters) represent common problems. Poor hygiene led also to infections of staff members. Four shelters indicate that at least one employee suffered from trichophytosis. In two cases one of the caretaker was reported to have the cat-scratch-disease. Wound infections (n=4) occur frequently, caused by cat bites and scratches.

Animal welfare and cat-housing in animal shelters

The questioned facilities can provide shelter for an average of 49 cats. In average they took care of 203 cats in 1995, and 232 cats in 1996 (projected from July to June 1996). Even if more than half of the cats found a new owner (1995 ca. 40% and 1996 ca. 60%), the animal shelters would be clearly over-crowded. In no case the demands of the federal chamber of veterinarians (1996) and Leyhausen`s expert opinion (1981) regarding group size and individual welfare could be realised. Each cat requires approximately 2 m2 and the group size shouldn’t extend 5 cats per ward.

Conclusion

The results pointed out that due to the increasing number of free-living and wild cats the animal shelters are in average 100% over-crowded. Low rates of neutering, the release of unneutered or neutered but unmarked cats prohibits control the feline population and leads to a distinct hygienic risk for humans, conspecifics and other animals. Insufficient quarantine wards, varying duration and varying reasons why to stay in quarantine, the formation of large cat-groups in quarantine with close contact to each other, the use of unsuited equipment in the wards, and irregularly cleaning and disinfection lead to epidemic infections of the cat shelter-population, affecting predominately the young animals (crowding disease).

These problems can be solved by veterinarians which should not only treat animals responsibly but also advise the cat owners and the shelter-staff. It is therefore necessary to inform the cat owners regarding consequences of free-living and unneutered cats and further training of the shelter-staff about hygienic measures in the shelter and the prevention of infectious diseases. Further veterinarians should support neutering and marking of cats in the shelter using tattoo or microchips. The use of microchips offers several advantages since cats do not need sedation for implantation, chips can not be removed easily, and the information on the chip is accessible for control using a scanner without even manipulating e.g. a feral cat.

The regulation of feline populations and the resulting problem of feral cats require systematic measures. Fighting the underlying reasons, neutering and marking of all cats are to be considered the most important factors. Trapping and neutering feral cats has implications to animal welfare and animal hygiene and is therefore dependant on public support and systematic handling by the responsible officials.

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Importance of sandpits for the epidemiology of Toxocara spp.

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Summary

Homekeeping of carnivores is increasing day by day in towns. Discussions about removal of animal excrements in streets and places developed in this time.

We investigated the occurence of helminthes ova in sandpits of 50 playgrounds in Rostock and 50 in five other towns near by Rostock (10 per town). We collected 8,000 sand samples at a 2-week interval over the year 1996.

The first results show nearly the same contamination of the sandpits of Rostock with *Toxocara spp*. (10 %) like in 1994 (7.4 %). The rates of contamination in the other towns are different (20 %, 10%, 40%, 30%, 10%). It has been found that the rate of detection of *Toxocara spp*. was the highest in late summer. The question, if there is a dependence on season, will be discussed. Eggs of hookworms, hairworms, human-mawworm were detected, too (approximately 18 %, 1 % and 2 % of all 100 sandpits). Furthermore, eggs of plant pathogen-, soil-nematodes and insects, which made the diagnostic more difficult, were also found.

Key words: parasite, nematode, protozoa, Toxocara spp., cat, dog, sandpits, playground

Introduction

The most common nematode observed in our studies (reported in another paper by DIBBERT et al.) was *Toxocara spp.* (22.1 % in cats, 17.1 % in dogs).This result is of immense public health significance. *Toxocara spp.* causes visceral larva migrans syndrome in humans. The dogs defecate on streets, in parks, public places and playgrounds. Cats prefer to defecate in loose sand or soil. People, especially children playing in such places, may become infected by ingesting the sand contaminated by faeces containing eggs of *Toxocara spp.*. Discussions about removal of animal excrements in streets and places developed in this time.

Material and methods

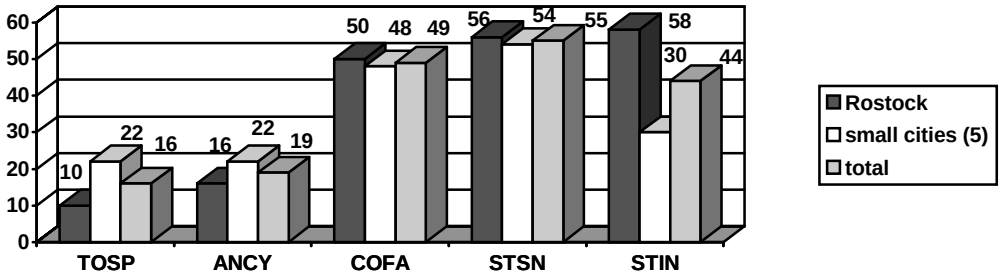
Fifty playgrounds were chosen in Rostock. In 5 other towns near by Rostock (Bad Doberan, Bützow, Güstrow, Ribnitz-Damgarten and Teterow) 10 sandpits were selected per town. The authors preferred sandpits with high frequency of children.

Per playground 5 sand samples (400 g - 500 g) were collected at a 2-week interval over the year 1996 (80 per playground, a total of 8,000). If faeces of dogs or cats were there, the authors took them, too (24 faeces samples of dogs, 96 faeces samples of cats).

Parasite-stages in the sand samples were detected by NaCl-flotation (density 1.195 g per ml). The samples were dried 1-3 days at room temperature. The sand (300 g, the rest were taken for other examinations) was filled into Erlenmeyer-glasses. NaCl-flotation-solution was poured in to the top. The foam was taken off after mixing and standing for a few minutes. After a new mixing and standing of the samples for half an hour the microscopically examination was done at a magnification of 100-600.

Results

The results of parasitological examinations in sandpits are shown in figures 1, 2 and 3.



legend:		COFA	sandpits contaminated by faeces
TOSP	Toxocara cati / canis	STSN	stages of soil nematodes
ANCY	Ancylostomatidae	STIN	stages of insects

Figure 1 Percentage of evidence of *Toxocara spp*., *Ancylostomatidae*, contamination by faeces and other soil organism in sandpits

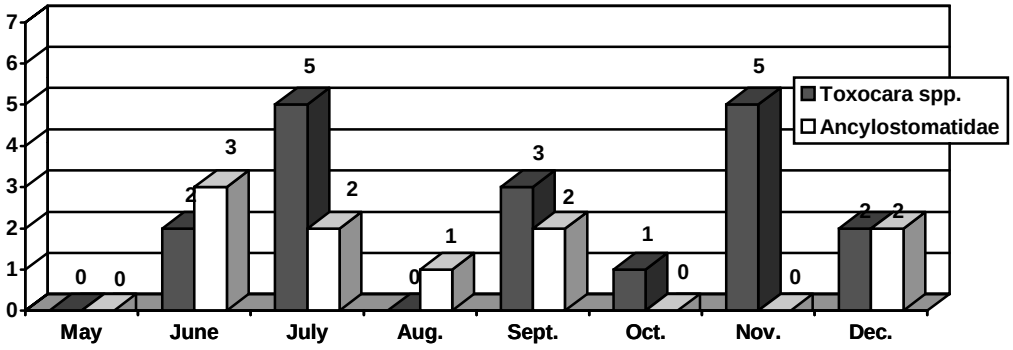


Figure 2: Number of faeces samples (total 120) containing parasite eggs

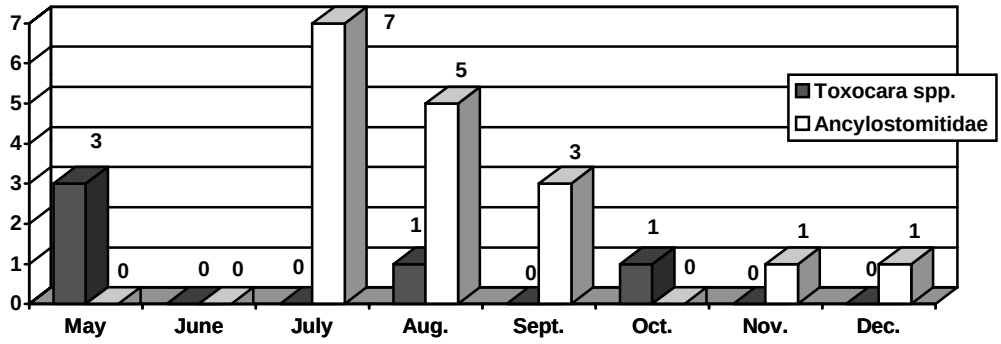


Figure 3: Number of sand samples (total 8000) containing parasite eggs

Discussion and Conclusions

Eggs of *Toxocara spp*. were found in 16 % of all tested sandpits. In Rostock the rate of registered *Toxocara* was 10 % (7,4 % in 1994). These results are comparable. In the small cities *Toxocara*-eggs were observed in 10 %-40 % of the sandpits. In other parts of Germany *Toxocara*-eggs were recorded in 22.2 % (KNAUS et al. 1987, Cottbus), in 10 %-100 % (HÖRCHNER et al. 1981; SENGEBUSCH 1989; KÖHLER et al 1980, Berlin), in 79 %- 100 % (DÜWEL 1983, München) and in 63,5 % (HORN et al. 1990, Hannover).

In 1996 the authors observed in 19 % of sandpits eggs of *Ancylostomatidae* (0 in 1994). Eggs and larva-stages of soil-nematodes were registered in 55 % (20 %-80 %), while stages of insects were found in 44 % (20 %-58 %) of sandpits.

The main contamination of playgrounds with *Toxocara spp*. is caused by cats (96 defecations of cats to 24 defecations of dogs, rate of *Toxocara*-infestation 22.1 % to 17.1 %). The observed eggs of *Toxocara* were in 1-egg-stage for the most part. The other eggs showed all development stages.

It seems, that seasonal dependence was found in our study. It can be connected with the birth of the whelp of cats in April-May and August-September. The probability of detection of parasitic stages in sand samples is between 40 % - 70 % (TEICHMANN 1992). The true contamination of playgrounds is surely higher than registered.

If dogs defecate in playgrounds this is visible, but you cannot see the hidden faeces of cats. Cats have free access to playgrounds and are highly infested. Transmission to humans is possible because the faeces of carnivores containing maw- and hookworm-eggs are present in playgrounds or lawns. The best prevention of contamination is to avoid the defecation. It can be reached by cover up the sandpits and make other attractive places for cats.

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Endoparasites in cats and dogs of animal homes

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Summary

The number of homekept cats and dogs are increasing incessantly. So the number of animals found and handed over to animal homes is rising, too.

From April 1996 to February 1997 we examined the occurence of endoparasites in faeces of 222 cats and 422 dogs of animal homes. In the investigation of 1994 we tested 15 cats and 55 dogs only. The native-phase-contrast-preparation was used to detect *Entamoebidae* and *Hexamitidae*. The flotation-procedure was used to show the stages of helminthes.

The results showed the evidence of *Toxocara spp*. (22.1 % in cats and 17.1 % in dogs) are higher than in 1994 (7 % in cats and 9 % in dogs). The registered hookworms (2 % and 7 %) were not so different to 1994 (7 % and 5 %). The rate of infestation with other parasites shows more or less variations.

Key words: parasite, nematode, protozoa, Toxocara spp., cat, dog, animal home

Introduction

At the beginning of the 1990's in Germany about 3.8 million dogs and about 4.2 million cats were being kept as pets (VET-REPORT 1993). In the Rostock district 6,247 dogs were registered in January 1997 (HARTMANN 1997). The number of cats being kept in flats is unknown. This number may be the same or more than that for dogs. The number of homekept cats and dogs is increasing incessantly. The population of stray cats has been estimated at 7,000. Straying dogs are rare. So the number of animals found and handed over to animal homes is rising, too. This was the reason why we decided to record the occurence of endoparasites in faeces of dogs and cats in the animal homes in Rostock and near by Rostock.

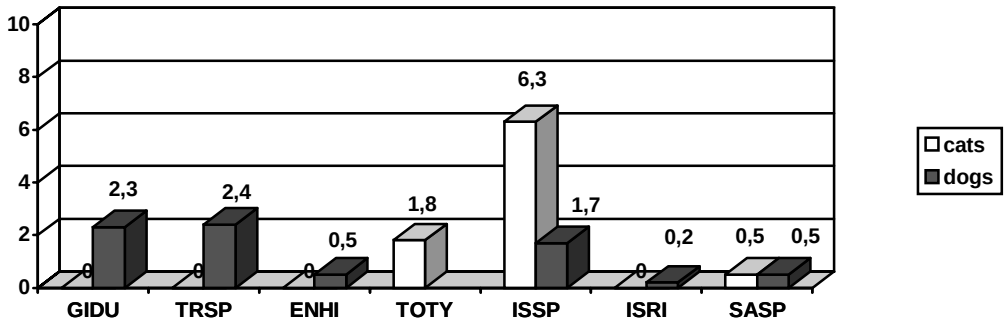
Material and methods

From April 1996 to February 1997 we examined 222 cats and 422 dogs of animal homes. In 1994 we tested 15 cats and 55 dogs of the animal home of Rostock only. The faeces of carnivores brought into the animal homes were collected by the staff. Animals were treated as a rule not with antihelmintic.

The native-phase-contrast-preparation was used to detect *Entamoebidae* and *Hexamitidae*. *Cryptosporidiidae* were made visible by carbolfuchsin. The modified NaCl-flotation procedure was used to show the stages of helminthes.

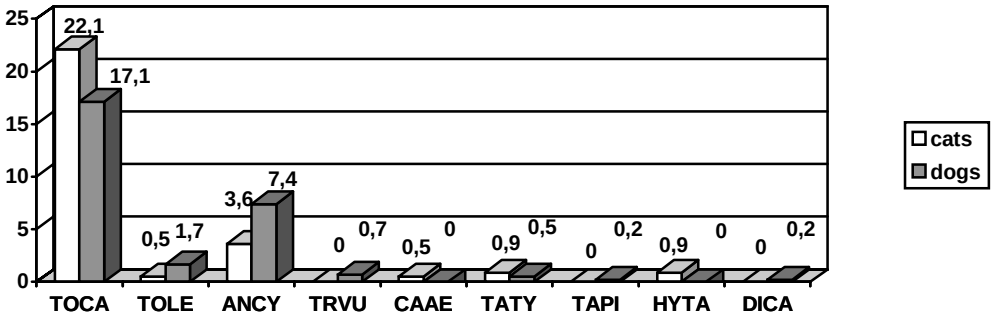
Results

We observed in 30.1 % of dogs (127 of 422) and in 32.4 % of cats (72 of 222) various kinds of helminth ova and / or protozoan oocysts in the faeces samples. Some animals were infested by more than one parasite. The results of parasitological examinations in cats and dogs are shown in Figures 1 and 2 . Table 1 shows the multiple infestations.



legend :		TOTY	oocystes of Toxoplasma-type
GIDU	Giardia-duodenalis-group	ISSP	Isospora felis / canis
TRSP	Trichomonas sp.	ISRI	Isospora rivolta
ENHI	Entamoeba histolytica	SASP	Sarcosporidia sp.

Figure 1 Detailed evidence of protozoa in cats and dogs in %



legend :		CAAE	Capillaria aerophila
TOCA	Toxocara cati / canis	TATY	eggs of Taenia-type
TOLE	Toxascaris leonina	TAPI	Taenia pisiformis
ANCY	Ancylostomitidae	HYTA	Hydatigera taeniaeformis
TRVU	Trichuris vulpis	DICA	Dipylidium caninum

Figure 2 Detailed evidence of helminths in cats and dogs in %

Table 1 Multiple infestations in percentage of cats and dogs

multiple infestations	cats	dogs
double protozoa	1.8 %	0
double nematoda	1.4 %	2.1 %
triple nematoda	0	0.2
protozoa + 2 nematoda	0.5 %	0

Discussion and conclusions

The most common nematode observed in carnivores of animal homes were *Toxocara cati* (22.1 %) and *Toxocara canis* (17.1 %). The rate of infestation was higher than in 1994 (7 % in 15 cats and 9 % in 55 dogs of animal home). These results are similar to the evidences of *Toxocara spp*. in the whole population including animals in private ownership (21.5 % of 739 cats and 16.5 % of 1555 dogs) in 1994. In Central Europe the evidence of *Toxocara spp*. in dogs ranged from 4.3 % in Austria (SCHENN 1986) to 20.4 % in Germany (SPROTTE et al. 1885). In Central Europe figures for cats infested with *Toxocara spp*. ranged from 5.1 % in Austria (SIXL 1975) to 67.1 % in stray cats in Germany (HANSEL 1980). The registered hookworms (3.6 % in cats and 7.4 % in dogs) were not so different to 1994 in the animal home population (6.7 % and 5.4 %). This result is comparable to results in cats ranged from 0.0 % (WALTER 1979, Germany) to 3.0 % (SUPPERER a. HINAIDY 1986, Austria) and in dogs from 3 % to 6 % (DEPLAZES et al. 1993, Austria). Eggs of *Toxascaris leonina* were a little increased in 1996 (0.5 % and 1.7 % to 0.3 % and 1.0 %). Cysts of *Giardia-duodenalis*-group were not seen in cats (0.3% in 1994), but detected in 2.3 % of dogs (0 % in 1994). *Giardia*-cystes have been obtained from 6.5 % to 10.3 % of dogs in Austria (SUPPERER et HINAIDY 1986, ECKERT 1992), from 1.3 % to 4.2 % in Germany (REITH et WEBER 1989). The rate of infestation with other parasites remained below 1 % like in 1994.

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Mycotoxin contamination of feeds in Hungary

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Summary

For the last 6 years 3116 cereal, feed ingredient and compound feed samples have been investigated for the presence and quantity of 8 mycotoxins (T-2 toxin /T-2/, HT-2 toxin /HT-2/, Zearalenone /ZEA/, Desoxynivalenol /DON/, Nivalenol /NIV/, Fusarenon-x /FX/, Diacetoxyscirpenol /DAS/ and Ochratoxin-A /OH/) that have practical importance in Central Europe. Each samples have been graded and grouped into one of the 3 groups (good, use it with care, excluded) on basis of the suggestions by the Hungarian Feed Code (1990) and by the authors previous experiences. Data analysis has allowed to draw some general conclusions.

Introduction

Relevant literature contains abundant data on mycotoxin contamination of cereals, feed ingredients and feeds of livestock and poultry. However, most of the papers have been restricted to report only the (natural) occurrence of different kinds of mycotoxins on variety of substances, sometimes the geographical and territorial occurrence of mycotoxins is analysed and in some of the cases the occurrence of a toxin is completed with its lower and upper concentration. Very few, if any, attempts have been made to analyse the natural mycotoxin contamination of feeds and feed ingredients from the viewpoint of animal husbandry.

The goal of the present paper is to report the natural occurrence of the most important fusariotoxins in feeds and feed ingredients in Hungary and evaluate the contaminated feeds from the point of their further use for feeding.

Materials and methods

HPLC and capillary gas chromatography has been used for regular analysis of feed ingredients and compound feeds for the presence and concentration of 7 fusariotoxins (T-2 toxin /T-2/, HT-2 toxin /HT-2/, Zearalenone /ZEA/, Desoxynivalenol /DON/, Nivalenol /NIV/, Fusarenon-x /FX/, Diacetoxyscirpenol /DAS/) and Ochratoxin-A /OH/ for feed mills and farms. The selection of these 8 mycotoxins has been based on earlier observations and study of field cases, which had indicated that due to climatic conditions home produced cereals are free from aflatoxins.

Between 1st of January, 1991 and 1st of April, 1997 altogether 3116 samples were tested for the presence and quantity of 8 mycotoxins that have importance in Hungary. The samples were sent in for routine examination by feed mills and primary livestock and poultry producers, therefore they may represent the average situation.

The Hungarian Feed Code (Budapest, 1990) summarises the maximum permissible levels of mycotoxins in feed ingredients and compound feeds for farm animals and birds. Because, as rule, the feeds are simultaneously contaminated with more than one mycotoxin each feed sample has to be evaluated individually. On basis of suggestions by the Hungarian Feed Code and by our previous experiments and experiences the feed ingredients and feed mixtures tested were assorted into one of 3 groups.

Group A: the samples are either free of mycotoxins or may be contaminated but both composition of mycotoxins and their summit concentration is considered harmless, therefore the feed ingredients or feeds equivalent to the samples analysed can be **unconditionally used** for processing or feeding.

Group B: In this case the sample does contain one or mycotoxins at different concentrations. However, feed ingredients may be used with certain care for compounding (e.g.

use in decreased proportion, dilution with non contaminated cereals, incorporated into feeds for animals that are not sensitive to the mycotoxin in question etc.). Feed mixtures of this group can also be used with sufficient care (e.g. dilution, re-formulation etc.). The member of group B can be **conditionally used.**

Group C: Due to extreme contamination the feed ingredients should be excluded from formulation of feeds. Compound feeds are unsuitable for feeding to animals or birds the feed had been prepared for. The member of this group therefore should be **excluded from further use.**

Results and discussion

Fig 1. shows terrific data. It is seen that only 32.4, 8.1 and 14.0 % of the maize, wheat and soybean samples tested proved to be free from either of the mycotoxins we had looked for. The main contaminant mycotoxin in maize was the T-2 toxin, while wheat and soybean samples were contaminated mainly by DON. ZEA contamination was also considerable.

Fig 1. Mycotoxin contamination of maize, wheat and soybean samples

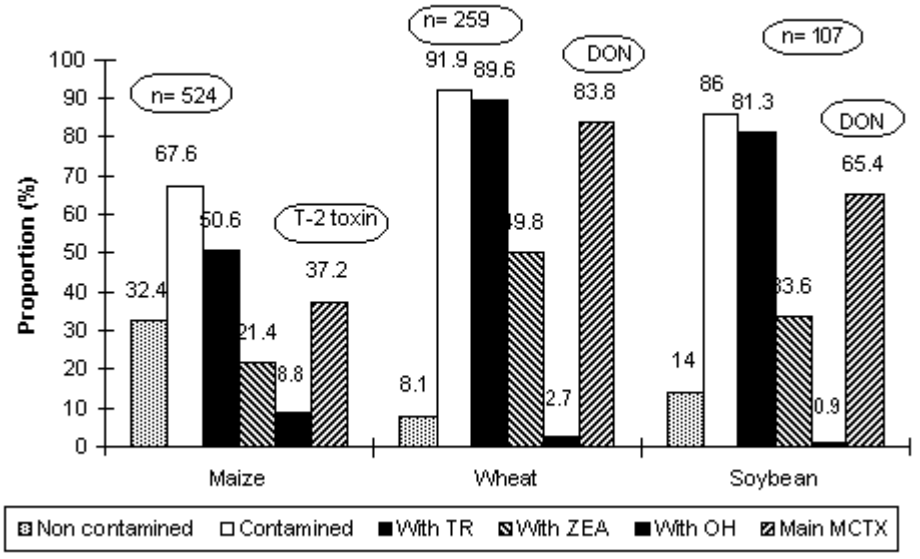

Fig. 2. Grading of feed ingredients

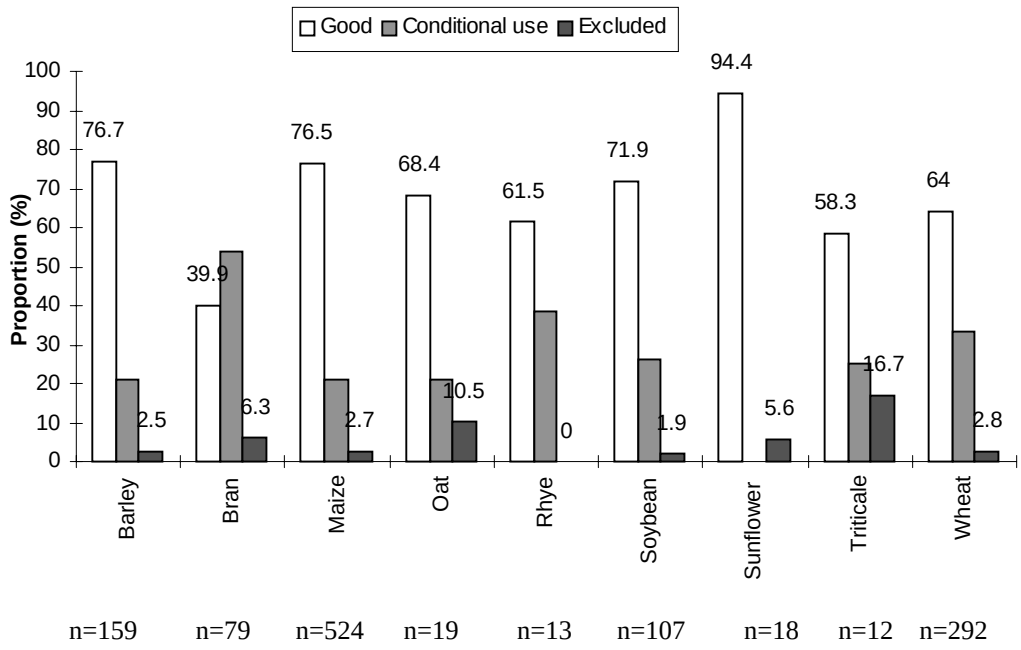

Fig. 2. indicates much better position. It is seen that only 2.7, 2.8 and 1.9 % of the maize, wheat and soybean samples, respectively had to be excluded from further processing. The considerable higher rate of exclusion of bran is of self-explanatory (fusariotoxins accumulate on and directly beneath the epidermis of grain seeds). More than 10 % of the oat samples were graded for exclusion. This is explained by the fact that oat fields are hardly, if ever, treated with fungicides during the period of vegetation. Triticale has been proven genetically more sensitive to fusarium infections than any other grains. It is also known that efficiency of treatments with fungicides in the period of vegetation depends on the genetic resistance of the plant. This is the explanation of the very high rate of C grading of the triticale samples.

Further analysis of our data has revealed that in the period of 1993 and 1996 the proportion of B + C graded maize and wheat samples has increased from 21.9 to 32.0 and 5.9 to 38.2 %, respectively. The considerable worsening of wheat grading might be connected to the gradual decrease of use of fungicides over this period.

Table 1. Grading of pig feeds

Feeds	No of samples	Good	Use it with care	Excluded
All	**332**	**98 (29,5 %)**	**21 (6,3 %)**	**213 (64,2 %)**
Piglet	116	49 (42,3 %)	12 (10,3 %)	55 (47,4 %)
Grower	36	7 (19,4 %)	3 (8,4 %)	26 (72,2 %)
Fattening	45	20 (44,4 %)	2 (4,4 %)	23 (51,2 %)
Sow/boar	135	22 (16,3 %)	4 (3,0 %)	109 (80,7 %)

Fig. 2. Grading of domestic fowls feeds

Feed	No of samples tested	Good	Use it with care	Excluded
Poultry (all)	451	214 (47,5 %)	5 (1,1 %)	232 (51,4 %)
broiler	21	20 (95,2 %)	0	1 (4,8 %)
layer	430	194 (45,1 %)	5 (1,2 %)	231 (53,7 %)
Duck (all)	61	16 (26,2 %)	0	45 (73,8 %)
starter	6	3 (50,) %	0	3 (50 %)
grower	7	2 (28,6 %)	0	5 (71,4 %)
finisher	8	0	0	8 (100,0 %)
layer	40	11 (27,5 %)	0	29 (72,5 %)
Goose (all)	322	94 (30,2 %)	6 (1,9 %)	222 (68,9 %)
starter	20	7 (35,0 %)	1 (5,0 %)	12 (60,0 %)
grower	41	15 (36,6 %)	0	26 (63,4 %)
finishing	17	11 (64,7 %)	0	6 (35,3 %)
layer	229	58 (25,3 %)	4 (1,8 %)	167 (72,9 %)
other	15	3 (20,0 %)	1 (6,7 %)	11 (73,3 %)
Turkey (all)	154	94 (61,0 %)	2 (1,3 %)	58 (37,7 %)
starter	92	63 (68,5 %)	27 (29,3 %)	2 (2,2 %)
grower	32	17 (53,1 %)	0	15 (46,9 %)
finisher	22	10 (45,5 %)	0	12 (54,5 %)
other	8	6 (75,0 %)	0	2 (25,0 %)

Mycotoxin grading of feed mixtures are summarised in Tables 1 and 2. The high rate of defected feeds (Grade C) is surprising. It follows that every effort should be taken to prevent the economic loss due to mycotoxin contamination of animal feeds.

Mycotoxins- Health and Economic Risk Factor, Prevalence in Lithuania

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Introduction

Mycotoxicosis- a livestock, poultry and fish disease Which is caused by feeds contaminated with toxic fungi. Mycotoxicosis is caused by mold and parasitic fungi secondary metabolite compounds- mycotoxins. Animal organisms are not only poisoned by these fungal mold toxins, micelle cells, but also via the substrate on which the fungal decomposing products develop, causing poor products appearance and value.

Mold fungi in feeds decrease the feed's wholesomeness, increases feed consumption per unit of weight gain, decreases productivity and has a negative affect towards the animals general health. Mold fungi cause feed's structural decomposition, increase economic losses, reduces palatability, creates poor products appearance, increases environmental contamination and are risk factors for other bacterial diseases.

Methods:

Vicam immunoaffinity chrimatography, HPLC and VICAM fluorimetry, are used to detect mycotoxins.

Blood bacteriocidic tests: a microbial culture suspension in physiological solution is prepared using a s. Aureus (strain# 2090 Concentration of 10 000/ml. After 1/2, 1, 2 and 4 hours of cultivation 0,1ml of solution is sown in petri dishes. After 24 hours, the cultures are evaluated by using the number of grown colonies and then a calculated bactericidal coefficient is obtained.

A nonspecific resistance evaluation of phagocytic granulocyte activity is obtained using a lysosome-cation protein amount in granulocytes. Prepared blood smears are dried and stained with "green strong" dyes for 15 minutes followed by Azure-eosin stain for 20-30 seconds. The slides are examined via microscopy. Primary granules, which cation proteins dye green and other compounds- pink. Nonspecific resistance units are counted according to the to the cation protein quantity in granulocytes.

Test results:

Tests performed in Lithuania on problem cases showed that 98% of feeds were contaminated with mold fungi and their toxins. Over 200 feeds and their components were tested for aflatoxins, ochratoxins and DON. From the test results we have learned that aflatoxins range from 0.00073-0.045mg/kg, ochratoxins 0.0012-0.1mg/kg and DON 0.085-18 mg/kg.

In Lithuanian -origin feeds, a larger amount of ochratoxins and DON were found and aflatoxins dominated in imported feeds. After performing these pilot tests, we concluded that mycotoxin activity is synergistic.

The effect of mycotoxins in livestock and poultry was determined by performing experiments and clinical tests. Test results showed that ochratoxins and aflatoxins suppress the organism's immune system.

In poultry, 2 testing groups of birds were used: One group's feed ration included aflatoxins- 2ppm and other included ochratoxins- 2ppm. We found a distinct decreasing lysosome-cation proteins. Results show that the effect of ochratoxins is stronger after longer application period than aflatoxins. It was established that mycotoxins also suppress bacterial activity in blood.

Economic loses due to mycotoxins were calculated by using statistical-economic calculations which were performed at problem farms and poultry houses.

Test results showed, that the greatest economic losses were due to a decrease in laying and increased mortality. Graph 1 (Below) reflects feeds with Aflatoxin- 1.38ppm and ochratoxin- 2,21ppm.

Table 1.Non specific resistance in chicken after various terms of application of mycotoxins.

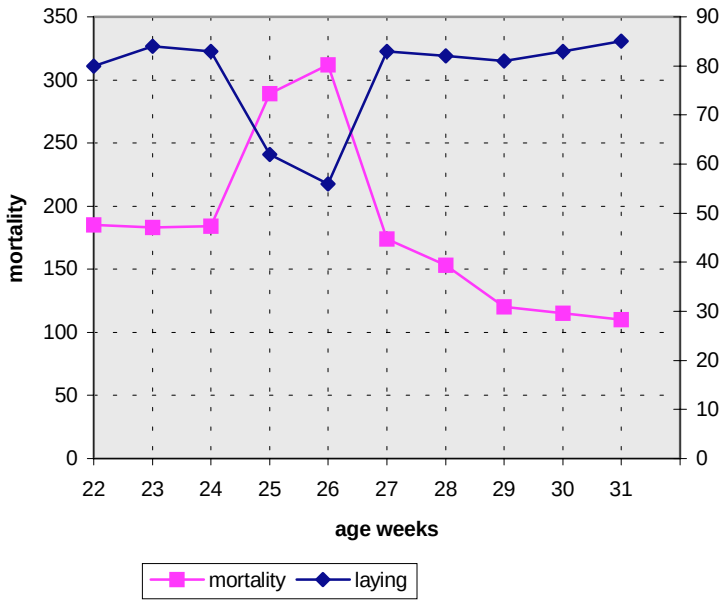
Time of application	control group	aflatoxin group	ochratoxin group
7 days	1.82	1.57	1.62
14 days	1.80	1.43	1.54
21 day	1.83	1.44	1.32
28 days	1.84	1.40	1.34

Table 2. Blood bactericidity index.

Time of application	control group	aflatoxin group	ochratoxin group
7 days	0.82	0.47	0.52
14 days	0.75	0.11	0.21
21 day	0.86	0.17	0.09
28 days	0.85	0.15	0.10

Performing economic calculations, we concluded that under Lithuanian conditions, feeding mycotoxin contaminated feeds creates an economic loose of $0.15/day/chicken.

Graph 1. Changes in mortality and laying of hens what was feeded with mycotoxin contaminated feed.



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The influence of mouldy fungi on the fodder of poultry on digestibility of nutrients and poultry weight

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Summary. On the basis of scientific data it has been proved that the effect of mycotoxins on health of animals and poultry, which are isolated by mouldy fungi, is adverse. Hovewer, in the literature the data on the use of mouldy fungi as protein admixture in feeds of poultry is available. The objective of the research is to clarify the effect of mouldy fungi and their mycotoxins on digestibility of nutrients of feeds given to poultry and on their weight increment. It has been determined that chickens which got feeds of good quality digest better nearly all nutrients. The weight increment of these chickens comprises from 18 to 90%.

Keywords. Mycotoxins, mouldy fungi, digestibility, the weight increment.

Introduction. Different data are available on the influence of mouldy fungi, on the quality of feeds and on animal health. In accordance with scientific data the effect of mycotoxins on health [3, 6] of animals and poultry, which are isolated by mouldy fungi, is negative. Currently, different opinions exist. It is possible to add from 1 to 3% of dry nontoxic Aspergillus niger fungi biomass in mixed feeds given to chickens aged from 1 to 50 days. Hovewer, admixture of 5% of this fungi biomass reduces the intensity of the growth and consumption of feeds for produsing [5] unit of production increases from 2.4 to 3.4%. The goal of our research is to clarify how various mouldy fungi affect digestibility of dry matter (DM), organic matter (OM), crude proteins (CP), crude lipids (CL), cude fibre (CF), ash and non-nitric extract substances (NES)of feeds as well as the weight increment of poultry.

Material and methods. In May - November five trials were established to test the effect of mycotoxins of mouldy fungi on chickens of the Heisex brown breeds. The chickens aged 24-58 days were divided into five groups. There were 5 chickens in a group. Each trial preparative and calculation periods lasting for five days have been determined. The feeds of chickens have been autoclaved at 1 atm. and contaminated by mouldy fungi. Also feeds moulded naturaly. In accordance with the methodology adopted in zootechnics feeds and isolated substances have been calculated and biochemical investigations conducted. In all trials feeds of high quality were given to poultry of control groups whilst contaminated feeds to chickens investigated. The characteristic of feeds for poultry is presented in **Table 1**.

Table 1 The scheme of trials

Trials	Group	The characteristic of ration
I	Control 1 investig. 2 investig.	70% of mixed feed for chickens aged 1-30 days + 30% of barley . 70% of mixed feed +30% of barley contaminated by Fus. spp. 70% of mixed feed +30% of barley contaminated by Aspergillus. ochraceus and Penicillum. verrucosum.
II	Control Investig.	Mixed feed for laying hens 70% of mix. feed for laying hens+ 30% contaminated by Fus. spp.
III	Control Investigat.	Mixed feed for chickens aged 1-30 days. 70% of mixed feed +30% of naturally moulded mixed feed.
IV	Control Investig.	Mixed feed for chickens aged 1-30 days. Naturally moulded mixed feed for chickens aged 1-30 days.
V	Control Investig.	Barley Barley contaminated by Penicillum verrucosum.

The results. While testing digestibility the chemical content of all feeds used has been determined. In accordance with the data of the analysis conducted by us the chemical content of qualitative feeds and that of feeds contaminated by mouldy fungi have not exhibited significant differences. However, the quantity of proteins in the latter feeds was less from 1.5 to 3.2% in all trials excluding the 5th one. In all trials the coefficients of digestibility of DM, OM, CP, CL, CF, ash and NES of feeds assigned from chickens investigated and control chickens have been calculated.

As seen from the Table 2, control chickens which were given unmoulded feeds consumed more of them. In the 1rst - 4th trials these chickens also better digested nearly all nutrients of feeds. In the 1rst trial control chickens digested OM better by from 1.65 to 2.5% and CP - from 4.82 to 5.86%, however, they digested ash worse by from 1.88 to 2.51% as compared to chickens investigated. In the 2nd trial control chickens better digested nearly all nutrients excluding CL. In comparison to chickens investigated, better digestion of DM by control chickens comprised 2.4%, that of OM and CP constituted 2.36 and 6.81%, respectively. Control chickens digested CL by 1.94% worse than chickens investigated. In the 3rd trial all nutrients were digested better by control chickens. Better digestibility of CP by control chickens constituted 4.70%. In the 4th trial control poultry digested only CF worse by 1.37% while OM and proteins better by 6.33 and 6.20%, respectively, as compared to chickens investigated. In the 5th trial control chickens were given barley of high quality. They consumed barley thrice more than poultry investigated, which

were fed by barley contaminated by Penicillum verrucosum. In comparison to the control, all nutrients excluding ash were digested better by poultry investigated: OM by 8.42% and CP by 6.5%.

Table 2. Feeds consumption (g) and the coefficients of nutrient digestibility (%)

Trials and group	Feeds consumed, g	DM %	OM %	CP %	CL %	CF %	Ash %	NEM %
I Contr.	63.16	70.58	74.10	82.64	92.10	20.50	79.00	75.33
1 Investig.	45.20	67.87	71.60	77.82	87.40	14.75	81.51	74.90
2 Investig.	54.80	69.54	72.45	76.78	89.14	17.70	80.88	75.20
II Contr.	71.00	70.70	73.30	63.10	72.03	15.43	34.40	80.42
Investig.	70.00	68.30	70.94	56.29	73.97	14.32	29.30	79.90
III Contr.	24.00	68.60	70.51	56.10	68.60	27.00	31.50	90.60
Investig.	24.00	65.50	67.65	51.40	67.58	25.80	29.31	78.90
IV Contr.	48.00	70.07	72.93	48.21	63.41	23.94	19.87	85.83
Investig.	42.00	63.04	66.60	42.01	59.57	25.31	19.51	84.37
V Contr.	36.00	66.73	66.60	35.70	52.25	21.90	67.60	77.80
Investig.	12.00	73.98	75.02	42.20	60.00	35.23	32.00	78.80

The influence of various mouldy fungi on the mass increment of chickens has been determined. In the 1rst trial the diurnal weight increment of chickens is 17g or 90.43% larger than that of poultry of the 1rst group investigated ($P>0.01$) and 10.2g or 54.26% larger than that of the chickens of the 2nd group investigated ($P>0.05$). In the 2nd trial the weight increment of control chickens is 0.4g larger as compared to that of chickens investigated. In the 3rd trial the weight increment of chickens is 2.4g or 18.46% larger than that of chickens of the group investigated..In the 4th trial the weight of control chickens increases 7.4g or 27% as compared to that of chickens investigated. In the 2nd - 4th trials the differences in the weight increment are statistically unreliable. In the 5th trial the weight increment of control chickens is 6g or 37.5% larger than that of chickens investigated ($P>0.01$, the data are statistically reliable).

Conclusions. In the 1rst - 4th trials chickens were given feeds of good quality. They digested better nearly all nutrients. In accordance with the investigations conducted in vitro by A.M.Abdelhamid and other researchers 10 nmol of aflatoxin, ochratoxin A, citrene, patulin, penicilin acid or sterigmatocystin reduced digestibility [1] of OM and DM of hay and straw. The weight increment of chickens investigated, which were given contaminated feeds, was less than

that of the control. After A.K. Lun and other researchers enterocytes of poultry craw can adsorb an transform deoxynivalenol, the process of detoxication of mycotoxins are observed in large guts of poultry [2, 4]. In the 5th trial the quantity of feeds consumed by chickens investigated was thrice less and digestion of nearly all nutrients by them was found to be better as compared to the control. It may be attributed to stress.In the 1rst, 2nd, 4th and 5th trials chickens consumed a more considerable quantity of feeds of high quality. In the 1rst - 4th trials chickens which got unmoulded feeds better digested almost all nutrients. In the 5th trial chickens investigated were given barley contaminated by Penicillum verrucosum. They consumed feeds thrice less and digested all nutrients excluding ash better than the control. The weight increment of the chickens which were given unmoulded feed was larger from 2.86 to 90.43%.

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Examination of contamination levels of ochratoxin A in feed and serum from regions with high percentage of nephropathy in pigs

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Summary

Toxicological investigations were performed in serum and feed samples coming from suspected for mycotoxic porcine nephropathy (MPN) regions with high frequency of changes as "enlarged mottled or pale kidneys" established during the meat inspection in Bulgaria. Ochrotoxin A (OA) was appeared to contaminate 100% of investigated 17 feed samples in levels 207.1 ± 65.1 ppb - mean for 1993 and 114,1 ± 35.8 ppb - mean for 1994. The percentage of positive serum samples and mean concentration of OA were significantly higher ($p < 0.05$) in spring (100% and 60.9 ng/ml ± 9.2) than the autumn (48% and 21,9 ng/ml ± 14.2 for 1994; 64% and 4.8 ng/ml ± 1.0 for 1993).

Key words: ochratoxin A (OA), mycotoxic porcine nephropathy (MPN), ochratoxicosis

Introduction

A nephropathy in pigs characterized by "mottled or pale enlarged kidneys" has been identified frequently during the routine meat inspection at slaughter time in Bulgaria. The frequency of this nephropathy has been found to vary from year to year, being especially high following wet harvesting periods. The nature of this nephropathy is not well known and it has not been nearly studied (Stoev and Stojkov 1993). The purpose of the present studies is to define the aetiological nature of the disease by toxicological investigations of serum and feed samples coming from suspected for MPN regions.

Materials and Methods

The studies were carried out during the period 1993 - 1994 and included feed, serum and urine samples collected from various farms with porcine nephropathy, which was identified during the meat inspection. For toxicological investigations 5 serum samples were collected from each more seriously affected farm during the autumn 1993 (a total 25 samples), spring 1994 (a total 25 samples) and autumn 1994 (a total 25 samples). In such way the serum concentration of OA was studied in dynamics. Also 5 urine samples were collected from some farms during the autumn

1994 (a total 15 samples). Various feed samples (a total 17) collected from the same farms and representatives for different long periods of time were also examined for OA. All samples were frozen at -20° C until toxicological examination, which was performed using HPLC technique.

Student's t-test was used to estimate significant differences between the mean values of various indexes.

Results and Discussion

As a rule the nephropathy was observed predominantly during the spring-summer period. The affected farms usually were had problems with correct storage of the feed. Sometimes the problems came from certain feed plants which have used the corn from the crops collected during moist and rainly days. All farms which had used feeds from the same plants had problems with poor growth in the pigs, but after changing the certain suspected feeds these problems disappeared. As a whole the frequency and duration of the observed nephropathy have depended on the duration of utilizing of various suspected feeds, stored in poor conditions and exceeded humidity.

Our surveys revealed that all investigated feed samples were positive for OA (Table 1). It was observed a significant ($p < 0.05$) fluctuation in the levels of OA in pig blood from certain affected farms during 1993 - 1994. The mean blood concentration of OA and percentage of contaminated samples were significantly higher in spring towards the autumn (Table 2). Also, high contamination levels of OA were established about urine samples (Table 3). The highest mean concentration of OA in blood during the spring-summer period indicates that the old crop contain higher contamination levels of OA than the new crop. A positive correlation was observed between the frequency of porcine nephropathy and the rate of OA in corresponding feed, serum and urine samples. The highest mean concentration of OA in blood during the spring- summer period indicates that the old crop contain higher contamination levels of OA than the new crop. A positive correlation was observed between the frequency of porcine nephropathy and the rate of OA in corresponding feed, serum and urine samples.

Table 1. Contamination levels of ochratoxin A (OA) in various feed samples collected during 1993 and 1994.

feed samples	percentage of positive	mean OTA levels (ng/g)	number of samples
mean 1993	**100%**	**207.1 ± 65.1***	**7**
mean 1994	**100%**	**114.1 ± 35.8**	**10**

* - SEM (standard error of the mean)

Table 2. Mean contamination levels of ochratoxin A (OA) and percentage of positives serum samples originated from different farms (a total 5) with porcine nephropathy during the autumn 1993, spring 1994 and autumn 1994.

origin of samples	autumn-1993 OA (ng/ml)	autumn-1993 % of posit.	spring-1994 OA (ng/ml)	spring-1994 % of posit.	autumn-1993 OA (ng/ml)	autumn-1993 % of posit	number of samples (total)
Dulovo	**3.4[2]±2.4***	**40%**	**75.8±19.6**	**100%**	**72.7 ±70.2**	**60%**	**5 (15)**
Sitovo	**6.2[3]±2.2**	**80%**	**48.3± 6.7**	**100%**	**1.8[3]± 1.8**	**20%**	**5 (15)**
Nojarevo	**6.4[3]±1.6**	**100%**	**53.4± 8.3**	**100%**	**5.7[3]± 4.1**	**60%**	**5 (15)**
N. Cherna	**1.4[1]±1.0**	**40%**	**84.2±41.2**	**100%**	**6.2 ± 3.9**	**40%**	**5 (15)**
Chernogor	**6.6[3]±2.9**	**60%**	**42.6± 3.6**	**100%**	**23.3 ±14.9**	**60%**	**5 (15)**
total	**4.8[3]±1.0**	**64%**	**60.9± 9.2**	**100%**	**21.9[1]±14.2**	**48%**	**25 (75)**

*** - SEM (standard error of the mean)**
[1] - significant difference ($p < 0.05$)
[2] - very significant difference ($p < 0.01$)
[3] - extremely significant difference towards the mean levels in spring ($p < 0.001$)

It is known that experimental pigs exposed to OA contaminated feeds in levels about 200 ppb (similar to those in our study) develope microscopic visible lesions in about a period of 3 months (Krogh 1974). On the other hand it is difficult to assess OA contamination of various feeds because of the pronounced variation in OA-levels in feedstuffs even in the same crop from the same area. The pronounced variation in results of monitoring feedstuffs for OA contamination may be due to the circumstance that the fungi invade only a minor fraction of feed particles with appropriate condition for a growth of fungi and formation of OA (Hald 1991). Thus, OA contamination of feedstuffs in two nearby situated places is markedly different. Also, it is necessary to find and mycological dimension of ochratoxinogenesis in observed nephropathy in Bulgaria.

Table 3. Mean levels of OA in various urine samples originated from 3 farms with porcine nephropathy during the autumn 1994

origin of samples	percentage of positive	mean-OA (ng/ml)	number of samples
Dulovo	**80%**	**7.2 ± 3.9***	**5**
Nojarevo	**60%**	**19.4 ± 11.7**	**5**
Cernogor	**80%**	**7.1 ± 2.6**	**5**
total	**73.3%**	**11.2 ± 4.2**	**15**

*** - SEM (standard error of the mean)**

In addition, the production of multiple toxins by a single strain such as A. ochraceus (producing OA and penicillic acid simultaneously), or by a mixture of fungi presents a problem

that has not been sufficiently investigated. Often, such mixtures of toxins have additional or sinergistic effects in farm animals greater than would be expected from the action of any single toxin. A such synergistic effect has been observed between OA and penicillic acid. Penicillic acid was shown to impaires mechanism of detoxification of OA in small-intestine, inhibiting carboxipeptidase activity (Parker 1982). In our case it is very likely that the feeds contain and other nephrotoxic compounds which enchance the toxicity or realize a synergistic interaction with OA, such a penicillic acid, citrinin, viomelein, xanthomegnin etc. Investigations in this direction are going to be carried out.

Conclusions

Based on the fact that the various investigated feeds fed to the pigs were contaminated with OA, a relationship between this nephrotoxic mycotoxin and the observed renal damages is suggested. The dissemination of the studied nephropathy is more pronounced during the spring-summer period, when the serum concentrations of OA are highest.

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New concept model for ecological risk assessment

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Summary

The concept model of Kendal et al. (1996) for ecological risk assessment was developed using free-living animals. Assessment was carried out on the basis of changes in behavior, reproduction and multiplicity of population.

On the basis of data from experiments performed to study the influence of heavy metals upon farm mammals and birds, a new concept model was developed for ecological risk assessment including quantitative criteria: immune response of the organism as measured by Phagotest and Bursttest, ecological bioenergetics assessed by the energy expenditure necessary for production of a unit biomass and a unit of energy in the secondary biological production as well as the reproduction of the population judged by the number of newborn.

The new concept model was aimed at improving the bioecological monitoring by using farming animals and quantitative criteria.

Key words: Ecological risk, immunity, reproduction, ecological bioenergetics, lead, cadmium

Introduction

Ecological risk assessment is a topic of numerous studies containing recommendations related to environmental management /McCarthy and Power, 1997; Renner, 1996, Suter, 1993 etc./. The conceptual model of Kendal et al. /1996/ assessed ecological risk for pollution with lead by reproduction, viability and morbidity of free living birds. According to the Recommendations of the World Health Organization farming animals should also be included in the number of pollution indicators. According to data of the Ministry of Environment, in Bulgaria large territories have been polluted with lead and cadmium. Besides air, water and soil control the use of bioecological monitoring was recommended in the study of the dynamics of environmental pollution. The aim of the present work was to create a conceptual model for ecological risk assessment by using farm animals and to introduce quantitative criteria for assessment.

Material and methods

The studies were carried out in an artificial ecosystem of the "mesocosm" type with farm populations of sheep + ram and broilers. Three groups of animals equal in age and biomass were bred under uniform conditions: control - fed on standard fodder rations, 1test group - standard fodder ration with lead and cadmium content 50 fold the Highest Permissible Concentration (HPC) and 2 test group fed on standard fodder ration + lead and cadmium content 100 fold the HPC.

The quantities of lead and cadmium in the fodder, drinking water, blood and secondary biological production were determined following the standard methods of Jorchem /1993/ by AAS "Varian-40 Zeeman" on the 1st, 45th and 90th day from the beginning of the experiment in sheep and on the 1st, 15th, 30th and 50th day in birds. Immunity was assessed by phagocytosis activity and intracellular killing of polymorphonucleares /PMN/ and monocytes /Mo/ of sheep using "Phagotest" and "Burstest" produced by "Orpegen", Pharma Co. The ecological bioenergetics was assessed by criteria introduced by us /Baykov et al., 1995/. Fertility index and gestation index according to Fitzhugh /1978/ and Semkov et al. /1989/ were used for reproduction assessment.

Results

In the development of the new conceptual model for ecological risk assessment the following requirements were taken into account:

1. Methods for quantitative assessment indicative of the status of the studied organism should be used

2. The constellation of methods should reflect the functional status of the organism as a function from the pollution of environment with lead and cadmium

3. The methods should be applicable in the conditions existing in the laboratories of the National Veterinary Service - local veterinary institutions and stations

The performed studies indicated that the quantity of lead and cadmium in the blood of the test animals and in the secondary biological production could not be used as criteria for lead and cadmium pollution. Till the present moment the studies performed with broilers indicated that no correlation existed between lead and cadmium concentrations in rations and the level of bioaccumulation in the secondary biological production used for human consumption /Baykov et al., 1996/. The study on the concentration of lead and cadmium in the blood of the test sheep and rams showed phase correlations in lead and cadmium content as well as lack of correlation

between the quantity of both elements in the rations and their concentrations in blood and milk **/Table 1**/.

Table 1. Average values of lead and cadmium in the blood and milk of sheep /mg/kg/

Groups	**n**	**blood**		**milk**	
		Cadmium	**Lead**	**Cadmium**	**Lead**
			beginning		
Control	12	0.016	0.072	0.007	0.022
I group	12	0.018	0.083	0.009	0.036
II group	12	0.020	0.096	0.009	0.030
			45 days		
Control	12	0.010	0.015	0.009	0.091
I group	12	0.046	0.246	0.010	0.180
II group	12	0.025	0.163	0.020	0.187
			90 days		
Control	12	0.016	0.039	0.009	0.069
I group	12	0.031	0.074	0.010	0.080
II group	12	0.020	0.079	0.010	0.093

The data in Table 1 and the published till the present moment data concerning the bioaccumulation of lead and cadmium in the organism indicate that the quantity of both toxic elements in the blood and the secondary biological production could not be used as an element in the conceptual model for ecological risk assessment.

The phagocytic activity of PMN of sheep fed on rations with increased lead and cadmium content /50 fold HPC/ did not change significantly statistically while in animals fed on rations containing both metals in 100 HPC dose, suppression of phagocytic activity was observed. Between the separate groups of animals significant were the differences in the phagocytic activity of monocytes and the suppression of phagocytic activity correlated with the contents of both toxic elements in the ration. The study of intracellular killing of microorganisms by macrophagues and PMN by “Burstet” indicated the lack of statistically significant differences concerning the degree of degradation of microorganisms depending on the quantity of lead and cadmium in the ration.

The performed studies on fertility did not give grounds to recommend this criterion for ecological risk assessment.

Till the present moment the performed studies on the ecological bioenergetics of birds were indicative of a reliable influence of the studied heavy metals on ecological bioenergetics /Baykov et al., 1997/.

Conclusion

The performed studies under model conditions indicated that the following quantitative criteria for ecological risk assessment of pollution with lead and cadmium could be applied:

change in the immune status assessed by the phagocyte activity of monocytes and the changes in ecological bioenergetics: quantity of energy for obtaining one unit biomass and quantity of energy for obtaining 1KJ energy in the secondary biological production.

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ATTEMPTS AT USING ULTRASOUNDS AND BIRD VOICES (GUINEA-FOWL) TO DRIVE AWAY RODENTS - MICE AND RATS

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Introducing newer and newer deratting preparations is not always effective because of the high "group intelligence" of rats directed at species preservation.

What is more, the remnants of chemical preparations and dead rodents constitute a certain danger for the environment. Sometimes the use of chemical poisons could be dangerous because of the presence of children and domestic animals or because of the danger of contaminating the food kept in the store-rooms.

To drive away rodents the ultrasonic generators of the frequency from 22 to 35 kHz and the pressure from 80 to 108 db were used. In view of the danger they present for people and animals they were used during the night in the absence of people. On the other hand, the simulators of the guinea-fowl voices were used both night and day. In both cases the effective driving the rodents away from the investigated sites was obtained. The use of simulators of the animal voices is safer for the human and animal health than the use of ultrasounds.

Key words: rodents - mice and rats, ultrasounds

Introduction

The use of newer and newer derating preparations in the control of rodents is not always effective because of a high degree of the "group intelligence" of rats directed at species preservation. Sometimes it also happens that the use of chemical poisonbaits may be dangerous because of the presence of children, domestic animals or the possibility of food contamination in the storehouses or big shops. What is more, the remnents of the derating preparations and dead rodents constitute certain environmental hazard (Przyborowski 1958, Kolbuszewski, Rokicki, Fabirkiewicz 1996). Numerous ovservations carried out by animal breeders confirm that the presence of guinea-fowl on a farm prevents the appearance of rodents on that farm.

The aim of the present investigation was a trial at cpmparing the possibility of driving rodents away from the farm buildings using ultrasounds or the simulators of the guinea-fowl voices.

Material and methods

The investigation was carried out on a pig farm and in a food store-house. Rats were become accustomed to consume the weighed multi-component baits (fish, grain, flakes) every day laid out in the deratting nests. When the consumption of baits stabilized at a constant daily level the generators of the GU-03 B type emitting ultrasounds of 22 to 35 kH frequency and the acoustic pressure of 80 to 108 db were turned on. The range of action of one generator is up to 80 m^2. In view of a harmful effect of that frequency range ultrasounds both on man and animals (Akamatsu, Sekiba 1977) the generators were turned on in the manure channels and in the attic only at night. The guinea-fowl voice simulators (Fabirkiewicz et al. 1966) switched on automatically for 15 sec. every 25 sec. during the night time. The drop in the bait consumption in the investigated buildings was the proof of driving the rodents away.

Results and discussion

Fig.1 shows the increased consumption of baits laid out in the deratting nests in a pig house which reached its peak on the 3rd and 4th day. On the 5th day of the experiment the ultrasound generations of the 35kH emission and 108 db acoustic intensity were switched on. As a result the consumption of baits drastically decreased reaching the zero level in 3 days. **Fig.2** demonstrates the increased consumption of baits in the food store-house. The increase was slower than in the case of the pig house, reaching its peak between the 6th and 8th day. Since the 8th night the simulators of the guinea-fowl voices had been switched and two days later the consumption of baits was stopped which was tantamount to the driving the rodents away. The results of investigations carried out in the pig house and food store-house presented in this paper confirm similar investigations performed by the authors in the cowsheds, stables and supermarkets.

There is an opinion, which appears in professional literature (Kowalski 1996) that rodents driven away with the help of physical phenomena come back after some time and that they may even become used to the ultrasounds. However, Akamatsu and Sekiba (1997) demonstrated harmful effects of ultrasounds on rodents which led to their death in case of a long-lasting

exposure. Thus the come back of the same rodents to the place from which they had been driven away is not very probable.

Conclusions

1. The use of simulators of the guinea-fowl voices is equally effective as the use of the ultrasound emitting generators.
2. The use of the guinea-fowl voice simulators is environmental friendly as compared with the use of generators emitting ultrasounds which are harmful for both man and animals.

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Fig. 1 The effect of ultrasound emission on the bait intake during succesive days of observations

Fig. 2 The effect of emission of the guinea-fowl voices on the driving the rodents away from the food store-house

Fig. 1 The effect of ultrasound emission on the bait intake during succesive days of observations

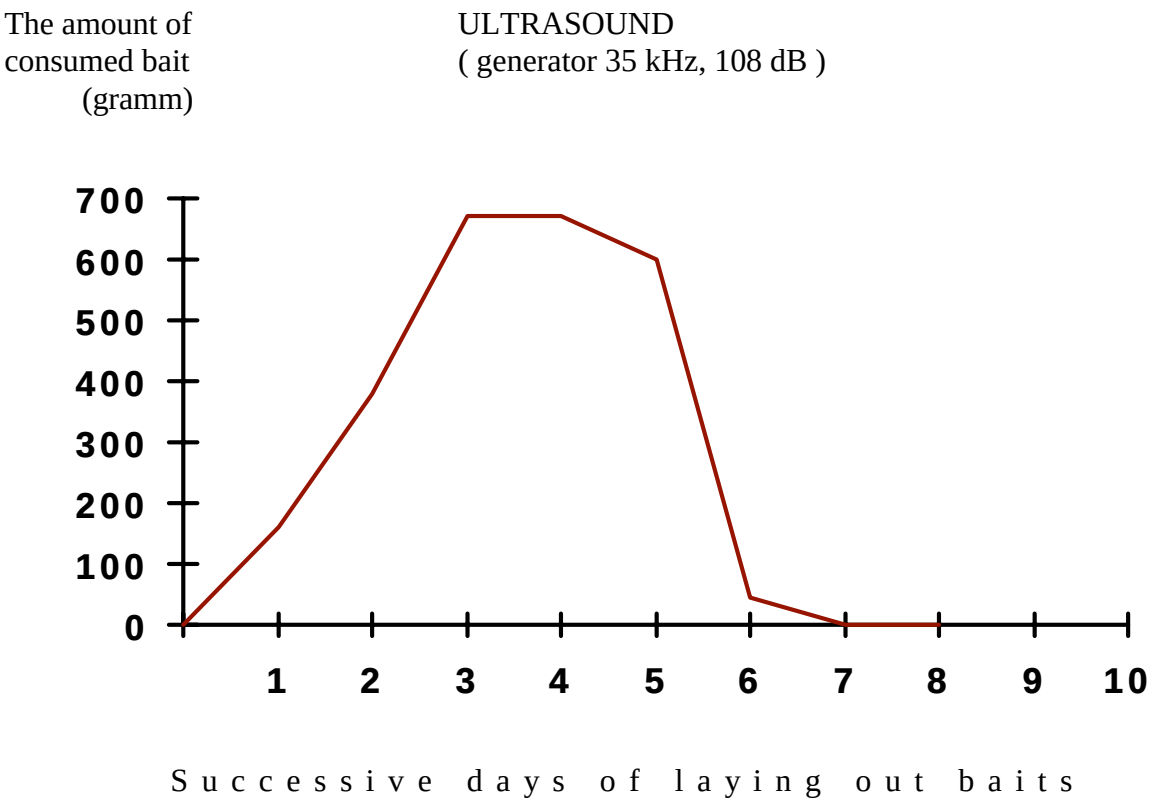
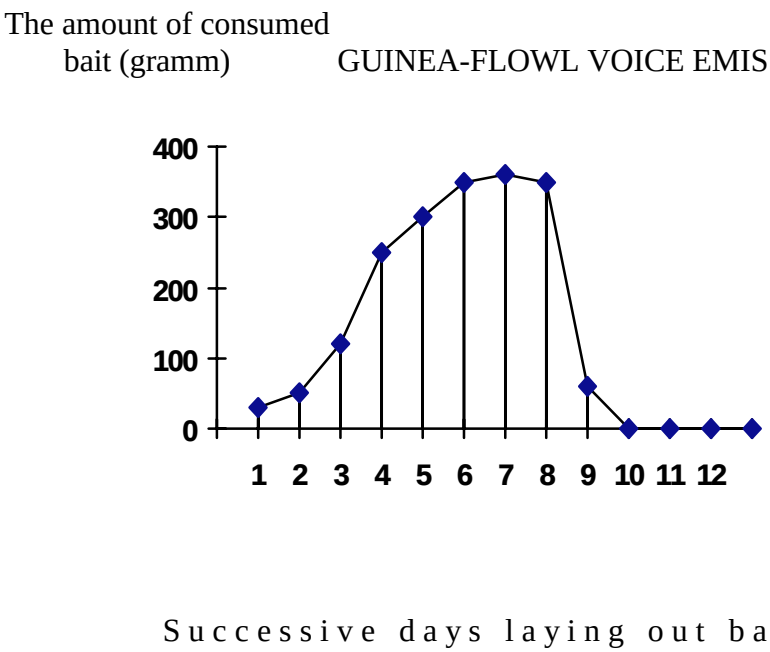


Fig.2. The effect of emission of the guinea-fowl voices on the driving the rodents away from the food store-house



MOLECULAR TYPING OF *E.-COLI* FOR DETECTING SOURCES OF FECAL POLLUTION OF GROUNDWATER

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Summary

Molecular fingerprinting methods based on RAPD and rep-PCR were used together with plasmid profiling to determine the source and relatedness of *E. coli* clones isolated from ground water wells. Possible sources of the pollution were investigated. Among the very high heterogeneity in the fingerprint patterns, 67 phenons could be generated from the 87 isolates investigated, only 1 *E. coli* clone with identical features in all methods used, isolated from the river water as well as from the ground water wells, was found.

Introduction

Molecular epidemiological tools like Random Amplification of Polymorphic DNA (RAPD), PCR of repetitive DNA sequences (rep-PCR), and plasmid profiling have been used for several years to characterise pathogenic bacteria. In the investigations described here we used these three methods to determine the source of a contamination of ground water wells by *E. coli*. In a first step coliform strains were collected from sites suspected to be the source of contamination like river water, the outflow of springs and fountains as well as samples from farm land and feces from wild animals. The isolates were proved to be *E. coli* strains by a multiplex PCR with primers of the lacZ and uidA gene, known to discriminate between *E. coli* and coliform Genera (Bey et al., 1991). In a second step fingerprints of the genomic DNA were generated with the M13 universal sequencing oligonucleotide as a random primer. The fingerprints were compared between the strains of each source as well as between each source and the strains of the ground water wells by a computer assisted fingerprint analyser. In a third step only strains showing an equal pattern in the so called M13-PCR were analysed further by a rep-PCR, using primers specific for REP-sequences and ERIC-sequences, respectively (Versalovic et al., 1994). In the last step strains with identical fingerprints in this rep-PCR were compared further by plasmid profiling. After combining the results of all investitgations we were able to discriminate an identical couple of strains with one strain originating from the river water and the other from the ground water wells.

Materials and methods

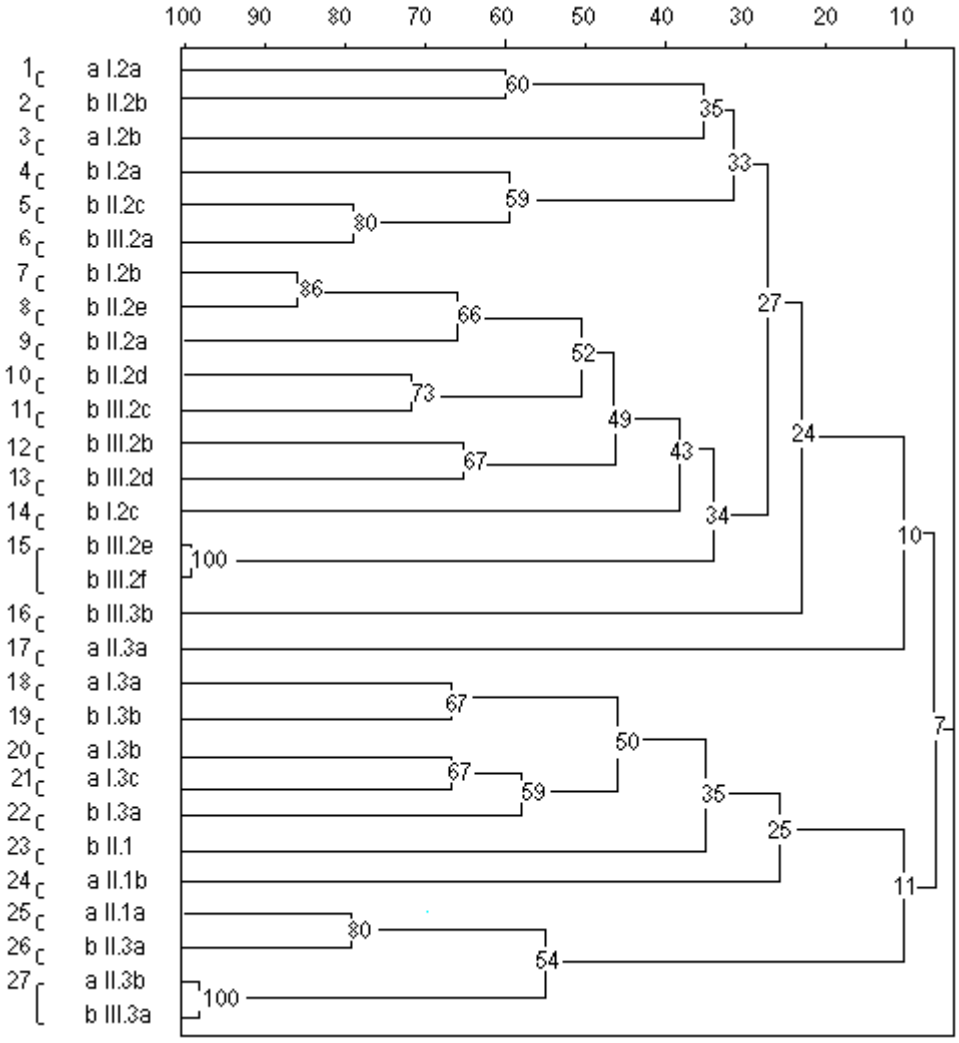
Bacterial strains were isolated by standard bacteriological methods using filtration techniques for the water samples as well as selective enrichment techniques for the other materials or in the case were the filters have been negative. Crude DNA preparations were made from pure bacterial cultures. Briefly, a loopful of the colonies from an agar surface was lysed in sterile water at 99 °C for 5 min and the DNA isolated by extraction with chloroform. The multiplex PCR was done with lacZ primers and with uidA primers (Bey et al., 1991). Thirty cycles were run with a 100 µl standard premix containing 1,5 mM $MgCl_2$, at 55 °C as annealing temperature. The RAPD was done with M13 primer: 5'-tatgtaaaacgacggccagt-3', annealing at 55 °C. The volume of the PCR mix was lowered to 50 µl and the „Hot start" method containing a waxbead (Ampliwax^{Tm25}, Perkin Elmer) was applied. The rep-PCR was done with primers BOXA1R (annealing at 55 °C), ERIC1R/ERIC2 (annealing at 55 °C), and REP1R-I/REP2-I (annealing at 40 °C) according to the published sequences (Versalovic et al., 1994). The PCR was carried out as described for the M13 primer. The amplicons generated by M13-PCR and rep-PCR were separated by gelelectrophoresis in a 1 % agarose gel containing ethidium bromide. The band patterns were compared with the software module „Fingerprint analyser", vers. 2.0 (Cybertech Berlin) using the Dice formula for weighted peak comparison and clustered by the UPGMA method. Plasmid DNA was prepared by the method of Kado and Liu (1981) with slight modifications. The

plasmid profils were compared with plasmid DNA of known molecular weight in a 0.6 % agarose gel.

Results

In Fig. 1 a dendrogramm generated by M13-PCR is shown comparing all strains from the river water (9 strains with an „a“ as the first character) with strains originating from the ground water wells (20 strains with a „b“ as the first character). The dendrogramm demonstrates a very high diversity within the strains of one source. The same was true for all other sources tested. There has been only one couple of strains, the one originating from river water and the other from the ground water wells, having a similarity value of 100 %. No other fingerprint, originating from a strain of any other source could be found being 100 % identical with a fingerprint of a strain from the ground water wells. The DNA from the strains aII.3b and bIII.3a was further analysed by rep-PCR with REP- and ERIC-primers. As shown in Fig. 2. identical fingerprints could be generated by these primers, too. The comparison of the plasmid profils yielded also an identical pattern between the two strains. In a second case, where a strain originating from a spring showed a high similarity to the M13 fingerprint of a strain from the ground water wells their diversity could be clearly demonstrated by rep-PCR and plasmid profiling (results not shown).

Fig. 1. Clustering of all the strains originating from the river water and from the ground water wells. See text for explanations.



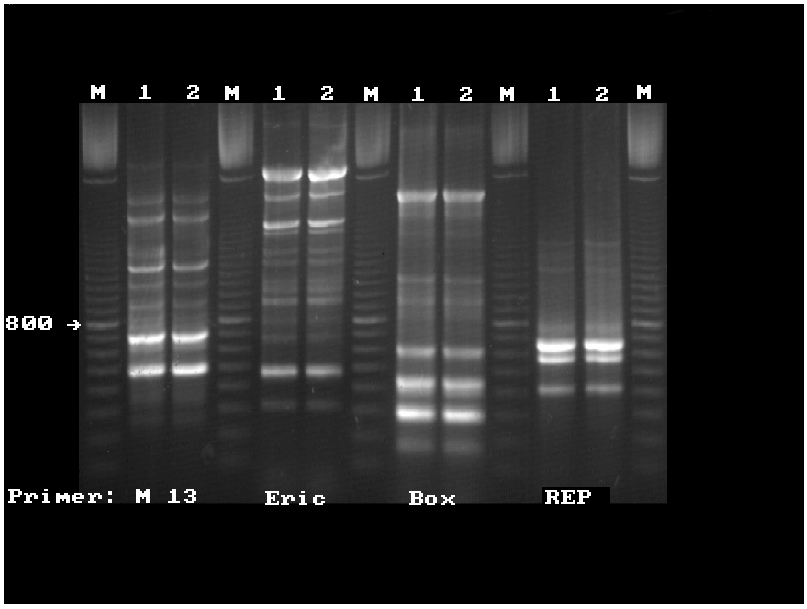


Fig. 2.
Comparison of fingerprints of strains aII.3b and bIII.3a
M: 100 bp standard
For the explanation of primers see text.

Discussion

The RAPD fingerprinting method with M13 sequencing primer proved to be a valuable tool to discriminate between *E. coli* strains on the basis of their diversity at the DNA sequence level. The huge diversity between strains of the same source was surprising but indeed there was no more homogeneity among the strains of the same source than between the strains of different sources. Among the 87 strains of *E. coli,* 67 different phenons could be demonstrated by M13-PCR. In only one case a 100 % identity could be found between a strain from the groundwater wells and another strain from the river water. The identity of these strains was proved also by rep-PCR and by plasmid profiling. In a second case the high similarity in the M13 fingerprint of two strains was disproved by the two other methodes. This example emphasizes the necessity to confirm the results of one fingerprinting method by another method which should be based on different molecular structures. The combination of a RAPD method with two rep-PCR fingerprints highly improved the discriminatory power. In general, the generation of fingerprints with random primers which cannot be ruled out to prime not only at the chromosomal DNA but also at plasmid sequences, needs the determination of the plasmid profile as a prerequisite for the epidemiological interpretation of RAPD results. On the other hand the plasmid profile alone is a wellknown, valuable tool for the differentiation of bacterial isolates, as long as plasmids are present at all. In the present study the results of the fingerprints, according to either the identity or the difference of highly similar strains, could be supported by the plasmid profiles.
The evidence of a couple of strains with identical fingerprints in M13-PCR, rep-PCR with two different primer target sequences, and an identical plasmid profile strongly suggests the contamination of the ground water wells by the river water. On the other hand a possible route of contamination by any other source is not disclosed by these methods.

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Disinfectant testing for veterinary purpose in Europe, state of discussion and preliminary standards

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Summary

The testing of disinfectants for veterinary purposes in until now mainly defined by CEN TC 216 pr EN 1040, pr EN 1275 pr EN 1656 and pr EN 1657. The two first mentioned preliminary standards are describing quantitative suspension tests for evaluating basic bactericidal and fungicidal activity of disinfectants as well antiseptics. The latter are describing quantitative laboratory suspension tests under more difficult conditions as low temperature (10 °C), organic soiling (0,3 % bovine serum albumine (BSA) or 1 % skimmed milk or 1 % BSA + 1 % yeast extract). Test microorganisms in the basic tests are Pseudomonas aeruginosa and Staphylococcus aureus or Candida albicans and Aspergillus niger. In pr EN 1656 Proteus vulgaris and Enterococcus hirae have to be used as additional strains. In pr EN 1656 + 1657 standardized hard water must be used for the dilution of the disinfectants instead of distilled water as in the basic tests. In bactericidal testing 5 log steps in reduction of the bacterial count must be achieved, fungicidal testing requires only a 4 log-step reduction. A good concept for a surface test is still lacking and the discussion about a method for virucidal-testing started last year. The CEN-methods have to be regarded as a compromise, they will need some improvement and completition in future.

Key words: disinfectant-testing, suspension-test, CEN-TC-216

Introduction

All disinfectants sold or distributed in the EU should fullfill the same requirements in future which will be defined by a CEN-standard. The European Standardization Organization CEN therefore established a technical committee with the number 216 (CEN-TC 216) to do this work. The organisational structure is, that four working groups have to elaborate those standards. The horizontal working group (HWG) is responsible for the coordination of the activities and for general questions. The working group 1 (WG 1) should cover the requirements of human medicine in testing disinfectants and antiseptics. WG 2 is responsible for the veterinary field and WG 3 is concerned with disinfectant testing for food and institutional areas. In addition HWG has formed a number of so called „ad hoc“-groups dealing with special questions of designing a

surface-test, a sporocidal-test, and a virucidal-test as well as one dealing with terminology. The methods presented by TC-216 first have the status of a preliminary standard (pr EN) and are valid for five years. The following methods which are or will be soon pr EN and which are of importance for the veterinary field are:

- Basic bactericidal test pr EN 1040
- Basic fungicidal test pr EN 1275
- Quantitative bactericidal suspension-test for veterinary purposes pr EN 1656
- Quantitative fungicidal suspension-test for veterinary purposes pr EN 1657

Principles of testing disinfectants and antiseptics in Europe

The whole procedere for testing disinfectants and antiseptics is divided into three phases

- Phase 1: Basic tests e.g. „fungicidal“ or „bactericidal-test“
- Phase 2: Laboratory tests taking the special field of application into account
  * Step 1: Quantitative suspension-test under conditions interfering with the action of the disinfectants
  * Step 2: Surface - or carrier-test
- Phase 3: Field-tests

The basic-test as well as tests in phase 2, step 1 are carried out as quantitative suspension-tests with a total volume in the test of 10 ml. The tests are running in principle as follows:

1 ml of a standardized suspension of microorganisms plus 1 ml of sterile distilled water (basic-tests) or a preconcentrated protein solution are mixed together and filled up with 8 ml of a preconcentrated disinfectant solution to 10 ml. After the desired or obligatory exposure time. 1 ml is taken out of this mixture and transferred into 8 ml of neutralization liquid filled up with 1 ml of distilled water to 10 ml. After 5 min a representative volume (1 ml) from this suspension will be taken for the determination of the amount of serviving microorganisms. The germ-count is determined either by the pour-plate technique (obligatory in basic-tests), by the surface-method or by membrane-filtration.

The method of the basic-test

In testing the bactericidal activity the following test strains have to be used:

- Pseudomonas aeruginosa ATCC 15442
- Staphylococcus aureus ATCC 6538

The bacterial test suspension harvested from the surface of Tryptone Soy Agar (TSA) must have a final bacterial count of 1,5 x 10^8 to 5 x 10^8 cfu/ml.

For fungicidal testing the following test strains must be used:

- Candida albicans ATCC 10231
- Aspergillus niger ATCC 16404

The test suspensions prepared from cultures grown on Malt Extract Agar (MEA) must be finally adjusted in the range of 1,5 x 10^7 to 5 x 10^7 cfu/ml.

In the basic-test 8 ml of disinfectant solution (20 °C) are mixed with 1 ml of sterile distilled water (20 °C) and 1 ml of the microbial test suspension (20 °C) in a tube placed in a water-bath and kept at 20 °C $\pm$ 1 °C during the exposure-time. In the basic test the disinfectant is diluted with sterile distilled water. After 5, 15, 30 or 60 min (the exposure-time may be chosen from those given figures) an aliquot of 1 ml will be taken and neutralized (see above). The number of surviving microorganisms must be determined by the pour-plate technique in the basic test or if neutralization by the given method is not possible, by membrane-filtration of 0,1 ml out of the reaction-mixture. Those 0,1 ml have to be transferred into 50 ml of neutralizing rinsing liquid, which are sucked through a membrane filter (47 mm to 50 mm diameter, pore-size 0,45 µm). The filter is rinsed with at least 150 ml and a maximum of 500 ml rinsing liquid and transferred to the surface of a suitable nutrient agar. In bactericidal-testing TSA and in fungicidal testing MEA has to be used. The test is passed if a 5 log reduction in bacterial count or a 4 log reduction in fungal count had been achieved at the chosen exposure time.

Suspension test for veterinary purposes

In addition to the bacterial test strains of the basic test two more strains have to be used:

- Enterococcus hirae ATCC 10541
- Proteus vulgaris ATCC 13315.

The fungal strains are the same as in the basic test. The test in phase 2 step 1 differs from the basic test mainly in the following points

- The test is generally performed at + 10 °C (4 °C, 20 °C and 40 °C may be additional tested) or at 30 °C for teat-disinfectants
- The test is generally done under organic soiling
- The disinfectant is generally diluted with water of standardized hardness (WSH)
- The obligatory exposure time is 30 min (1 min, 5 min and 60 min may be used in addition)

- The germ-count may be determined by the pour-plate method or by the spread plate technique (1 ml at least to the surface of 3 plates)

According to the intended field of use, a low organic soiling (0,3 % bovine serum albumine, BSA) or a high organic soiling (1 % yeast extract + 1 % BSA) may be used. The low soiling is regarded as representative for veterinary clinics etc., the high organic soiling for animal houses. For testing teat-disinfectants 1 % of reconstituted skimmed milk is used. The requirements concerning the log-reduction are as in the basic test

Final remarks

The methods agreed on until now are not fulfilling all requirements which could be attended due to the state of science. For example no information about the minimal inhibitory concentration of disinfectants are available. A surface test is still lacking, without surface test using a representative surface the whole testing procedure is invalid for giving recommendations for practical use. The virucidal test is still not lacking and must be developed on soon.

A profile of serum albumin, albumin globulin ratio and total leukocytic count in prepubertal buffalo calves.

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Summary

One hundred and fifty blood samples from 100 healthy and 50 diseased buffalo calves, were collected from buffalo calves of day 1 to 5 months of age. The samples were analyzed for total serum protein (TSP), serum albumin (SA), serum globulin (SG) and total leukocytic count (TLC). The mean TSP values for healthy buffalo calves ranged between 5.407 to 7.446 g/dl. A gradual increase in TSP level was observed with an increase of age. The TSP value for diseased buffalo calves varied as compared to healthy calves. A gradual increase in values of SA. SG and TLC was observed with the increase of age in healthy and diseased buffalo calves.

Key Words: Total serum albumin, globulin, albumin globulin ratio, total leukocytic count, prepubertal buffalo calves.

INTRODUCTION

Following bacterial diseases, gamma-globulin often increases and in the earlier stages of diseases and globulin fraction rises while albumin decreases. Total leukocytic counts are also affected under diseased or inflammatory conditions (Benjamin, 1978; Siddiqui et. al., 1989). Little information is available regarding the level of TSP, ,SA, SG and TLC in young buffalo calves. This study was formulated to figure out the profile of TSP, SA, SG and TLC in the serum of healthy and diseased buffalo calves of day old to 5th months of age.

MATERIALS AND METHODS

A total of 150 buffalo calves were selected randomly amongst animals of livestock farm Bahadar Nagar, Okara, Pakistan. Among these calves 100 were apparently healthy, and 50 were not healthy and were suffering from various disorders**.** Based on age in months, they were divided into 5 groups (A-E) each comprising 20 healthy and 10 diseased buffalo calves. The values in groups A, B, C, D and E were of 0-1, 1-2, 2-3, 3-4 and 4-5 months of age respectively.

Total leukocytic count was made using the counting chamber technique as described by Coles, 1980. The serum protein and serum albumin were determined by Biuret method with commercial kits (proti) using a spectrophotometer as adopted by Douman et al., 1977. Serum globulin was estimated by subtracting the serum albumin value from total serum value.

RESULTS AND DISCUSSION

Mean values for TSP, SA, SG and TLC are shown under Table 1. A gradual increase in values in all groups was observed with the increase of age from (0-5 months in health and diseased buffalo calves. The mean TSP values for healthy calves ranged between 5.407 to 7.446 g/dl. Total serum protein level was lower in diseased calves as compared with healthy calves. The difference of TSP values among group B and C was non-significant ($P>0.05$) while among others the differences was significant. The difference in values when compared with E was highly significant ($P<0.01$) except group D where it was significant ($P<0.05$). Increase in TSP values with the increase in age has also been noticed by other workers (Robert et al. 1968; Shirley el al. 1982). This increase with the increase in age may be due to increasing demand for

proteins for the tissues of growing animals exhibiting optimum metabolism (Green et al. 1982). The decrease in TSP values in diseased calves may he due to diarrhoea, heat stroke or fever and other disorders including parasitic infestations (Benjamin 1978; Borg 1981; Kishtwaria, et al. 1983: Siddiqui et al. 1989).

A significant difference ($P < 0.05$) hesween SA values was observed hi all groubs both in healthy and diseased calves except 5th month of age. Serum albumen levels decreased in diseased cases and a significant difference was observed between values from healthy and diseased groups. Decrease in SA level was observed in diseased cases which were suffering from diarrhoea, heat stroke and round worth infestation.

An increase in mean SG levels was observed in diseased buffalo calves as compared to healthy calves in all age groups. The increase in diseased calves was observed infestation. This increase in SG has been pointed out to be due to decrease in SA in diseased conditions (Benjamin 1978; Siddiqui et al., 1989).

The values for TLC were higher in diseased calves as compared to healthy buffalo calves. The increase in TLC was observed in calves suffering from diarrhoea, heat stroke and round worm infestation. This increase in TLC may be a result of immediate mobilization of neutrophils those have marginated in small blood vessels and are flushed into larger vessels with the increased blood flow. The increase in TLC in diseased cases have also been noticed by other workers (Benjamin 1978; Siddiqui et al., 1989).

Table 1: Total Serum Protein (TSP), Serum Globulin (SG), Serum Albumin (SA), (mean, g/dl) and Total Leukocytic Count (TLC) (TLCx 10^3) values in healthy and diseased buffalo calves (0-5 months of age).

Parameter	Particulars	Groups				
		A	B	C	D	E
TSP	Healthy	5.4	6.05	6.19	6.94	7.44
	Diseased	5.78	5.86	5.83	6.69	7.24
SA	Healthy	2.25	2.68	2.76	2.89	3.35
	Diseased	1.98	2.3	2.33	2.59	3.02
SG	Healthy	3.21	3.36	3.41	4.03	4.08
	Diseased	3.79	3.55	3.49	4.09	4.36
TLC	Healthy	8.75	8.97	9.32	9.56	9.61
	Diseased	9.99	10.42	10.7	11.17	11.2

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STUDIES ON THE POSSIBILITY OF TECHNOLOGICAL CONTROL OF THE POPULATION IN POLYPOPULATIONAL TECHNOGENIC ECOLOGICAL SYSTEMS

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The animal farm is a technogenic ecological system:

- animals make biologic transformation of /vegetable and synthetic / forage in products / necessary and valuable for mankind/,

- biological processes are being developed in a created by man ecological niche as a result of the adaptive behaviour of man - labour,

- labour makes the system stable.

The exploiting population of man and the exploited population of productive animals participate in the ecological system mainly.

An ecological niche for synanthropic, saprophyte, parasitic and pathogenic populations is being developed as well. That is what legitimates the technogenic ecological system - farm as polypopulational. The additional for anthropocentric aims and technological processes ' populations influence the biologic and economic farm efficiency. This causes the necessity for these populations to be included in the technologic algorithm management. The parasitic populations are of extreme importance, such as:

- consumers of resources created through labour - changing the nature of resources created through labour	competitors of useful population biological aggressors
- endo- and ecto-parasites on useful population - pathogenic toward productive population	parasites and bio-agressors toward useful product
- consumers of useful product - changing the nature of useful product	reductors
- consumers of non-commodity products - reorganisers of non-commodity products	saprophytes, mutualists sanitary dangerous;
- synanthropic populations - populations producing biologic toxicants	biologic and mechanic mediators

Management of Anthropogenic Ecological System - Farm is applying of adaptive human behaviour for optimising of biotic and abiotic factors and for reaching ecological system stability. The management of biocenosis and populations is a part of technologic management for ecological system stabilization. /fig 1/.

Technologic adaptation is being done for:

- nature factors
- resources
- anthropocentric goals.

Biocenotic and technologic information is being taken through labour - diagnostic methods. Normative model is built by intellectual labour on the set anthropocentric goals, technologic economic possibilities and available resources with the help of knowledge and farmer experience. The decisions for labour influence are made on the basis of intellectual analysis of differences between normative model and the existing condition of ecological system. Model application of management on sinanthropic rats population in swine farm /fig. 2/. Goal of technogenic influence - minimal desity of parasitic population. The trophic way of influence is used, we have added two types of biotoxicants to forage and applied different regimen:

- 1, periodically included acute toxicant in poison spots,
- 2, permanent poison spots with chronic cumulative toxicant. /fig. 2

The registered biologic effect showed priority for the second variant toward the set goal. There the tendency for parasitic population density minimising is lasting and steady. The first variant has a shotterm effect. The parasitic population restores its initial density. The process is unpredictable and that is why it has to be watched technologically.

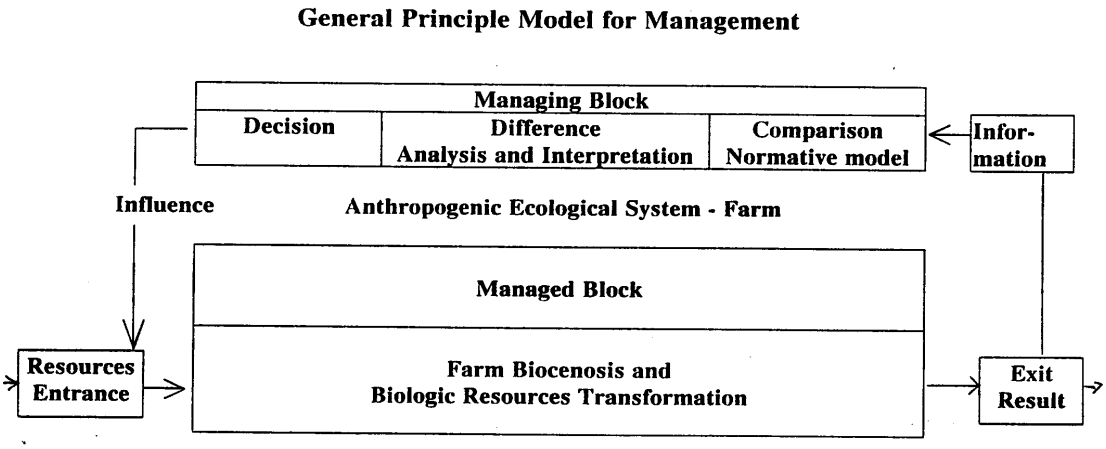
Other methods for influence on population level for suppressing and minimising of parasitic population density are necessary to be studied and found.

This model for approach can be used in the management of other populations in the technogenic ecological system - farm.

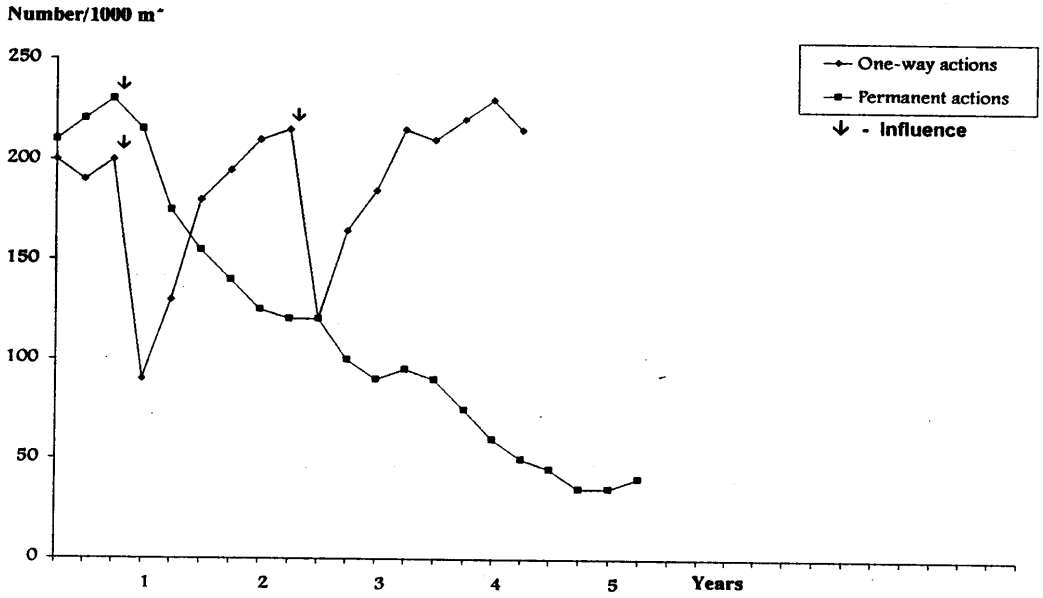
Fig 1. General Principle Model for Management
Fig 2. Rat Population Density Dynamics in Tvo Different Influence Regiment

General Principle Model for Management

Fig. 1.



/ fig 1 /



/ fig 2 / Rat Population Density Dynamics in Tvo Different Influence Regiment

Fig 2. Rat Population Density Dynamics in Tvo Different Influence Regiment

A profile of serum albumin, albumin globulin ratio and total leukocytic count in 6-10 months old buffalo calves

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Summary

One hundred and fifty blood samples were collected from 100 healthy and 50 diseased buffalo calves of 6 to 10 months age. The samples were analyzed for total serum protein, (TSP), serum albumin (SA), serum globulin (SG) and total leukocytic count (TLC). A gradual increase in TSP values was observed with an increase of age. The TSP value for diseased buffalo calves varied as compared to healthy calves. There was a gradual increase in values of SA, SG and TLC in health and diseased cases with the increase of age from 0 to 5 months. Serum globulin and TLC values in discased calves were higher than healthy calves whereas SA values were lower in diseased calves as compared to healthy calves. Increase in SG or TLC values and decrease in SA values in diseased cases were observed in those which were suffering from diarrhoea, fever and fascioliasis.

Key Words: Total serum protein, serum albumin, serum globulin, total leukocytic count, prepubertal buffalo calves.

INTRODUCTION

Total serum protein levels in the body is affected in severe burns, metabolic or other disorders and alter huemorrhage due to excessive loss of protein and is a bad prognostic sign for normal health (Robert et. al.. 1968; Shirley et al., 1982). Protein level vary in many diseases such as liver dysfunction, terminal stages ill cancer, parasitism and renal disorders.

Following bacterial diseases, gamma-globulin often increase and in the earlier stages of diseases like fascioliasis or nematode infestation total protein rises, the globulin fraction being increased and the albumin decreases. In physiological states, such as pregnancy protein values particularly albumin decline. Not much literature is available regarding the level of total serum protein, serum albumin, serum globulin and TLC in buffalo calves. The present study was therefore designed to asccertain the profile of TSP, SA, SG and TLC in healthy and diseased buffalo calves.

MATERIAL AND METHODS

A total of 150 (100 normal and 50 diseased) buffalo calves were selected randomly amongst animals of livestock ferm Bahadar Nagar, Okara Pakistan. Based on age in month. They were divided info 5 groups (A-E) each comprising 20 healthy and l0 diseased buffalo calves. The values in groups A, B, C, D and E were of 6, 7, 8, 9 and 10 months of age respectively.

Total leukocytic count was made using the counting chamber technique as described by Coles, 1980. The serum protein and serum albumin were determined by Biuret method with commercial kits (proti) using a spectrophotometer as adopted by Douman et al. 1977. Serum globulin was estimated by subtracting the serum albumin value from total serum value.

RESULTS AND DISCUSSION

A gradual increase in TSP, SA, SG and TLC values was observerd with the increase of age from 6-10 months in healthy and diseased buffalo calves (Table l). The difference of TSP values among group B and C was non-significant ($P>0.05$) while among others the differences it was difference ($P < 0.05$). However, a highly significant difference ($P < 0.01$) in TSP values was noticed between values of all groups and values of E except D where it was significant ($P<0.05$).

Increase in TSP values with the increase in age has also been reported by other workers (Robert et al. 1968; Shirley et al. 1982). This increase with the increase in age may be due to increasing demand for proteins for the tissues of growing animals exhibiting optimum metabolism (Green et al. 1982). The decrease in TSP values in diseased calves may he due to malnutrition, diarrhoea, fever and other disorders including parasitic infestations as observed by many other workers (Benjamin. 1978: Borg 1981; Kishtwaria, et al. 1983: Siddiqui et al.1989). In some diseased calves an increase in TSP level (5.79 anti 7.57 g/dl) were observed during 6th anal 10th month of age as compared to healthy calves (5.41 and 7.46 g/dl). This increase was observed in calves having dehydration signs and is therefore attributed due to dehydration.

Total leukocytic count was higher in diseased animals as compared to healthy animals. The increase in TLC was observed in calves suffering from diarrhoea and fever. This increase in TLC may be a result of immediate mobilization of neutrophil that have marginated in small blood vessels are flushed into larger vessels with the increase blood flow. The increase in TLC in diseased cases have also been noticed by other workers (Benjamin 1978; Siddiqui et al. 1989).

Table 1: Total Serum Protein (TSP) Serum Globulin (SG) Serum Albumin (SA), (mean g/dl) and Total leukocytic Count (TLC) (TLC x 10') values in healthy and diseased buffalo calves (6-10 months of age)

Parameter	Particulars	Groups				
		A	B	C	D	E
TSP	Healthy	5.41	6.06	6.20	6.94	7.46
	Diseased	5.79	5.88	5.96	6.72	7.57
SA	Healthy	2.45	2.72	3.18	3.21	3.44
	Diseased	2.01	2.34	2.44	2.68	3.10
SG	Healthy	3.21	3.41	3.46	4.05	4.14
	Diseased	3.84	3.61	3.53	4.13	4.36
TLC	Healthy	8.76	8.93	9.36	9.58	9.65
	Diseased	10.02	10.55	10.82	11.36	12.08

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Studies on the influence of the increased concentration of Cd and Pb in rations on the immune status of sheep

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Summary

As a criterion for determining the effect of lead and cadmium in concentrations 50 and 100 fold higher than the Highest Permisiible Concentrations (HPC) on the immunity and respectively the resistance of organisms, the changes in the functions of a mononuclear phagocytic system was used. By application of "Phagotest" and "Bursttest" the phagocytic activity and intracellular killing by polymorphonuclears were assayed.

Key words: lead, cadmium, mesocosm, sheep, blood, phagocytic activity.

Introduction

For Bulgaria, as well as for the countries of Eastern Europe the increase in the concentrations of the heavy metals lead and cadmium in lithospheric areas transformed into agroecological systems consists a serious ecological and public health problem (WHO, 1989; WHO, 1992). Besides the control on the concentration of the toxic factors via their direct determination in the air, water and soil, organismal bioindicators are used for pollution assessment. The World Health Organization (1989) advises on using farm animals and their production as bioindicators for heavy metal and toxic xenobiotics. The developed by Kendall (1996) conceptual model for ecological risk assessment indicates that the changes in resistance of organisms could be considered indicative for the pollution of soil with Pb and Cd in sublethal concentrations. Data exist indicating that the changes in immunity take place for doses much lower than those causing visible symptoms of intoxication (Dean, 1989). This means that the immune system could be used successfully as a specific indicator for toxicological characterization of certain pollutants.

Two mainstream approaches we found outlined in the reference literature: influence of heavy metal bioaccumulation along the trophic chain on the immune status of farming animals and outlining the opportunities for application of immunobiological indices in bioecological monitoring.

Material and methods

Animals. An artificial ecosystem of the "mesocosm" type (Odum, 1984) including populations of sheep Tzigai breed equal in age and weight was created by controlling the major abiotic factors (temperature, humidity, velocity of air movement and toxic gases).

Diet regimen. Three groups of animals were object of study (1 control and two test groupe) each containing 12 sheep fed for 180 days on standard concentrated fodder

supplemented with Pb and Cd as follows: I group (control); II group - standard fodder blend containing Pb and Cd 50 fold above HPC; III group - standard fodder blend containing Pb and Cd 100 fold above HPC.

Analytical assays. The concentrations of Pb and Cd on the entrance of the ecotechnical system (fodder) and their concentration in blood was assayed by AAS "Varian-40 Zeeman" using the standard methods of Jorchem (1993) on the 1st, 90th and 180th day of the experiment in the taken 20 samples fodder and 12 samples blood for each group.

Immunological assays. The mononuclear phagocytic system and its functional changes were used as a criterion for the effect of Pb and Cd on immunity and therefore on the defense mechanisms of organism. "Phagotest" and "Bursttest" produced by Orpegen, Pharma Co. were used to study the phagocytic activity and intracellular killing by Polymorphonuclears (PMN) and monocytes (Mo).

Results and discussion

The mean values of the studied chemical elements in sheep blood in the beginning of the experiment, on the 90th and 180th day are given in table 1.

Table 1. Mean values of lead and cadmium in sheep blood (mg/kg)

Groups	n	Cadmium	Lead
		beginning	
Control	12	0.016 ± 0.002	0.072 ± 0.012
I group	12	0.018 ± 0.003	0.083 ± 0.017
II group	12	0.020 ± 0.002	0.096 ± 0.024
		90 days	
Control	12	0.010 ± 0.001	0.015 ± 0.002
I group	12	0.046 ± 0.010	0.246 ± 0.037
II group	12	0.055 ± 0.015	0.263 ± 0.031
		180 days	
Control	12	0.016 ± 0.002	0.039 ± 0.009
I group	12	0.031 ± 0.005	0.194 ± 0.022
II group	12	0.079 ± 0.010	0.309 ± 0.024

The lower concentration of both toxic metals on 180 day in the blood of sheep from the first group was most probably related to the induction of metalthioneins which play a detoxifying role. The theory of Reimers (1994) could provide an explanation for these phenomenon in the first group on the 180 day, concerning the mechanism of adaptation to toxic effectors. According to this theory the third period under study coincides with the second phase of adaptation when activation of non-specific systems against unfavorable influence occurs. Therefore the defense mechanisms depend on the duration of treatment and the concentration of the contaminator. The immunological studies performed were in accordance with the recommendations for studying

the changes in phagocytic activity as most sensitive criterion of the unfavorable action of the chemical pollutants.

The liquid citometer FACScan, Becton Dickinson (530 nm) differentiates well PMN and Mo of sheep. The phagocytic activity of PMN and Mo in sheep fed on standard fodder ration (control) was 70% and 26%, respectively. When animals were fed for 180 days on rations containing 50 fold HPC no significant changes were observed in the phagocytic activity of PMN. Monocites posses lower phagocytic activity in this case compared to the control. When sheep were fed on rations containing Pb and Cd 100 fold above HPC the phagocytic activity of both PMN and Mo was strongly influenced. Data from this group shoed a significant decrease which suggested the suppressive action of high concentrations.

It is well known that the in intracellular killing of microorganisms by macrophagues and PMN most active part play the free oxygen radicals (H_2O_2 and O_2) together with the hydrolytic enzymes. In these cases the respiratory "Burst" increases in the phagocytic cells. As a result of this intracellular killing which is caused by activation of phagocytes could be assessed by the "Bursttest". The test also opens an opportunity to trace the effect of certain preparations compared to the stimulator fMP which consists of peptides as chemotractant and is a weak stimulator of "Burst" in the cells. It was determined that the percentage of oxidation (indicator of microorganismal degradation) in PMN from control untreated animals and in vitro treated with fMP was 56.78 and 54.55, respectively. Mo showed lower values - 55% and 26.36%. No suppressive effect was observed for the higher concentrations. A very weak, statistically insignificant increase of PMN activity was observed in animals treated with lower dosage compared to in vitro treated cells with fMP. The higher doses of Pb and Cd did not possess any influence on PMN. No changes in the respiratory "Burst" was observed also for Mo for both studied doses of heavy metals. The results from the present study indicate that both tests applied routinely for humans and laboratory animals ("Phagotest" and "Bursttest") are well applicable for studies of the immune status of farm animals. It is also advisable to study the dynamics of the immunobiological indices for a long period which should be combined with patologoanatomical studies of the parenchim organs.

Acknowledgements

This work was supported by a grant CC-428 from the National Fund for Scientific Investigations of Bulgaria.

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Examination of a steam cleaning apparatus upon its hygienic efficiency based on microbiological and parasitological tests

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Summary

Steam cleaning was tested with regard to microbicidal and parasiticidal effects. The tests were carried out with the test germs Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans as well as undeveloped eggs from Ascaris suum. The germ carriers used were tile, wood and carpet. The undesired effect of a swirl of germs caused by steam pressure was taken into consideration in the test set-up.

The experiments showed log reduction factors of at least 5.0 in the germ count at a steaming time of 5 seconds and a steaming distance of 2.5 cm for all three test germs on all three germ carriers. The swirl of germs caused by steam pressure could considerably be reduced by covering the steam outlet nozzle with cloth.

The steam stopped the development of ascarid worm eggs to 100 %.

Key words: steam cleaning, disinfection, microbicidal, parasiticidal

Introduction

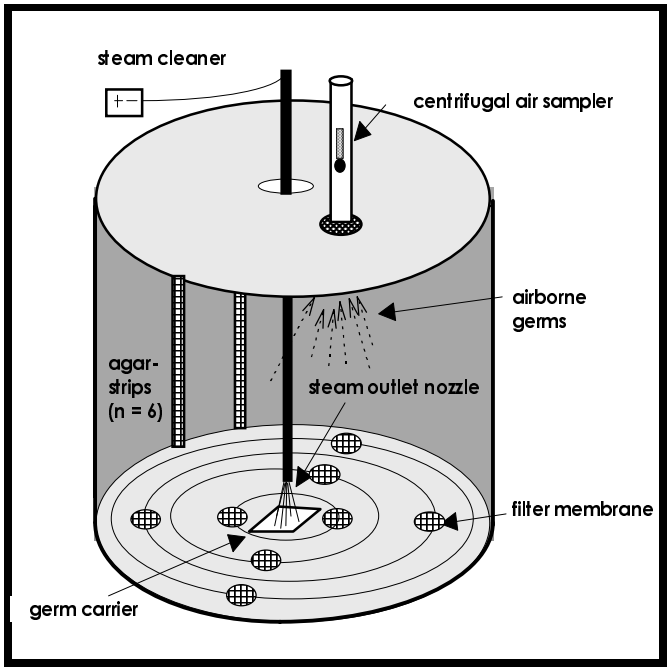
Aim of this study was to look into the question as to whether steam cleaning has effects which make this method suitable to control hygienic problems in keeping farm animals, pets and companion animals, but also in the veterinary practice (especially small animal practice). As bacteria and parasites increasingly become resistant to chemical disinfectants and antibiotica respectively antiparasitaria, and as there is a constant danger of allergic reactions for cleaning and nursing staff, who have regular contact with these chemical agents, it is necessary to find alternative cleaning and disinfection methods. Several research teams recommend steam cleaning for the control of house dust mites (Coloff et al. 1995, Janko et al. 1995). A reduction in the use of chemical disinfectants would also be welcome for ecological reasons.

Material and methods

The steam cleaning apparatus tested works at a boiler pressure of about 5 bar and a maximum temperature of 150 °C in the boiler. The tests were based on the DVG (German Veterinary Society, 1988) guidelines for testing chemical disinfectants.

Microbiological tests

Fig. 1: Test set-up



In order to determine parameters with which optimum results can be achieved by steam cleaning, three different germ carriers (limewood, smooth tiles, polyamide carpet), three different test germs (Staph. aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Candida albicans (ATCC 10231)), different steaming times and distances were tested. For the assessment of germ quantity the steaming was carried out in a closed container (Fig.1). The germ carriers (size: 3x3 cm) were contaminated with 0,1 ml of germ suspension, dried and steamed at in the centre of the test container. The reduction in germ counts could be determined by a comparison with an untreated carrier as a means of control. Germ swirl in the test container was measured with filter membranes at the bottom and agar strips on the wall of the container. The air inside the container was checked for germ content with a centrifugal air sampler immediately after the steaming process. Finally, the quantities of germs found were summed up and the logarithmical reduction in colony count was calculated in comparison with the original colony count.

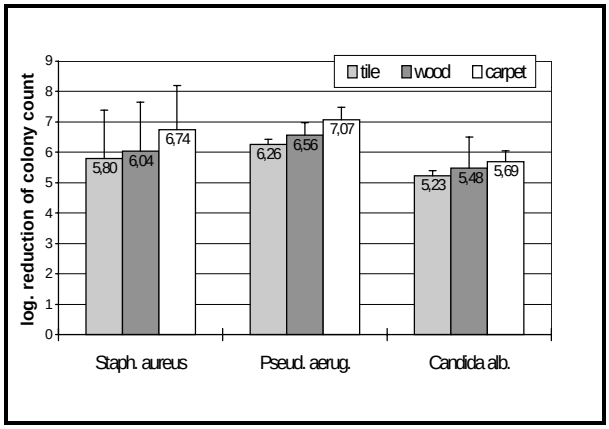
Parasitological tests

The parasitological tests were carried out on undeveloped eggs of Ascaris suum. Efficiency criterion was the eggs` capacity to develop after the steaming process. In repeated tests three different germ carriers (tile, wood, carpet) were steamed at for 2 respectively 5 seconds at a distance of 2.5 cm, after contamination (tile on its rough back) and drying. Ascarid worm eggs were recovered mechanically with toothbrushes. The result of light microscopical examination (300 eggs at a time) of the capacity to develop after 20 days incubation at 26 °C was compared with the capacity to develop of control eggs (about 97 %), which were exposed to the same procedure, except for steaming.

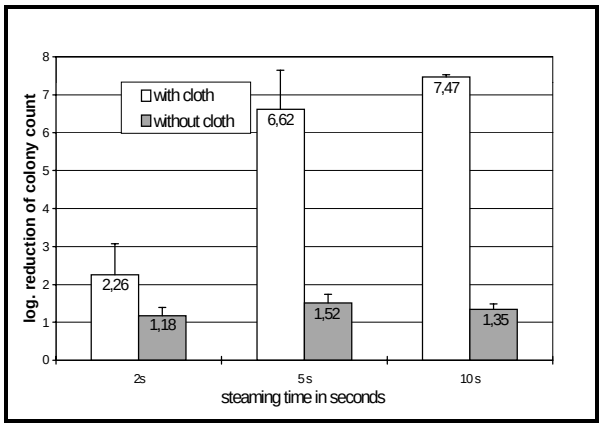
Results

Microbiological tests

The logarithmical reduction factor obtained at a steaming time of 5 seconds and a steaming distance of 2.5 cm for all three test germs on all three germ carriers was at least 5.23 (Fig. 2 on the right; mean of 10 repeated tests). This means, that with steaming, results similar to chemical disinfectants can be achieved.



The swirl of germs caused by steam pressure could considerably be reduced by covering the steam outlet nozzle with cloth. Without cloth, due to swirl of germs, a higher reduction of germ quantity could not even be achieved with a prolonged steaming period. Fig. 3 on the right shows logarithmical reduction of colony count of Staph. aureus at a steaming distance of 2.5 cm with and without cloth. With cloth the reduction in colony count was considerably higher. Without cloth steam velocity was 8.38 times higher than with cloth. This caused a strong mechanical swirl effect. With cloth the germs are exposed to the thermal influence of steaming for a longer time period.



Parasitological tests

The light microscopical examination of the treated Ascarid worm eggs at a steaming time of 5 and even 2 seconds at a distance of 2.5 cm showed a 100 % destruction of the eggs on all three germ carriers. In contrast to untreated eggs there were no signs of development during the incubation period. The untreated eggs` capacity to develop was between 96-99,5 %. The light microscopical examination of the eggs in the petri dishes show that the zygote inside the steamed eggs is a relatively dark lump. In comparison with the untreated eggs in the original egg suspension a slight shrinkage and a rougher granulation could be noticed at a larger magnification as a microscope slide preparation.

Conclusions

The good results of colony count reduction in microbiological tests and prevention of development in undeveloped Ascarid worm eggs even on very porous surfaces such as wood and carpet show that steam cleaning has very favourable thermal effects. In order to check whether this method can be applied to large surfaces, and whether these surfaces remain unharmed, further tests will be necessary. Except for a swelling of the germ carrier wood (caused by humidity) no obvious harm of the test surfaces could be noticed. A study concerning virucidal effects of the method is still to come. If the rules for application mentioned above (steaming distance 2.5 cm; steaming time 5 seconds; cloth) are followed, and if these experimental results can be confirmed under practical conditions with an acceptable expenditure of time, steam cleaning can be used as a valuable, alternative and ecologically friendly method to control hygiene problems.

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Use of a three stage dust sampler in two horse stables

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Summary

High dust concentrations in horse barns seem to affect the respiratory health of the animals and contribute to diseases such as the chronic obstructive pulmonary disease (COPD). The measurement of peak concentrations is difficult because most instruments are using filtration as the sampling principle for dusts and particles which delivers average values over the sampling time, only. Quick changes in particle pollution can't be detected. This paper reports of a new portable three stage personal sampler (Respicon TM) which combines the gravimetric and the optical measurement technique. The instrument samples three fractions of the dust according to the particle size: Inhalable, thoracal and respirable. The optical system delivers continuous results in mg/m³ on the increase and fall of all three dust fractiones during the measuring period. The three filters sample the dust particles for gravimetric analysis in the conventional way. The highest dust concentrations were found during mucking out and when the new straw bedding was prepared (18 to 40 mg/m³). During feeding the airborne dust ranged between 1 and 3 mg/m³. Measurements in an open group box barn revealed dust concentrations below 0.3 mg/m³. The new sampler seems to be a useful tool for air quality measurements in horse barns.

Keywords: three stage dust sampler, horse barn, air quality

Introduction

Airborne dust in animal housing is supposed to affect the respiratory health of animals and man and contributes to diseases such as COPD (cronic obstructive pulmonary disease) (Zeitler-Feicht et al 1988, Jaggy et al 1997). The measurement of airborne dust is usually carried out by instruments which are using filtration as the sampling principle. The disadvantage is that these methods can't deliver a concentration profile over the sampling time. This paper reports on a new portable three stage dust sampler which combines the gravimetric and the optical measurement principle. The instrument can also be used as a personal sampler. Some recent results are reported about measurements in two different horse barns (Jaggy 1996).

Material and Methods

The measurements were carried out in two different stables where the animals were either kept in boxes (area 10.5 m) which were part of a confined building or were accomodated in a group stable with open laying boxes and access to an outdoor paddock (Piotrowski 1984). The instruments were positioned in both stables above the feed trough beyond of the direct reach of the animals. Fig. 1 gives a schematic view of the instrument.

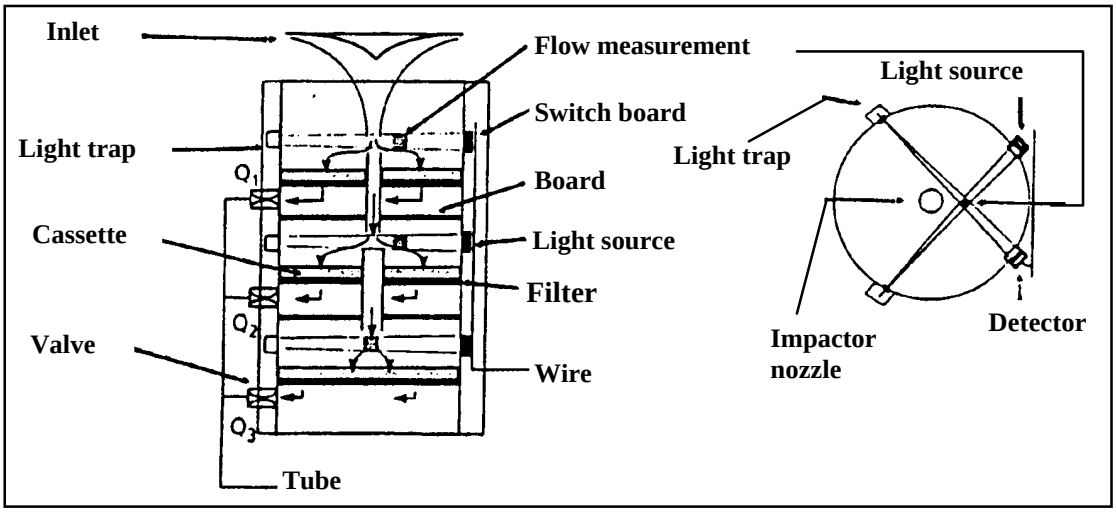

Fig. 1: Schematic view of the Respicon TM

The instrument (Respicon TM, Fima Hundt, Wetzlar, Germany) consists of a two step impactor with three light scattering photometer and three glass fibre filters. Three fractions of the dust are separated: The inhalable fraction, the thoracal fraction and the respirable fraction. A constant air flow of 3.11 l/min is provided by means of a battery powered pump (SKC Ltd., UK). Filters and light scattering deliver parallel results. The size of the Respicon TM is 5.0 x 6.0 x 11.0 cm. The weight is 390 g. The weight of the pump is 900 g, the size of the pump is 12.5 x 11.5 x 4.5 cm. The glass fibre filters are weighed before and after sampling. The photometers deliver curves of the dust concentrations of the three fraction continuously over the time. Figure 1 gives a schematic view of the instrument.

Results and Discussion

Fig. 2 shows the curve of the dust concentration in a confined horse barn during the morning activities (6 h) such as feeding and mucking out by means of the photometric device. The highest dust concentrations were reached when the straw bedding was worked up. Peaks of 40 mg/m³ were observed.

Fig. 3 shows the aquivalent measurements in the group stable (4 h). Highest total dust concentrations are seen when the straw was renewed.

Table 1 gives the average values of 8 samplings in each barn taken by filtration. The dust concentrations in all three dust fractions were distictly lower in the group stable than in the confined barn. This is probably due to ventilation conditions and animal activity.

It seems that the recently developed three stage dust sampler offers new opportunities in air quality measurements not only in horse barns and animal housing but in other areas such as the working environment of humans, too.

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Table 1: Dust fractions in the two horse barns (mg/m³). x = mean.

		dust concentration		
type of barn		respirable	thoracal	inhalable
confined box	x	0.061	0.187	0.428
(n = 8)	s	0.025	0.083	0.153
open box	x	0.049	0.135	0.279
(n = 8)	s	0.016	0.045	0.112

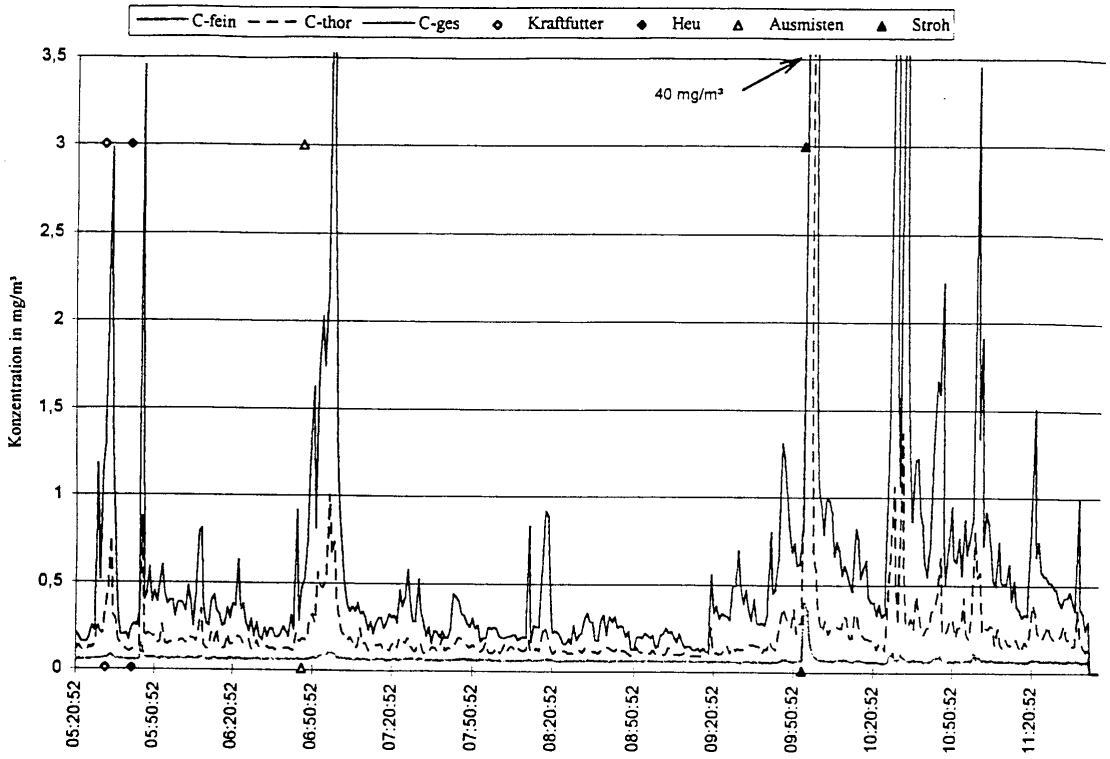

Fig. 2: Curve of the dust concentration in the confined barn

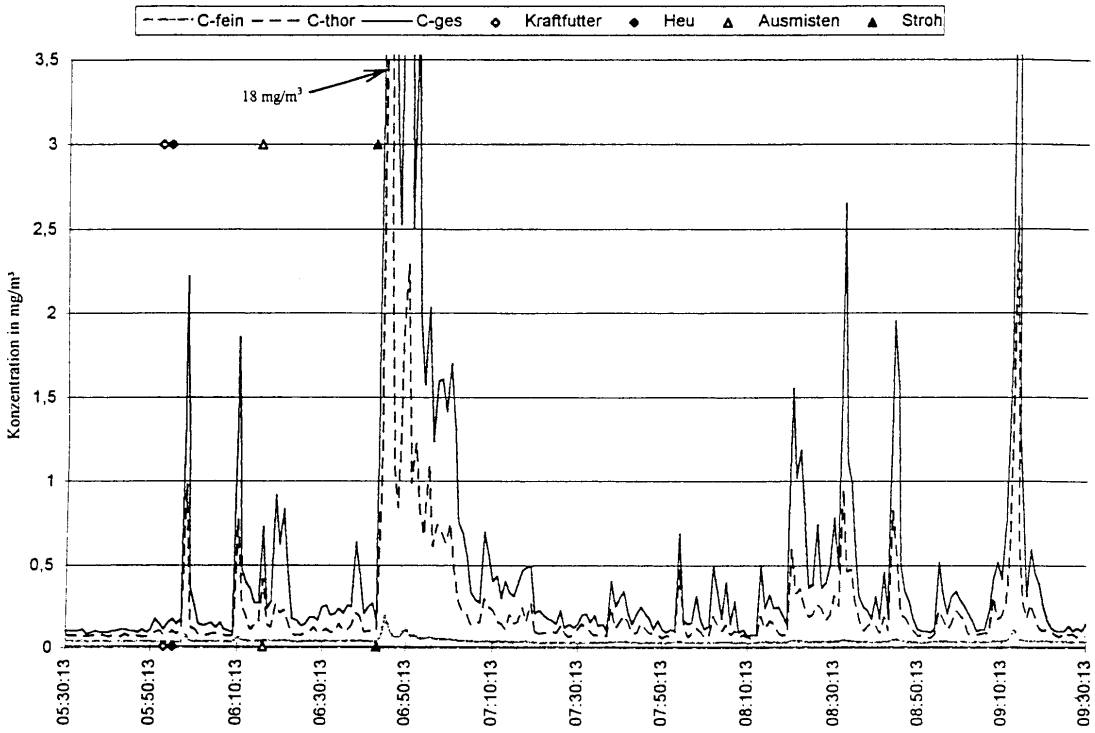

Fig. 3: Curve of the dust concentration in the group stable

Parasite populations of synantropic rodents in the intensive animal farms

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Summary

Nevertheless, what the production activities of the intensive animal farms are yearly losses sustained as a consequence of harmful rodents. That is reason to make a study of the factors, determined the level of parasitism and the mode for upset the balance of the population density.

The control on the density of harmful rodents in the animal breeding farms can be activated by long lasting disturbance of the intrapopulational, sexual, age and reproductive balance. An essential method for control and management of the parasitic populations is the systemic, technological application of the deratization technics and means.

Key words: rodents, deratization, populations, parasitism

Introduction

The big territories of the farms, the presence of nourishing food and constant microclimate make them reliable and cosy constant biotops for harmful rodents. That is the reason to make a study of the factors, determing the level of parasitism and the mode for upset the balance if the populations density.

Materials and Methods

The investigations were carried out during the period 1981-1990. Species structure of harmful rodents in big farms has been determined annually during 5 years by some somatometric measures and biological indications. Populate spatial disposition of dominant and discrimination species has been determined in 3 intensive farms, in every production sector.

Seasonal dynamic of reproductive process in the rodent populations in 2 pig-farms, 2 poultry-farms and 1 cattle-farm, and sheep-farm, has been studied. The correlation between females, and weigh-age been studied every season. All this investigation was carried out in farms, where deratisations have not been organised during 2-3 years period.

The influence of the sporadical and permanent exterminating actions on the populations dynamic of the rats for a 7 year period has been determined in 1 pig-farm occupation of Black rats (Rattus rattus) in high density.

Results and Discussion

It has been found that in nearly all farms the rats are dominant species, while in North Bulgaria Brown rats (Rattus norvegicus) are dominant and in South Bulgaria - Black rats. Clear subordination between climatic factors of every region and dissemination of the two rat-species has been noticed. This species dissemination is due to the fact that the use of the anticoagulants has been started considerably earlier in the farms in South Bulgaria, while many years people have worked with anticoagulants of first generation. In this way the populations of the Brown rats have become sparse, and their place has been occupied by resisting to this preparates Black rats. These facts (for stock farming) are fundamentally different from the data for populated of the settlements (towns and villages) /1, 2/, where for many years the domestic mice are dominant species.

There exists immediate string between accessibility and quality of the fodder in the respective sector and density of the rats no matter what the production direction /fig.l/.

The conditions, which the farms afford an opportunity for nest and lasting population in the buildings are second condition for numerous populations of synantropic rodents. The higher The density of rats in one lodging, the bigger the rat´s family is. This shows that the degree of

tolerance in the families is higher when the rivalry between them reinforced reason of the area. In these conditions of unlimited access to food, rival hierarchical relations reduced their meaning /3, 5/.

The average values of indices determined dynamic of reproductive process in rat-population in farms is reflected on fig 2. No matter what the production direction is, in all farms seasonal conditioned dynamic in reproductive process of the rat-population has been determined, which is weakly seasonal dependent in pig-farms and more strongly dependent in sheep-farms and cattle-farms. However rat-population in pig-farms is in relatively steady sexual and age balance annually /l, 3, 4/.

When applying of anticoagulants as a permanent present in premise's poison baits, the numbers of rats net only go down, but continual trend for future reduction of the values may be remarked /Fig 3./1, 3 /.

We consider, that continued and permanent deratisation of animal farms lead to disturbance population balance among roddens, gradually destroying and wastage of reproductive populations potential and that being the case the population lose integrity, social structure and hierarchy. There appear disturbance of interrelation between population grouping, separate families and between individuals. In that way a hard rarefied population gradually dies due to impossibility to reproduce it self and to exploit the environment. In this way permanent intervention measures lead to averting parasitism of synantropic rodents in unnatural ecosystem - animal farms.

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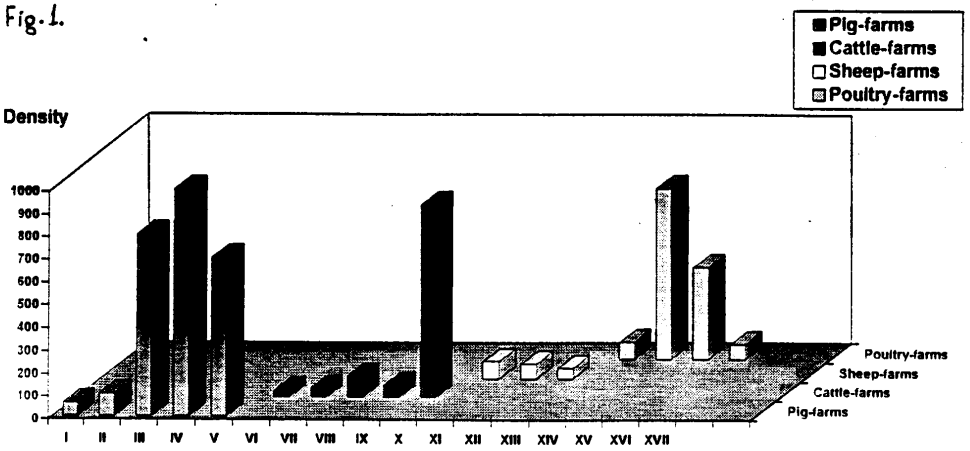
RAT POPULATION DENSITY IN BUILDINGS WITH DIFFERENT PRODUCTION SECTORS **Fig. 1**
INFLUENCE OF ONEFOLD DERATIZATION ACTS AND PERMANENT DERATIZATION ON THE NUMBER OF RATS IN INTENSIVE SWINE FARM **Fig. 3**.
SEASON DINAMICS AND RAT POPULATION STRUCTURE IN INTENSIVE ANIMAL FARMS WITHOUT DERATIZATION ACTS **Fig. 2a**
SEASON DINAMICS AND RAT POPULATION STRUCTURE IN INTENSIVE ANIMAL FARMS WITHOUT DERATIZATION ACTS **Fig. 2b**
SEASON DINAMICS AND RAT POPULATION STRUCTURE IN INTENSIVE ANIMAL FARMS WITHOUT DERATIZATION ACTS **Fig. 2c**
SEASON DINAMICS AND RAT POPULATION STRUCTURE IN INTENSIVE ANIMAL FARMS WITHOUT DERATIZATION ACTS **Fig. 2d**

Fig. 1

RAT POPULATION DENSITY IN BUILDINGS WITH DIFFERENT PRODUCTION SECTORS

Fig. 1.



I-improved pregnancy; II-pregnant; III-farrowing; IV-growing ups; V-fattening; VI-non-milking cow; VII-maternity; VIII-milking cow; IX-heifer; X-fattening; XI-mothers; XII-lamb; XIII-rams; XIV-growing up parents; XV-parent forms; XVI-broiler;XVII-incubator

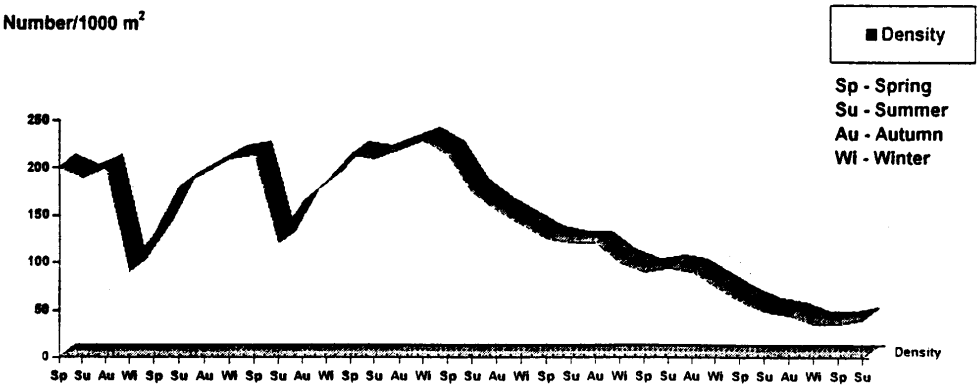
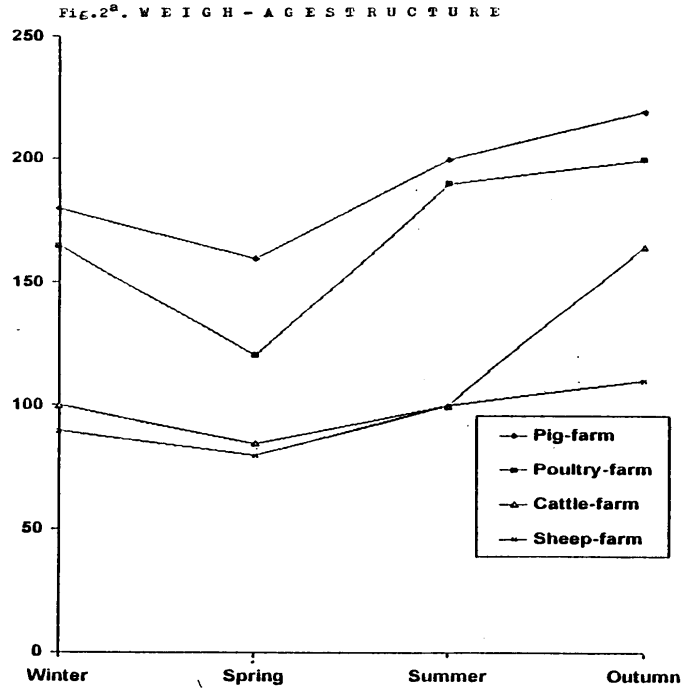


Fig. 3

INFLUENCE OF ONEFOLD DERATIZATION ACTS AND PERMANENT DERATIZATION ON THE NUMBER OF RATS IN INTENSIVE SWINE FARM

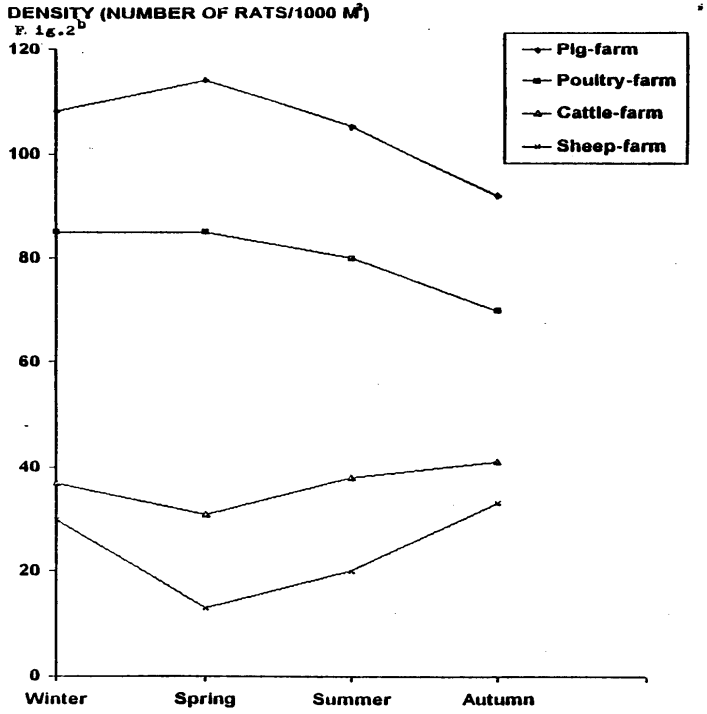
Fig. 3.

Fig 2a



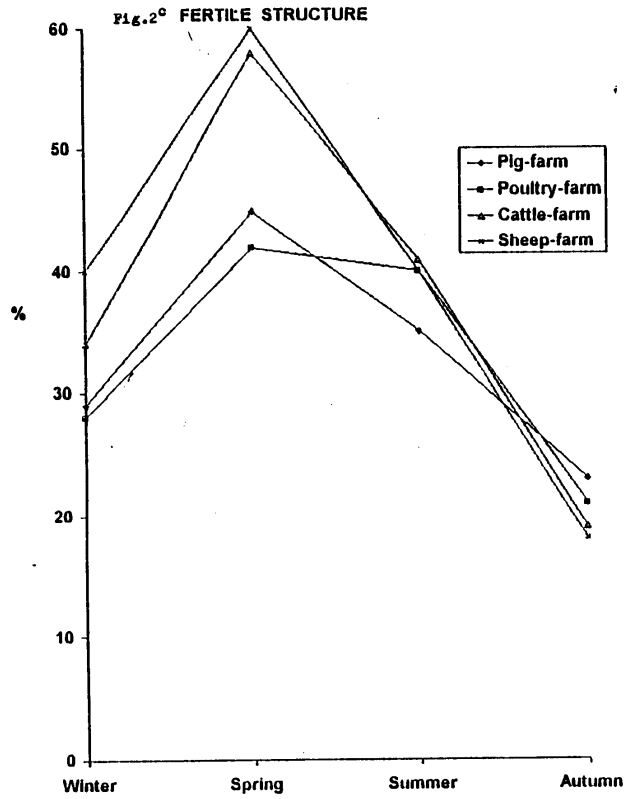
SEASON DINAMICS AND RAT POPULATION STRUCTURE IN INTENSIVE ANIMAL FARMS WITHOUT DERATIZATION ACTS

Fig 2b



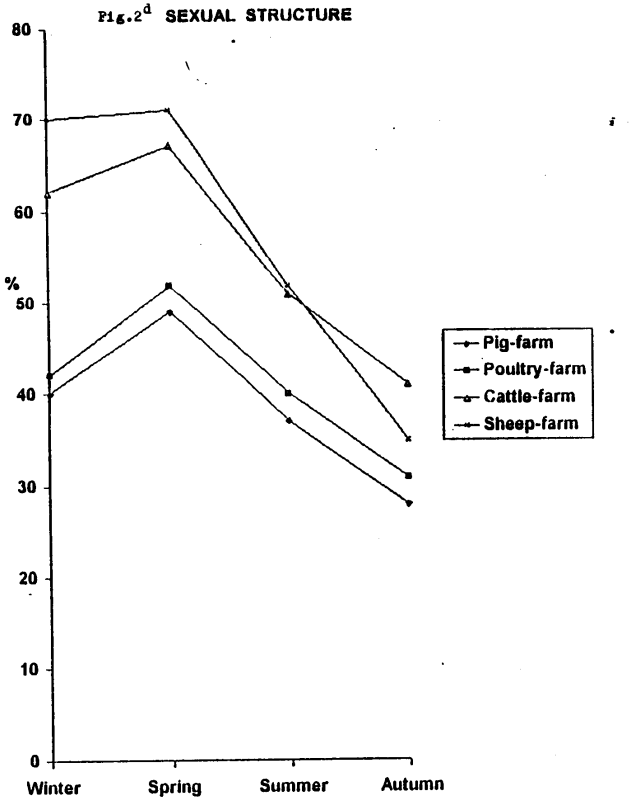
SEASON DINAMICS AND RAT POPULATION STRUCTURE IN INTENSIVE ANIMAL FARMS WITHOUT DERATIZATION ACTS

Fig 2c



SEASON DINAMICS AND RAT POPULATION STRUCTURE IN INTENSIVE ANIMAL FARMS WITHOUT DERATIZATION ACTS

Fig 2d



SEASON DINAMICS AND RAT POPULATION STRUCTURE IN INTENSIVE ANIMAL FARMS WITHOUT DERATIZATION ACTS

Epidemiological, serological and Hematological investigations in an outbreak of foot and mouth disease in district Lahore, Pakistan.

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Summary

After an outbreak of a vesicular disease in Lahore district a demoninator based active surveillance was conducted in four villages. During and after the epidemic the homesteads were visited and interviewed from door to door. The total number of animals kept by the farmers (N=1537) and the affected number of animals (n=1384) by age, sex and species were enquired from the farmers and recorded on a questionnaire proforma. The clinical signs of the affected animals and their sequence were recorded. Epidemiological investigations revealed that morbidity rate of 66.94% (n=867/1537) was higher in buffaloes than cattle which was 48.34% (n=117/242). Young animals of both species were more susceptible than adults. Morbidity rate in young cattle was 50.64 (n=39) in 77 animals but in case of adult cattle amongst the total population of 165 morbidity rate was 47.27% (n=78). In case of young buffaloes 290 (69.37%) out of 418 animals while in case of adult buffaloes morbidity rate was 63.51 % (557/877). Case fatality rate was observed higher 42.85 % (363/847) in buffaloes. Frequency of clinical signs observed were found as depression (63.30%), mucosa diffusely red (61.87%), anoretic (61.48%), muzzle hyperemic encrusted (61.09%), erosions (58.49%), laminitis (56.37%), temperature 101-105°F (54.26%), drooling of saliva (53.35%), shivering (44.82%), mastitis (38.51%), temperature 101-103°F (4.35%), temperature 105-107°F (6.18%), polypnea (5.79%), cough (3.38%), diarrhoea (2.40%), subnormal temperature (0.71%) and abortion (0.65%).

Key words: Epidemiology, foor and mouth, disease, outbreak, hematology, Lahore, Pakistan, frequency, clinical, signs.

INTRODUCTION

Foot and mouth disease (FMD) is an acute, highly communicable infection of cloven-footed animals, domesticated and wild. The disease is associated with the formation of vesicles in the mouth, teats and feet.

The disease is caused by a virus first isolated in 1897. It is classified with the enteroviruses as a member of the family Picornaviridae genus Apthovirus. There are 7 immunologically distinct types of virus identified as Types O, A and C, Southern African Territories (SAT) 1, 2, 3 and Asia-1 (Cottrol and Callis 1975). Within these types are over 60 subtypes, which have been designated by complement fixation (CF) tests. New subtypes are emerging occasionally and there are indications that they mutate spontaneously in the field conditions. The virus is exceedingly resistant to environmental influences. It will survive drying and can survive for prolonged periods in meat and lymph nodes.

This project was designed to study the epidemiology, clinical signs and economic losses to the farmers due to this outbreak.

MATERIALS AND METHODS

Denominator based active surveillance was conducted in the four villages affected with an outbreak of FMD around the border area of District Lahore. All the homesteads were visited from door to door. The total number of animals of various species kept by the farmers and the affected animals by age, sex and species were enquired from the farmers and recorded on a

questionnaire proforma. The clinical signs of the affected animals and their sequence was recorded. Each questionnaire was meant for one household only.

Different auction markets of livestock and fairs were visited in and around the affected area in terms of price of livestock at various age groups and different sexes to evaluate the economic losses in the affected area during the said outbreak.

RESULTS AND DISCUSSION

Buffalo: An incidence (morbidity) rate of 69.37% (n=290) among 418 buffalo calves less than 24 months of age had been recorded. Whereas in adult buffaloes (25 months and above) it was 63.51% (557/877). The Mortality rate in buffalo calves was 39.23% (164/418) while in adult buffaloes, it was 22.69% (199/877). The case fatality
rates for calves and adult buffaloes were 56.55% (164/290) and 35.72% (199/557) respectively.

Cattle: Among the total population of calves (n=77), morbidity, mortality and case fatality rates were 50.64% (n=39), 19.48% (n=19) and 38.46% (15/39 respectively). In adult cattle the total population was comprised of 165 and their morbidity, mortality and case fatality rates were 47.27% (n=78), 13.33% (n=22) and 28.20% (22/78) respectively.

S.No.	Epidemiological rats	Buffalo		Cattle	
		Calves	Adults	Calves	Adults
1.	Morbidity rate	69.37 %	63.51 %	50.64%	47.27 %
2.	Mortality rate	39.23 %	22.69 %	19.48 %	13.33 %
3.	Case Fatality rate	56.55%	35.72%	38.46%	28.20%

During this epidemic total buffaloes (1295) and cattle (242) population which surveyed was 1537. From the above results it can easily be concluded that the morbidity rate of 66.44% was higher in buffaloes than cattle which was 48.95%. The present study is in accordance with the study of Dutta et al. (1983). The reason behind may be that the cattle population kept in the area belonged to Sahiwal breed (local breed) which is a bit resistant to foot and mouth disease.

Young animals of both species (Buffalo and cattle) were found more susceptible than adults and it is in accordance with the findings of Sharma et al. (1981). The high mortality in the calves was due to cardiac involvement and frequent deaths occurred due to acute heart failure.
Frequency of clinical signs was observed and found it as depression (63.30%), mucosa diffusely red (61.87%), anorectic (61.48%), muzzle hyperimic encrusted (61.90%) erosions (58.49%), laminitis (56.73%), temperature 101-105°F (54.26%), drolling (53.35%), shivering (44.82%), mastitis (38.51%), temperature 105-107°F (6.18%), polypnea (5.79%), cough (3.38%), diarrhoea (2.40%) and abortion (0.65%). Theses findings are partially in accordance with Hajela and Sharma (1978) and Sharma et al. (1984). Heavy economic losses had to face during the outbreak. The economic losses due to FMD outbreak were recorded Rs.5.286 million both in cattle and buffaloes.

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The development of resistance in the housefly (*Musca domestica L.*) after the selection with pyrethroids under field conditions in piggeries

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Summary

The insecticidal selection pressure acting at the repeated use of preparations with the same active ingredient in practice poses a risk of rapid development of resistance. The increased resistance of flies to synthetic pyrethroids resulted in corresponding decrease in the efficiency of insect control. The alternative use of organophosphates and pyrethroids decelerated the development of resistance within a three-year period and resulted in a successful control of insect.

Key words: housefly, resistance, insecticidal pressure, pyrethroids, organophosphates

Introduction

Considerable numbers of houseflies *(Musca domestics L.),* up to 99% of the total insect fauna, can be found in piggeries. Losses in production of meat amounting to 5-10% were recorded in animal houses overrun by houseflies (Rae, 1980). The transfer of pathogens by flies to feed, working utensils, open wounds, mucous membranes, etc., represents an important indirect way of disease spreading.

The most extensively used way of killing flies is the chemical treatment of places of their occurence with contact insecticides. However, the long-term repeated use of insecticides with identical chemical structure results in the development of resistance.

Materials and methods

The development of resistance of houseflies to synthetic pyrethroids was investigated following two subsequent treatments of a delivery room with COOPEX 25 WP (a. i. permethrin) and K-OTHRINE 25 WP (a.i. deltamethrin), and after the alternate

treatment with the preparations mentioned and organophosphates ALFACRON 10 PLUS (a.i. azamathiphos) and Bi-58 (a.i. dimethoate). All the preparations mentioned were applied in doses recommended by the producer.

A method of tarsal contact (Rupeš et al., 1975) was used to determine the LC_{50} and LC_{95} values. The ratio of the values mentioned determined in wild housefly populations tested and that obtained for the sensitive strain SRS/WHO was used to calculate the factors of resistance which were evaluated according to Keiding (1980).

Results

The examination of houseflies during the first year showed a moderate resistance to permethrin (resistance factor for LD_{50} was 15). Two subsequent applications within 8 weeks of preparations based on permethrin and deltamethrin resulted in a marked increase in the factors of resistance (Fig.1). After winter and spring seasons, the values of resistance were in the range from moderate to a high level.

The alternate use of organophosphates based on dimethoate and azemethiphos and the pyrethroid permethrin resulted in a moderate level of resistance which was maintained for three years (Fig.2).

Similar results were obtained by alternating azamethiphos and permethrin (Fig.3).

Fig.1 Development of resistance after the practical application of pyrethroids
Fig.2 Development of resistance to permethrin after the rotating of insecticides
Fig. 3 Development of resistance after the rotating of insecticides

Fig. 1

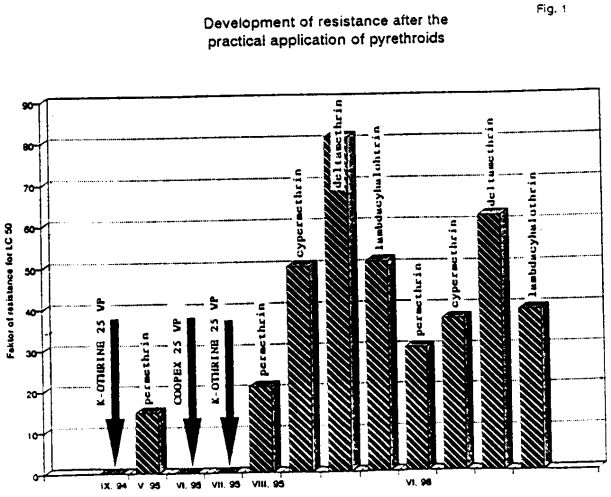

Fig. 2

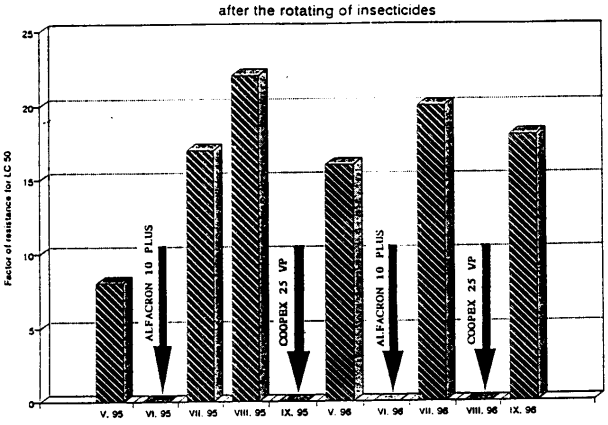

FIG. 2

Fig. 3

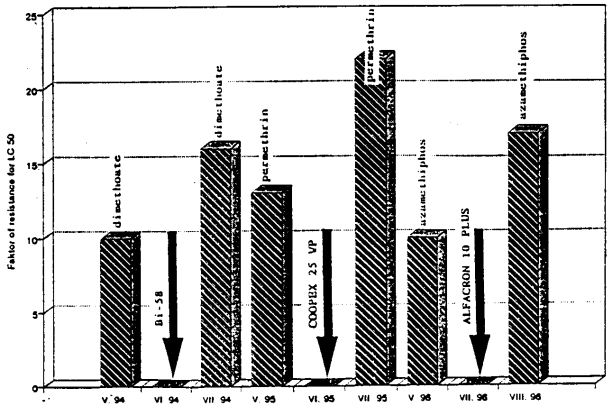

FIG. 3

Discussion and conclusions

The development of resistance in wild populations of insect is affected by a large number of interacting factors of which the key factors are frequency and dominance of resistant alleles, number of generations and offspring in one generation over a year, mobility of population and its character and persistence and frequency of application of insecticides (Georghiou, 1994).

The alternate use of preparations is based on the assumption that the frequency of occurence of individuals resistant to one insecticide will decrease during the application of the alternate one. The alternate application of Bi-58, COOPEX 25 WP and ALFACRON 10 PLUS in our experiments maintained the resistance at a moderate level for three years (Fig. 2 and 3). However, the alternate application of ALFACRON 10 PLUS and COOPEX 25 WP resulted in a decreased residual effect of the preparation based on azamethiphos, most likely due to the development of RR homozygous population. The subsequent use of pyrethroids resulted in more rapid development of resistance (Fig.1) in comparison with that at the rotating approach. The results obtained are in an agreement with those of Roush (1989) according to whom the rotating approach is the best strategy in combating resistance. The alternate use of organophosphates and pyrethroids in residual spraying appears as particularly advantageous.

The results presented in this paper were obtained within solving the project No. 95/5195/575 and 1088/94.

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ENTEROSORBENT-V, natural mineral corrector of animal health

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Summary

ENTEROSORBENT-V preparation was produced from natural aluminum silicates and has the appearance of flaky gray-white powder without taste or odor, particle size ranges from 0.5 to 3.0 mm.

We studied its chemical composition, porous structure, sorption capacity for certain harmful substances (cholesterol, carbamide, creatinine), as well as its detoxication effect in case of mercury and lead poisoning of animals.

Key words: ENTEROSORBENT-V, harmlessness, sorption capacity, detoxication effect

Introduction

Extracellular toxins entering the bodies of animals, and other altering impacts frequently result in critical conditions, with endogenic toxicosis being one their leading pathogenic syndromes [Marusanov *et al*, 1995]. This makes relevant the application of efferent detoxication techniques (*efferens* — efferent, Latin) [Belyakov, 1994]. The enterosorption technique based on binding of extracellular and intracellular toxins, supermolecular structures, and cells, and their evacuation via the alimentary tract, is one those [Belyakov, 1991].

A whole range of sorbents differing in properties and selectivity is available at present. Still, the successful solution of a number of problems related to sorption therapy calls for production and analysis of new sorbents with combined sorption effect from natural mineral raw stock [Tarasevich, 1988]. This is why we deemed it important to study the porous structure, harmlessness, and certain sorption properties of ENTEROSORBENT-V preparation produced from natural aluminum silicate at Department of Animal Health, St.Petersburg Academy of Veterinary Medicine, and its detoxication effect for mercury and lead poisoning of animals.

Research Materials and Methods

The presence of toxic inorganic contaminants, and sorption of mercury and lead ions in solutions were studies at SPECTROSCAN X-ray fluorimeter.

The porograms of ENTEROSORBENT-V samples under analysis were taken on Porosimeter-2000 of Carlo Erba (Italy).

The sorption of carbamide, creatinine, and cholesterol was studied by technique proposed by Levanova (1992).

The enterosorption technique was simulated on males of white rats for 20 days. The animals from experimental group received ENTEROSORBENT-V parenterally with food at the rate of 1g per kg of body weight. Blood was taken for biochemical analyses at the end of the experiment, after decapitation of animals.

Males of white rats underwent poisoning by salts of lead and mercury, with the animals divided into experimental and control groups. The control animals were given, in one case, lead acetate in the amount of 200 MAC, and mercury-chloride sublimate in the amount of 1/50 LD_{50}, in another case. The rats in experimental groups received with food, in addition of heavy-metal salts at above dosages, ENTEROSORBENT-V preparation in the amount of 0.5 g per kg of body weight. The experimental exposure took 35 days.

The contents of heavy-metal salts in body organs and tissues were determined at the end of experiment: lead by voltamperic method on instrument SVA-IBM, mercury — on mercury potentiometer YULIA-2. The biochemical analysis of blood was performed in automatic mode on SYNCHRON-4 (USA).

Results and Discussion

The mercury porometry yielded the total void volume (V_E) of 1.316 cm^3/g. Data obtained from integral and differential curves indicate the polymodality of sorbent under study, with predominant size of pores 10^3 - 10^5 A. The microporous structure exhibited the presence of micro- and submicropores with total volume 0.026 cm^3/g.

Absence of toxigenic inorganic contaminants is one of criteria of enterosorbent's harmlessness. It was established that Ti, V, Sr, Hg, As, Pb, Cd, V, Mo, Se were present in ENTEROSORBENT-V samples in concentration $1 \cdot 10^{-3}$%; Mn — $3 \cdot 10^{-3}$%; Ga — $2 \cdot 10^{-3}$%. This level does not has any adverse effect on the macroorganism, and does not exceed its MAC.

In vitro experiments established the following sorption capacity: salts of lead (at concentration $5 \cdot 10^{-3}$g · ion/g)=0.072 g · ion/g, mercury (at $5 \cdot 10^{-3}$g · ion/g)=0.012 g · ion/g, cholesterol (at 8.80 mmole/L) — 45%, carbamide (at 9.60 mmole/L) — 56%, creatinine (at 0.16 mmole/L) — 20%.

No negative changes in biochemical homeostasis was detected in the course of enterosorption simulation in animals. The blood serum content of substances determining the severity of endogenic toxication course decreased: creatinine, carbamide, cholesterol (Table 3). This indicates the correction of the body's metabolic processes.

The experimental poisoning of animals by salts of mercury and lead has revealed a detoxication effect of the preparation application (Tables 1, 2). The detoxication mechanism occurs through the following proposed scheme:

$$(-\underset{|}{\overset{|}{\underset{|}{\overset{|}{Al}}}}-O-\underset{|}{\overset{|}{Si}}-)_{2n}Mg + nPb^{+2}\,(nHg^{2+}) \rightarrow (-\underset{|}{\overset{|}{\underset{|}{\overset{|}{Al}}}}-\underset{|}{\overset{|}{O}}-\underset{|}{\overset{|}{Si}}-)_{2n}Pb\ n(Hg_n) + nMg^{2+}$$

Table 1

Mercury content in rat organs and tissues (as per GOST 26927-86. Mercury.)

		mg/kg
kidneys	experimental	48.111
	control	83.982
liver	experimental	2.322
	control	6.780
lungs	experimental	1.037
	control	1.351
skin with hair	experimental	0.537
	control	0.812
stomach wall	experimental	0.274
	control	2.007
small intestine wall	experimental	0.313
	control	0.776

Table 2

Lead content of rat organs and tissues (as per GOST 26932-86. Lead.)

		mg/kg
kidneys	experimental	29.90
	control	41.83
liver	experimental	1.43
	control	026.8
bone tissue	experimental	0.88
	control	2.30

Table 3

Certain indices of rat blood serum in the course of enterosorption simulation

		experimental	control
carbamide	mmole/L	9.6±0.47	10.3±0.48
creatinine	mmole/L	$0.056\pm1.87\cdot10^{-3}$	$0.066\pm3.22\cdot10^{-3}$
cholesterol	mmole/L	0.86±0.053	1.00±0.061

Conclusion

Being polyselective sorbent and sorption depurant, ENTEROSORBENT-V has general detoxication effect. The preparation promotes elimination of extracellular and intracellular toxins recirculating in organs and systems of animal bodies, it is able to form complexes with lead and mercury ions and evacuate them into environment.

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Prevalence of camel tuberculosis by using short thermal test and identification of organism from lymph nodes

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Summary

In the present study an attempt was made to estimate the prevalence of tuberculosis in camels by applying short thermal test and post mortem examination of reactors and non-reactors in the Lahore abattoir. Gross and microbiological examination of various lymph nodes of reactors and non-reactors was carried out. Heat concentrated synthetic medium tuberculin (HCSM) was used parenterally at a dose rate of 4 ml subcutaneously and rectal temperature was recorded after 2, 4, 6 and 8 hours post inoculation. A total of one hundred weak, thin and emaciated camels were randomly selected and tested. Only three animals gave positive reaction to short thermal test and also positive to culture and isolation test, while no false positive and/or false negative case was recorded. Similarly no case of miliary T.B. was recorded. A total of 2 out of 3 positive reactors, showed macroscopic lesions primarily mediastinal, lymph nodes. Out of these one showed characteristics lesions of T.B. and another showed only pin head point lesion in early stages. While the 3rd showed only Mycohacterium tuberculosis on bacteriological examination and proved no visible lesions.

Key words: Tuberculosis, prevalence, short thermal test, camel, slaughter house.

INTRODUCTION

Among the bacterial zoonoses tuberculosis is still a stigma in the society of under developed, developing and some developed countries of the world. It has been recorded since the ancient civilization like Egyptian Pharaohs (Stable forth and Galloway, 1959). The relationship of cattle and human tuberculosis was pointed out by Chauvean in 1868, who postulated that the disease could be transmitted through the digestive tract and, therefore, warned against the consumption of raw of semi cooked meat (Moore, 1913, Marshal, 1932). In Pakistan no work has been done for the investigation of tuberculosis in camels by short thermal test. Most of the work done is confined to the incidence of tuberculosis in the farm animals as detected by the tuberculin test. The object of this project is to determine the prevalence of camel tuberculosis in the abattoir, situated in Lahore. Detection of prevalence will aid to understand distribution and thus leading to control the disease in the field.

MATERIALS AND METHODS

A total number of 100 apparently healthy camels were, selected randomly from Lahore abattoir and were divided into three groups according to their general body condition as mentioned in the table.

Before selecting, animals were examined physically. It was a daily routine to select randomly 4 emaciated, 3 thin and 3 physically normal animals. Physical condition of the animals was assessed by prominency of rib cage and pelvic brim. The animals exceeding a rectal temperature of 101 °F were excluded from the experiment. The animals selected with special reference to temperature and body condition were inoculated with 4 ml. of heat concentrated synthetic medium (HCSM) tuberculin s/c on the right side of neck.

After inoculation of tuberculin, temperature was recorded frequently viz-a-viz 0 hour, 2 hour, 4 hour, 6 and 8 hour post inoculation. The animals showing a rise of 2°F or above than their initial temperature were recorded as positive to short thermal test. After slaughtering, the

visceral organs and carcasses were examined carefully for any visible or palpable pathological anomally. After observation of the reaction, post mortem examination was conducted and lymph nodes were collected for macroscopic pathological changes and bacteriological examinations.

RESULTS AND DISCUSSION

Only three out of 100 animals were found positive for tuberculosis by tuberculin test i.e., short thermal test (STT) and same were confirmed by isolation of Mycobacterium tuberculosis from lymph nodes and by observation of gross lesions from lymph nodes.

Table: Distribution of Positive Cases Relating to the Physical Condition

Group	Total No. of animals N=100	Physical condition of camels	Number of Camels Positive by		
			Short Thermal Test	Isolation of the organisms from lymph nodes	Gross pathological changes in the lymph nodes
I	n=30	Normal	0	0	0
II	n = 30	Thin	0	0	0
III	n =40	Emaciated	3	3	3

Maunder (1948), Gregory (1949), Seddon (1965), Rushford (1964), Mahoney (1959), Jong and Ekdhal (1969) and Randuz (1984) have also employed it as ancillary test. The duration of short thermal test was 6 hour during study where as some of the workers have conducted test up to 10 hr. The critical period appears between 6 and 8 hours observations. Rushford (1964) also supported 8 hours reading post inoculation. Similarly Seddon (1965), Gregory (1949) and Randunz (1984) recommended 8th hour reading for STT.

It has been concluded from the present study that:

- Short thermal test was fairly efficient when utilized as primary diagnostic test and was reliable at 6 hour post inoculation of tuberculin.
- T.B. was more prevalent among emaciated and aged animals.

It may be suggested that thorough examination of whole the carcass may be carried out to derive complete information. Identification and isolation of organism by culture and laboratory animal inoculation should be accompanied to coniform the diagnosis.

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STALOSAN F - Not a chemical disinfectant, but a very good hygiene substance

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Summary

The Danish preparation „Stalosan F“ was tested on the basis of guides for testing of disinfectants issued by the German Veterinary Society. „Stalosan F“ is to be applied as powder, but the test method is intended and suitable only for the examination of soluble chemical disinfectants. Therefore we test also the effect of „Stalosan F“ powder on contaminated wooden carriers.

„Stalosan F“ has no or low bactericidal, tuberculocidal, fungicidal and virucidal effects. But there is a very strong reduction (4-5 lg) of virus titer, probably also a reduction of a number of bacteria. The decreasing effect on microbial contamination of surfaces caused by adsorption of microorganisms, the binding of water and urea on floor, the reduction of ammonium emission and a little deodorization effect qualify „Stalosan F“ as a very good hygiene substance.

Keywords: disinfectant, testing of disinfectant, Stalosan F, hygiene substance

Introduction

According to reports from practice the preparation „Stalosan F“ produced in Denmark (by comp. Stormøllen) shows very goods effects on the animal health and environment when scattering it as a powder on animal house surfaces. The producer considers „Stalosan F“ to be a disinfectant. In Germany tested disinfectants are included into an official list and by this are recommended for application if their microbicidal effect has been proved true according to the Guidelines for the Examination of Chemical Disinfectants issued by the Disinfection Commission of the German Veterinary Society (DVG, 1988).

Material and methods

„Stalosan“ is a brown-red powder which mainly consists of phosphate compounds (85 %), Ca-, Cu- and Fe-sulphates (2.6 %), active chlorine (0.25 %), Perica oil (0.05 %) and Al-silicate (10.1 %). It is intended for the application as powder (up to 50 g per sqm). The test method prescribed by DVG refers only to aqueous solutions of chemical disinfectants. Therefore besides

the official methods for the examination of aqueous solutions a modified test procedure had to be applied to the powdery „Stalosan F“.

The examination of the microbicidal effect of the preparation (pH value 3.45 up to 2.51) was performed by suspension and germ carrier tests with the following strains:

- *Staphylococcus aureus* (ATCC 6538)
- *Enterococcus faecium* (Kulmbach Str. 2)
- *Proteus mirabilis* (ATCC 14153)
- *Pseudomonas aeruginosa* (ATCC 15442)
- *Mycobacterium avium* (Av 56)
- *Candida albicans* (ATCC 10231)

Appropriate deactivating agents were 3% Tween 80 (for gram-positive bacteria and Candida), 0.5% Na-thio-sulphate (for gram-negative germs) and 0.1% Cysteine (for Mycobacterium).

After pre-tests for toxicity and suspension tests had been performed the virucidal effect of 1% up to 5% dilutions of „Stalosan F“ was examined by germ carrier tests (on mull and wood) with following strains:

- ECBO virus (LCR-4)
- REO virus (type 1)
- *Newcastle Disease virus* (*Montana*)
- Vaccinia virus (*Orthopoxvirus commune, Elstree*)

Results

In suspension (without protein) aqueous solutions (dilutions) partly show bactericidal, tuberculocidal and fungicidal effects. But when adding a 20% cattle serum a microbicidal effect fails to appear even with high concentrations of the preparation (see Table 1).

It is true that „Stalosan F“ dilutions show no or only a very limited cytotoxic and virus-deactivating effect but cause a clear reduction of the virus titre of about 4 up to 5 lg in the suspension tests and 1.5 up to 3 lg in the germ carrier tests (Tab. 2).

Discussion and conclusion

1. „Stalosan F“ is no chemical disinfectant according to the binding DVG standards although the aqueous solution is strongly acidic.
2. By an adsorptive bond of the pathogens to the particles of the mineral-containing powder „Stalosan F“ in all probability causes a strong reduction of the number of germs (germ dilution) in suspensions and on surfaces of animal houses.

3. The combination of the germ-reducing effect with effects of absorption of moisture and the reduction of ammonia emission from the surfaces of animal houses qualifies „Stalosan F“ as a highly valuable hygiene agent.

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Table 1: Bactericidal effect of Stalosan F in suspension test (with and without protein loading, 20° C)

test bacterium	**Stalosan conc. (%)**	**without serum** affecting time (min)				**with serum** affecting time (min)			
		5	15	30	60	5	15	30	60
Staph. aureus	1 % phenole	-							
	0	+++							
	0,5	++	+	+	+	+++	+++	+++	+++
	1	++	ai. -	ai. -	ai. -	+++	+++	+++	+++
	2	ai. +	ai. -	ai. -	ai. -	+++	+++	+++	+++
	4	ai. +	ai. -	ai. -	ai. -	+++	+++	+++	++
	8	ai. +	ai. -	ai. -	ai. -	+++	++	++	+
	16	ai. +	ai. -	ai. -	ai. -	++	++	++	+
	32	ai. +	ai. -	ai. -	ai. -	++	++	++	+
Ent. feacium	1 % phenole	-							
	0	+++							
	0,5	++	+	+	+	+++	+++	+++	+++
	1	++	ai. +	ai. -	ai. -	+++	+++	+++	+++
	2	+ +	ai. -	ai. -	ai. -	+++	+++	+++	+++
	4	ai. +	ai. -	ai. -	ai. -	+++	+++	+++	+++
	8	ai. +	ai. -	ai. -	ai. -	+++	+++	+++	+++
	16	ai. -	ai. -	ai. -	ai. -	+++	+++	+++	+++
	32	ai. -	ai. -	ai. -	ai. -	++	++	++	++

legend:
+++ strong growth
++ middle growth
+ visible growth
ai. + growth after incubation on medium
ai. - no growth after incubation on medium

Table.2 Virucidal effect (reduction of titer) of 5% and 1% Stalosan dilutions in suspensions test

		reduction of titer (log 10 KID_{50})*				
test virus	**Stalosan conc. (%)**	**after different affecting times (min)**				
		0	15	30	45	60
ECBO	5	6,75	3	2,92	2,92	2,83
	1	6,75	3,58	3,58	3,5	3
REO	5	6,42	2,83	3	2,83	2,58
	1	6,42	3,17	3,25	2,83	3
Vaccinia	5	7,25	3,42	3,33	3,17	3,17
	1	7,25	3,42	3,5	3,33	3,25
Newcastle Disease	5	7,67	3,67	3,58	3,42	3,42
	1	7,67	4	3,83	3,66	3,5

* mean values of 3 tests

The biologic effects of some environmental pollutant in livestock

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Summary

A superphosphate factory emitted hydrofluoric acid, sulphur dioxide and sulphur trioxide gasses which pollute the environment. Combined chronic sulphur and fluorine intoxication on health condition of camels were studied and reported. The most prominent clinical Signs were emaciation, anaemia and dental lesions as mottling, brownish discolouration and wearing of teeth. Blood serum fluoride and sulphur were significantly elevated in animals at areas less than 2.5 km. far away from factory. Bone fluoride was generally elevated in affected Cases. Blood picture including RBCs, Hb, WBCs, copper, molybdenum, inorganic phosphorus and alkaline phosphatase were studied. Water samples showed increase in sulphur and drop in copper and molybdenum content.

Key words: Chronic fluorosis, Sulphurosis, Camels, RBCs, Hb, WBCs, F, S, Cu, Mb, P, Ap, water minerals.

Introduction

Industrial pollution is today a serious problem among many countries in the world. Pollutants are harmful for people, animals and plants when emitted in atmosphere from industry. Industrial fluorosis in livestock is today a disorder well known in all industrial countries (Ender,1969 and Crimson et al . 1979). Weeth and Capps, (1972) described sulphur poisoning in heifers.

The aim of the present study was to investigate the clinical signs, some blood constituents in combined fluorosis and sulphurosis in camels.

Materials and Methods :

Animals: 40 toxicated male camels, 4 - 6 Years old, belonged to various areas near the superphosphate factory and 12 healthy animals from Manfalout region as control.

Blood samples : Used for determination the values of RBCs, WBCs, Hb, F, S, Cu, Mb, P and Ap.

Bone samples : For determination of fluoride.

Water samples : For determination of S,Cu and Mo.

The methods used were according to Stockholm and Koch (1923), Fray and Taves (1970) and Coles (1980).

Results:

The clinical signs were loss of appetite, rough hair, emaciation, weakness, cachexia, paleness of m.m. and general poor health. Dental lesions include mottling, brownish discolouration, Pitting, Fast wearing and attrition of permanent incisor teeth (fig. 1 &2).

The estimated parameters of the blood , bone and water samples were recorded in tables 1,2 and 3.

FIG. 1 FIG. 2 Table 1. Table 2. Table 3.

Conclusions:

It could be concluded that camels present in the highly polluted areas were affected by the toxic fumes and showed the signs of intoxication.

It could be added that wastes of superphosphate factory have harmfull effects on the animal health and causes great economic losses.

It also emphasized the importance of blood, blood serum and bone analysis as means of diagnostic methods for fluorosis and sulphurosis.

The manufacturing units of the factory and methods of wastes disposal must be readjusted and minimize as greatly as possible, air, water and pasture pollution.

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Table 1: some blood parameters in toxicated camels at different localities .

Place and distance /blood parameters	Gaz El-Akrad 100 m.	Ezb mohamed 1.5-2 km.	Gaz El-Tawabia 0.5-1 km.	Manquabad 1-2 km.	Manfalout 25 km.
F (ppm)	12.5± 0.7**	8.9 ± 1.6**	4.5 ± 1.2	5.9 ± 0.3*	3.0 ± 0.16
S (mg%)	1882.1 ±261.76**	2011.3 ±218.10**	2273.8 ±448.4**	1732.8 ±293.5**	503.4±67.8
RBCs (T/L)	7.6±1.4*	7.4±0.7*	6.02±0.17**	9.6±1.2	10.1±0.6
Hb (G/L)	114.0±5**	105±4.3**	115±10.2**	104±5**	153±4
WBCs (G/L)	15.2±2.3	16.8±3.1	13.8±3.8	10.7±0.4	12.3±1.2
Cu (ug%)	86.1±3.0**	92.1±4.1*	83.3±3.5**	106.3±6.2*	150.6±6.9
Mb (ug%)	2.7±0.15**	3.01±0.02*	2.9±0.4*	2.81±0.21	5.31±0.01
P (mg%)	6.1±0.3	5.1±0.5	5.9±0.4	5.1±0.2	6.3±0.2
AP (IU/L)	134.0±2.4**	60.4±8.9	80.5±10.3	52.5±4.9	74.2±3.6

* Significant at P< 0.05 + Stand. error.
** Significant at P < 0.01

Table 2: The values (range and mean of bone fluoride, ppm) in clinically healthy and toxicated camels.

Name of bones	Clinically healthy camels		Clinically toxicated camels	
	prox.Ext.of bone	shaft of bone	prox.Ext.of bone	shaft of bone
Metacarpal	824±42 590±1240	775±48.5 590±1240	4046±360** 2945±6200	3695±446** 2015±6200
Metatarsal	902±78	757.5±53.3	5215±645**	4167±433**
Ribs	717±33.4 620±868	828±70 682±-1550	3513±246** 2790±4340	3996±588 2170±7750
Mandible	9525±104 713±1085		4433±757** 2015±9300	

Table 3: Sulphur, copper and Molybdenum content of canal Water at some selected areas

	Gaz. El-Akar-ad 100 m.	Ezb Mohamed 1.5-2 km.	Gaz El-Tawabia 0.5-1 km.	Manquabad 1-2 km.	Manfalout 25 km.
S (mg%)	801.92±63.33	908.45±75.42	725.34±68.1	207.33±4.92	70.23±18.14
Cu (ug%)	344.82±18.2*	298.31±17.5*	512.71±21.5*	411.28±31.54	861.12±34.31
Mb (ug%)	132.81±12.1*	165.33±13.1*	175.72±15.23	235.71±19.32	271.51±14.23

Figure 1:

Figure 1:

Figure 2:

Figure 2:

New approach to the growth of homoiotherms and its modelling

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Summary

The described selfregulating model of growth enables the calculation of growth velocity and growth curve for any homoiotherme. The input values employed: the daily feeding dose, the initial body mass and the thermoinsulating properties of the organism, and the cooling power of the environment (all are defined in the SI units). This methodical approach opens new ways for the evaluation of thermal microclimate as in the breeding of the farm animals, se well as breeding of the wild animals.

Key words: growth curve, modelling, selfregulating model

Introduction

Modelling the body mass growth, in relation to the nourishment, motion activity, and the influence of various stressing factors, represents very important question so far the body mass development of a man and animals are concerned. The sigmoid form of the growth curve, originally derived for the growth of a population, was applied by Robertson and Donaldson to the growth of the individual organism (ROBERTSON, 1908, DONALDSON and ROBERTSON, 1915). A comparison of three general growth models (Logistics, Gompertz s and Saturation Kinetics) was published by ROGERs et al. (1987). The Richards flexibile model of growth has recently been used by many authors - for the evaluation of the postnatal growth in chickens (KNÍ• ETOVÁ et al.,1991a,b), for comparison of two different feeding regimes on the heretability of growth curve parameters in Japanese quail (GEBHARDT-HENRICH and MARKS, 1993), in geese (KNÍ• ETOVÁ et al.,1994), and in turkeys (HYÁNKOVÁ et al.,1995) In Richards flexibile growth curves, most coefficients of the derived growth curves have no direct relation to the biological or physiological processes involved in growth (HYÁNEK and HYÁNKOVÁ,1995). It is the same problem as that of the coefficients of the polynomials employed in linear or nonlinear regression analysis employed by EMMANS (1981,1987) and MOUGHAN et al.(1995).

Methods and results

Selfregulating growth model (published by NOVÁK,1996) generates the growth curve not as a direct function of time, but as the differential of the body mass (**dG**), and its specific amount of the gross energy (**SGEG**). From the mathematical point of view, the equation of the body mass differential represents the application of the basic physical law of the mass and energy conservation, to the conversion of the foodstuff mass and energy to the organism´s body mass and energy. The body mass increase (**dG**) in general named the product, with the gross energy content (**SGEG**) denotes, not only the body mass change or the foetus growth in the pregnant mammals, but also the milk produced in mammals, or egg s laying in poultry. The products formation however could be performed only from that part of the mass and metabolisable feed energy engested, which was not transformed to the thermostatic heat (**THF**), neither to the heat produced under the influence of the various stressors (**THX**) Among the stressors (**X**) we consider for instance the influence of heat or cold, the motion activity, changes in the gravitational field, psychic stress and many others. The increase of body mass (of the product in kilograms per individual per day [kg/i/d], is then expressed by the equation:

dG = [(IMEF - (THF+THX)) / SGEG] . QGL [kg/i/d]

QGL - biological quality, the degree of the left potency for the body mass growth this quotient is generated automatically as the function of the actual body mass

IMEF - the resulting daily amount of metabolizable energy taken in food is given by the equation

IMEF = DFD .SMEF [MJ/kg/d]

DFD - daily feeding dose

SMEF - value of specific amount of the metabolizable anergy in the eaten food.

In the real time, expressed as the age of the modelled organism, the mass differential (**dG**) is calculated for the environmental temperature of the optimal product generation ($\mathbf{T_{op}}$), in a definded time interval (**t**). Calculated body mass changes e.g. increases, or in the case that the feeding is insufficient also decreases, are integrated to the actual body mass [**G(t)**]. The development of the body mass in the desired age points, G(t) values, are defined by the equation:

$$G(t) = G_o . \int_0^t dG . dt \qquad [kg/i]$$

For ilustration of this methodical approach on graph are presented the curves modelling the growth of cattle and pigs.

GRAPHS

Discussion

The calculation of the SGM growth curve is adjusted for the temperature of optimal production ($\mathbf{T_{op}}$). Calculation of $\mathbf{T_{op}}$ requires data about the body core temperature, thermal insulation and other measurable values (NOVÁK,1994 ab, 1995); such data are usually not presented in the current publications.

From the theoretical point of view the presented SGM is similar, to the Bertalanffy s model (BERTALANFFY, 1957), in its conception of the growth as a dynamic equilibrium of two antagonistic processes: anabolism and katabolism. The coefficients of the SGM are derived from well known allometric functions (MEEH, 1879, KLEIBER,1961) in combination with the known biophysical, biological and physiological rules governing the energy balance in homoiotherms.

Conclusion

The comparison of the experimental values of body mass growth, with the values calculated by the selfregulating model of growth, clearly demonstrates that the calculated values sensibly follow the influence of daily feeding dose on the body mass growth as it does the average value of body mass of the real experimental organisms.

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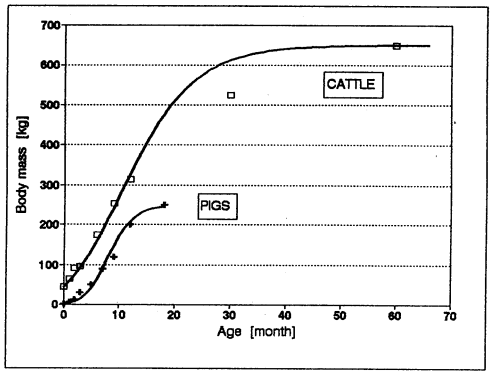

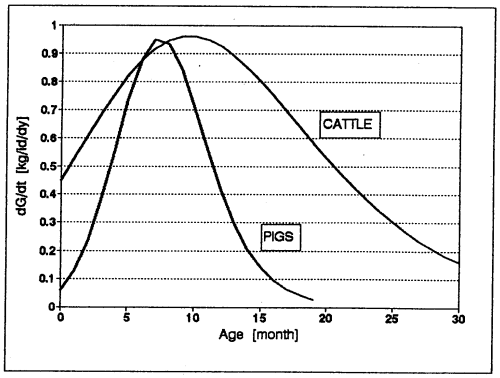

The influence of giving of Penstim, levamisole and isoprinosine on some nonspecific immunity indexes of nanny goats

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Summary

Females after the delivery are especially susceptable to infections caused by conditional pathogenic micro-organisms and also uteric infections induced by ascending way of penetrated microbes. Adequate nonspecific immunopreparation allows for avoiding diseases. The aim of our studies was evaluation of levamisole, isoprinosine and PENSTIM influence on some humoral nonspecific immunity indices of nanny goats. Studies included determination of lysozyme and ceruloplasmin activity, level of total protein and gammaglobulin in blood serum. Single immunostimulators application caused the increase of lysozyme activity and gammaglobulin level in goat blood serum.

Key words: goats, lysozyme, gammaglobulin, humoral nonspecific immunity, levamisole, isoprinosine, ceruloplasmin, total protein content.

Introduction

Females are strongly susceptible exposed to infection caused by conditional pathogens and bacteria getting inside the uterus by the ascending way in the first 10 days after delivery (Kudlać 1971). Intensive fetus growth, delivery and lactation are a significant burden for the female organism. Stratification of physiological and environmental burdens (feeding mistakes, incorrect supporting and care) often lead to reduction of organism defence forces. Early prophylactic application of on adequately well-chosen immunomodulator often creates possibilities to give up chemotherapy. For example levamisole, which was giving to cows, by the parenteral route at a dose of 2,5 mg/kg of b.w. for two weeks before delivery, caused a decrease from 9,3% to 3,7% of mastitis cases and reduced fetal mortality from 24,8% to 4,8%. Studies of Morin and Ballet (1982) showed that prophylactic isoprinosine application caused augmentation of some immunological parameters. No articles about reaction of the goat organism to levamisole and isoprinosine application were found in the available literature. The aim of our studies was

evaluation of the influence of levamisole, isoprinosine and PENSTIM preparation (contein isoprinosine) on some humoral immunity indices in goats.

Material and methods

The studies were conducted on 16 clinically healthy pregnant Polish white goats at the age of 3 to 5 years. The animals were kept in similar conditions and given the same feeding. Goats were randomly divided into 4 groups of 4 animals in each group. One control group and three experimental groups. Experimental groups were given immunostimulators. Two weeks before expected delivery, the first group got a single intramuscular injection of isoprinosine at a dose of 25 mg/kg of b.w., the second group got PENSTIM (preparation manufactured by VET-AGRO) at a dose of 20 mg/kg of b.w. and the third one got subcutaneous injection of levamisole at a dose of 5 mg/kg of b.w. Blood samples for examinations were collected from the vena jugularis before administration of immunostimulator on the delivery day and on 7th and 14th day after delivery. In blood serum indicated: lysozyme and ceruloplasmin activity using turbidymetric method (Siwicki A.K.1993), the level of total protein and gammaglobulin by spectrometric method (Lowry O.H.1980). Obtained results worked out statistically by two-factors method in nonorthogonal scheme using t-Student test.

Results and discussion

The results were statistically analysed and shown in **tab. 1** and **2**. Comparing results of examinations of lysozyme activity in blood serum on the delivery day with data before immunostimulation it is possible to suggest that it′s increase was: physiological in the control group, significant but not statistically in the group with isoprinosine and PENSTIM up to the 14th day of our studies. Levamisole administration caused the decrease of lysozyme activity and it′s increase was noticed on the 14th day after delivery. Gammaglobulin level decreased in the control group on the delivery day but was higher in experimental groups up to the 7th day of studies. This high level of gammaglobulins in the group with levamisole on the 14th day after delivery should be noted because it decreased in control group and the remaining experimental one. Blood serum biochemical examinations showed a little decrease of total protein level on the delivery day. In experimental groups it didn′t change. The highest increase of ceruloplasmin activity was observed after administration of isoprinosine and PENSTIM. It was probably connected with intensive activity of macrophages and neutrophils which have direct influence on ceruloplasmin releasing by hepatocytes.

Conclusion

Basing on obtained results it is possible to suggest that:

- single applications of isoprinosine, levamisole and PENSTIM caused the increase of lysozyme activity and gammaglobulin level in goat blood serum,
- a little increase of ceruloplasmin, after administration of examined immunostimulators may be the cause of increase of phagocytes activity and their influence on hepatocytes.

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Table 1. Some immunological indexes of goats (x, s)

Table 2. Some blood serum biochemical indexes of goats (x, s)

Table 1. Some immunological indexes of goats (x, s)

Specyfication	Goats 2 weeaks before parturition				Goats in parturition day				Goats 7 days after parturition				Goats 14 days after parturition			
	I	P	L	C	I	P	L	C	I	P	L	C	I	P	L	C
Lisozyme mg/l	0,54 0,28	0,50 0,08	0,56 0,17	0,42 0,20	0,97 0,20	0,75 0,22	0,35 0,23	0,57 0,21	0,80 0,42	0,72 0,40	0,36 0,30	0,28 0,08	0,90 0,39	0,35 0,21	0,45 0,25	0,20 0,07
Gammaglobulin g/l	3,75 2,25	1,99 1,06	4,31 2,17	3,5 1,36	3,83 1,68	2,52 0,91	4,81 2,33	2,33 0,71	4,23 1,99	4,08 3,47	3,93 3,10	3,20 1,67	2,01 0,80	2,22 1,25	5,85 3,08	2,54 2,21

Table 2. Some blood serum biochemical indexes of goats (x, s)

Specyfication	Goats 2 weeaks before parturition				Goats in parturition day				Goats 7 days after parturition				Goats 14 days after parturition			
	I	P	L	C	I	P	L	C	I	P	L	C	I	P	L	C
Total protein g/l	67,25 15,04	62 3,26	59 5,20	61,75 9,81	67 10,82	61,5 5,80	58,67 9,86	59 7,70	61,33 4,93	65,5 9,40	66,5 6,61	63,5 12,78	62,33 3,05	61,5 5,32	58,75 11,5	70,25 16,05
Ceruloplasmin mg%	13,58 6,49	10,47 4,13	9,80 10,25	11,17 6,44	16,37 6,79	12,15 6,10	7,88 7,23	6,61 7,34	12,69 6,04	11,06 8,91	4,1 2,96	4,61 4,84	8,35 6,20	3,89 3,04	6,09 4,64	1,53 1,96

Interpretation:
I - Isoprinosine group
P - Penstim group
L - Levamisole group
C - Control group

Aerosol of peracetic acid used in disinfection practice

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Summary

The disinfection effect of aerosol of peracetic acid was tested in battery houses for layers. The disinfectant Persteril (40% peracetic acid) showed sufficient bactericidal preventive effect comparable with formaldehyde aerosol in concentration of 12.5 $g.m^{-3}$ of preparation Formalin (40% formaldehyde).

Key words: aerosol, peracetic acid, disinfection

Introduction

The summary of disinfecting properties of peracetic acid, based on extensive experiments, were presented by Kretzschmar et al. as early as in 1972. The advantages observed consist mainly in a wide-spectrum effect at low concentration of the active ingredient ranging from 0.2 to 0.4%, short exposure time (20-30 min), and rapid self-inactivation. The corrosive effects of the preparation are smaller than those of chlorine preparations and the corrosive effects on aluminium are almost negligible. Besides the wide-spectrum effect of peracetic acid, Schliesser and Strauch (1981) stressed the possibility of use of this acid also at low temperatures without harmful effects on rubber, plastics, aluminium and steel. Peracetic acid is frequently used in Slovakia in the form of spray within the preventive programmes, particularly on specialised farms. The investigation of decontamination effectiveness of a number of chemical disinfectants in layer houses carried out by Rosocha et al. (1985) showed that peracetic acid had the best properties. When applied to the floors the top layers of which consisted of ceramic materials, concrete and asphalt, in the concentration of 0.4%, the plate counts of mesophilic bacteria per 1 cm^2 of floor area decreased from 10^{5-6} to 10^{1-2}. The spray disinfection in battery houses should be supplemented with aerosol disinfection and formaldehyde is the very substance suitable for this purpose. However, from the point of view of environmental protection, formaldehyde is not an ideal disinfectant and because of that we concentrated on the use of peracetic acid aerosol which is rarely, if ever, used in the practical disinfection. The aim was to compare disinfection efficiency of peracetic acid and of formaldehyde.

Materials and methods

The aerosol was produced by a thermodynamic reaction using preparations Formalin and Persteril with the identical content of the active ingredient (40%). Investigations were carried out in battery haouses for layers with concrete floors. Cement plaster formed the surface layer of peripheral walls. Aerosol disinfection was carried out immediately after spraying the floors, using the doses 7.5 $g.m^{-3}$ for Persteril and 12.5 $g.m^{-3}$ for Formalin. In the first case we started from the results obtained by a carrier-test and in the second case from disinfection practice. The preventive disinfection was carried out continuously in 4 + 4 houses at temperatures 15-18^{o}C and relative humidity of air 72-80%. After 4 h exposure time swabs were taken from 10 cm^{2} area, 10 from each of individual house-construction elements.

Results

Results indicating the effectiveness of Persteril aerosol on concrete carriers are presented in **Tab. 1**, and the results obtained after disinfecting battery houses with aerosols of Formalin and Persteril in **Tab. 2** and **3**.

Conclusion

Results obtained point to the possibility of application of peracetic acid aerosol within the disienfection preventive programme in battery technologies and also in the cases when application by spraying is not feasible for technical or some other reasons. Its favourable application is foreseen in food industry and municipal hygiene as well as in laboratories. Provided that the air is sufficiently humid and the houses can be tightly closed, this preparation can successfully replace the more toxic formaldehyde due to its disinfectant and deodorant effectiveness, simpler execution and negligible side effects.

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Table 1 Disinfecting effect of peracetic acid aerosol on concrete carriers, contaminated with E. coli (mean number of CFU. cm^{-2})

Exposure time min	Peracetic acid			
	$ml.m^{-3}$			
	1	2	3	4
0	8.10^4	8.10^4	8.10^4	8.10^4
10	6830	2500	900	12
20	3860	3400	330	60
30	830	120	0	0
60	130	142	0	0

Table 2 Disinfecting effect of peracetic acid aerosol

Construction element	Mean number od $CFU.cm^{-2}$	
	Before disinfection	After disinfection
technology lines	$4.8 .10^4$	127
peripheral walls	$9.8 .10^4$	$7.4 .10^2$
manip. passage	$1.1 .10^6$	
Persteril spray:		
- 0.5%		$1.7 .10^3$
- 1 %		66
Manure canal	$8.2 .10^7$	
Persteril spray:		
- 0.5%		$8.3\ 10^5$
- 1 %		$4,9\ 10^2$

Table 3 Disinfecting effect of formaldehyde aerosol

Construction element	Mean number od $CFU.cm^{-2}$	
	Before disinfection	After disinfection
technology lines	$1.7 .10^4$	49
peripheral walls	$3.4 .10^4$	71
manip. passage	$0.5 .10^6$	
Formalin spray:		
- 1%		$2.6 .10^2$
- 2 %		8
Manure canal	$2.2 .10^7$	
Formalin spray:		
- 1%		$4.4\ 10^5$
- 2 %		$1,6\ 10^2$

Effect of glucocorticoid alongwith antimicrobial drugs against haemorrhagic septicaemia in buffalo calves under field conditions

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Summary

Two long acting antibiotic preparations of oxytetracycline and amoxicillin, were used in these trials against haemorrhagic septicaemia, singly and alongwith a combination of synthetic glucocorticoids (Dexamethasone and prednisolone). Eighty buffalo calves suffering from haemorrhagic septicaemia were selected and treated under field conditions. Weighted clinical score was recorded before and after treatment in each case, on the basis of severity of clinical signs. Reduction in this score and recovery or death of animal was also noted. It was concluded that amoxicillin was more effective than oxytetracycline and the addition of glucocorticoids reduced the convalescence period and also increased the survival rate. Therefore long acting amoxicillin alongwith dexamethasone and prednisolone is recommended as the successful therapy for haemorrhagic septicaemia.

Key words: Oxytetracycline, amoxicillin, (glucocorticoid), dexamethasone, prednisolone, buffalo, calves, haemorrhagic septicemia, Pasteurella multocida. septicemia.

INTRODUCTION

Haemorrhagic septicaemia is one of the most common and major infectious disease of dairy animals in Pakistan having a mortality rate of above 70% and causing heavy economic losses worth more than 1.887 billion rupees annually to the country (Chaudhry and Khan, 1978).

Haemorrhagic septicaemia is a bacterial disease caused by *Pasteurella multocida,* a gram negative bipolar organism. *Pasteurella multocida is* classified serologically, on the basis of capsular antigens, as type A, B. D and E. Type B is mostly found in South East Asia while E is common in Africa.

Semi syntheti glucocorticoids like prednisolone and dexamethasone are potent anti-inflammatory agents which are used to subside the inflammation (Brander et at. 1982).
Keeping in view of the above factors the present progect was designed to:

i) observe the effect of glucocorticords in reducing the severity of disease alongwith antimicrobial drugs.

ii) compare the efficacy of long acting oxytetracycline and amoxicillin against haemorrhagic septicaemia in buffalo calves under field conditions.

MATERIALS AND METHODS

Eighty buffalo calves suffering from haemorrhagic septicaemia and twenty healthy one were selected for trials. Their feeding and management was under the normal field conditions. For this purpose eighty buffalo calves suffering from H.S. were treated under four treatment groups comprising twenty calves each i.e. A, B. C and D. Twenty clinically normal buffalo calves were also examined and kept under group 'E' as mentioned below:

S.No.	Groups	Number of Animals	Drugs Administered
1.	A	20	Long acting oxytetracycline (Terramycin LA, Pfizer, containing oxytetracycline 200 mg/ml).
2.	B	20	Long acting amoxicillin (Clamoxyl LA, Beecham). lt contains amoxicillin trihydrate 150 mg/ml
3.	C	20	Prednisolone + Dexamethasone (Opticortenol-S, Ciba), prednisolone 7,5 mg/ml, dexamethasone 2,5 mg/ml
4.	D	20	Drugs used in group B + Opticortinol-S.
5.	E	20	Control (Non treated).

RESULT AND DISCUSSION

Haemorrhagic septicaemia is primarily a disease of Cattle and buffaloes but equines and camels may also be infected (Ajmal et al. 1987; Momin, et al. 1987). The mortality rate is higher in young animals than adults (Kazimi and Haq, 1981). The disease is of great economic importance due to higher mortality rate (Chaudhry and Khan, 1978; Afzal and Rakhshanda, 1988).

In present research project long acting oxytetracycline (Terramycin LA, Pfizer) was used in group 'A' and long acting amoxicillin (Clamoxyl LA, Beecham) in group 'B'. As swelling of the throat region causes dyspnoea, so a combination of two glucocorticoids, dexamethasone and prednisolone (Opticortenol-S, Ciba) was added along with terramycin LA and clamoxy LA in groups C and D respectively.

On the basis of survival rate, the efficacy of groups A, B, C and D was 40%, 90%, 60% and 95% respectively. The result of this study are in line with lllahi and Afzal (1965) who treated experimental hemorrhagic septicaemia in cattle and buffalo with sulphadimidine and crystalline penicillin G very effectively. They reported that results of terramycin were somewhat discouraging.

The findings of the present study are different from those reported by Krishna and Kaushik (1965). They noted that Pasteurella multocida type B was sensitive to oxytetracycline, which is also effective in clinical cases of pasteurellosis. This difference might be due to difference in species. Pillai et al. (1980) studied that Pasteurella multocida was sensitive to oxytetracycline in-vitro. But in this study drug trials were carried out under field conditions.

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Helminthiasis: its dissemination and treatment with fenbedazole in the migration sparrows

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Summary

A study was undertaken on 400 wild migratory sparrows. Among randomly selected 200 were sacrificed and 129 (64.5 %) were naturally infected with two species of Nematode, Ascaridia galli (39.27%) and Heterakis gallinarum. The blood picture of these birds showed that red blood cells, haemoglobin level, lymphocytes and eosinophil were decreased whereas total leukocyte count and neutrophils were increased. No effect on monocytes and basophils was observed. Out of other 200 sparrows, 86 (43%) naturally infected were divided into two groups, one left as control and other was subjected to try efficacy of Panacur (fenbendazole) at the rate of 9.9 mg/kg body weight orally. The results were observed in terms eggs per gram of feces (EPG) counts after 24, 48 and 96 hours of medication. In the medicated group, the ova count was significantly as compared to the control group.

Key words: helminthiasis, immunology, nematodes, sparrows, poultry, feces

INTRODUCTION

Thousand of species of wild birds migrate to Pakistan, India and Srilanka in severe cold stormy dry weather from the Northern hemisphere for wintering and then go back to their original homes every year.

The migratory birds are a potential source for the spread of diseases from one region to another during the migratory process. Among the spectrum of diseases, the parasites include nematodes, cestodes and trematodes, posing a potential health problem as a result of transmission from wild birds to the poultry and commercially raised game
birds (Calnek et al., 1991).

The objectives of the study are to investigate:

1) The worm load of migratory sparrows.

2) The effect of worm infestation on blood picture.

3) Efficacy of Panacure (fenbendazole) as a dewormer.

The ultimate goals was to understand the role of migratory sparrows for the dissemination of parasitic diseases to the poultry in Pakistan.

MATERIALS AND METHODS

Four hundred live wild migratory sparrows were purchased from the professional hunters during the months of January and March. These birds were divided into two batches.

Batch 1: Two hundred sparrows were sacrificed to see the natural worn load and its effect on blood picture. For examining the worm load, each gut was incised longitudinally from oesophagus upto the cloaca. The intestines were washed in running water, the worms were picked up and were preserved in 70% alcohol. Then they were fixed in formoacetic fixative for 24 hrs. Hematological studies of the infected sparrows was done, whose blood was collected at

the time of slaughter and preserved with EDTA (ethylene diaminetetra-acetate) at the rate of 1 mg/ml.

Batch 2: Two hundred sparrows were taken and the faecal examination of each bird was done by direct smear methods, the positive sparrows were separated and divided into two groups one left as control and the other was treated orally with Panacur (fenbendazole) at the rate of 9.9 mg/Kg body weight, as suggested by Koblova, 1986. The results were observed in terms of eggs per am. of faeces by McMaster eggs counting technique (Gordon and Whitlock, 1939).

RESULTS AND DISCUSSION

The study was started in January 1994. For this experiment, 400 live wild migratory sparrows were purchased out of which 200 birds were sacrificed. Out of which 129 (64.5%) were harbouring endoparasites. Two species of nematodes such as *Ascaridia galli* and *Heterakis gallinarum* were found and their rate of infection was 39.5% and 25% respectively. No cestode and trematode could be observed. The presence of *Ascaridia galli* and *Heterakis gallinarum* has also been confirmed by Salfina et al., 1990, Fakae et al., 1991 and Haider, 1978. The study indicated that total leukocyte count and heterophils were increased whereas the total erythrocyte count, hemoglobin level, lymphocytes and eosinophils were decreased. The findings are in agreement with those of Sadun, 1950, Matta and Ahuwalia, 1982, Sekhar et al., 1986, Sekhar and Simah, 1985, and Humprey and White, 1970.

From the other 200 wild sparrows, a total of 86 (43%) birds were found naturally parasitized which were divided into two groups being one as a control and the other group was subjected to drug efficacy using Panacur (fenbendazole) at the rate of 9.9 mg/Kg body weight orally. The results were observed in terms of eggs per gm of faeces pre and post medication of 24, 48 and 96 hours. The control group showed progressive increase and the medicated group gradual decrease. The results of present study coincide with those of Koblova, 1986, Kovalenko, 1987 and Velichkin, 1984.

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HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF BLOOD SERUM IN RATS FORCED TO TRAINING

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Summary

The investigations were performed on 40 Wistar rats divided into the control and experimental groups. The experimental group rats were forced to run on the moving belt until physical exhaustion.

In the collected blood samples the following indices were determined: haematoctit value, haemoglobin level and the number erythrocytes and leucocytes. The biochemical survey of blood serum included the contents of total protein, the level of urea and glucose and the chosen mineral elements such as Ca, Mg, Na, K, and inorganic P. No statistically significant differences were observed between the experimental and control group in relation to haematologic indices. A highly significant increase of the urea level from 5.13 mmol/l in the control group to 9.1 mmol/l in the experimental group and the decrease of the glucose level were observed in the the blood serum. The observed differences between groups in relation to mineral elements were slight and not confirmed statistically.

Key words: rats, haematological indices, total protein, urea, glucose, Na, K, Ca, Mg, P-inorganic, training

Introduction

The behaviour of haematological and biochemical indices of blood serum as a physiological reaction of the organism to a single physical effort and the image of changes resulting from the action of adaptative physiological mechanisms of the organism in a long-lasting exercise are two basic trands of research in the accessible scientific literature.

The study of mechanisms of the physiological adaptation to physical exercises and training is necessary from the practical point of view in work, sport, military training and also in rehabilitation to reach optimal health (Spodaryk 1993).

The present investigation aimed at evaluating the haematological and biochemical indices of blood serum in rats forced to a single physical effort.

Material and methods

The investigation was carried out on 40 Wistar rats three months old of both sexes kept in the temperature of 20^{o}C and 75% relative humidity. The animals were divided into two groups: experimental I and control II.

The experimental rats were forced to a single run in the running wheal at a speed of 16 m x min^{-1} until physical exhaustion. Immediately after the effort blood samples were obtained by means of rat decapitation. In the collected whole blood the following indices were determined: haematocrit value, haemoglobin concentration and the of erythrocyte and leucocyte numbers. The haematological indices were determined by the means commonly used in clinical diagnostics. Total protein was determined in the blood serum by the buret method (Richterich 1971). Urea and glucose concentrations in the serum were determined using the Bio-Test reagents. The levels of Na, K, Ca and Mg in the blood serum were determined using the atomic absorption spectrophotometry (AAS) and the level of inorganic P according to the method by Bagiński et al. (1969). The differences between groups were analysed by the t-Student test.

Results and discussion

The performed haematological investigations demonstrated that the number of erythrocytes was slightly higher in the experimental group amounting, on the average, to 7.94 x 10^{12}/l versus 7.36 x 10^{12}/l the control group. According to Kozlowski (1976) physical effort leads to the growth of erythrocyte number following their increased relese from the spleen as the result of the sympathetic system stimulation. The same author reports that the number of leucocytes grows in a similar way up to 40-50 thousand/mm^{3}. In the present investigations a higher number of leucocytes was observed in the blood of rats forced to physical effort as compared with the control group.

The haematocrit value and haemoglobin concentration were similarly higher in the experimental rats. The differences between groups were not statistically confirmed. The results agree with those obtained by Monkiewicz (1992) who observed similar tendencies in young runners after each training. On the other hand, Spodaryk (1993) demonstrated that both short-lasting physical effort in rats and continuous training led to the significant decrease of the number of erythrocytes, haemoglogin concentration and haematocrit value with simultaneous significant reticulocytosis. TABLE

The performed biochemical investigations of the blood serum in rats revealed a slightly lower total protein level amounting to 69.8 g/l in the group forced to physical effort as compared

to the control group where that value amounted to 71.5 g/l. Also the glucose level was significantly lower a significantly in the experimental group as compared to the control group and the mean values amounted to 5.74 and 7.04 mmol/l, respectively. According to the opinion by Kozlowski (1976) during physical effort the level of glucose decreases as a result of its uptake by the cells of skeletal muscles where it is utilized. Contrary to total protein and glucose, the concentration of urea in is the blood serum of rats forced to effort was significantly higher $P<0.01$ amounting to 9.10 mmol/l as compared to the control group where that value amounted to 5.13 mmol/l.

The concentration of the investigated mineral components such as Na, K, Ca and Mg was slightly higher in the experimental group. The increased K level in the blood serum after physical effort Kozlowski (1976) explains by the appearance of intracellular K and the increased concentration of other mineral components in the blood serum he explains by blood concentration resulting from the organism dehydration during physical effort. It should be stressed that only the concentration of inorganic P in the blood serum was lower in the experimental group as compared to the control group. The differences concerning mineral components were not confirmed statistically.

Conclusion

Higher mean values of haematocrit, haemoglobin concentration, erythrocyte and leucocute numberwere nited in the group of rats forced to physical effort. The total protein and glucose concentrations were lower in the group of experimental rats and the urea contents was significantly higher in that group.

The levels of Na, K, Ca and Mg were slightly higher in the group of rats forced to physical effort, however, the level of inorganic P was lower in that group.

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AWF im. Bronislawa Czecha, Krakow.

Table 1 Haematological and biochemical indices of blood serum in rats

	Units	Group I n =20		Group II n =20	
		x	s	x	s
Haematocrit	l/l	0,34	0,02	0,31	0,09
Haemoglobin	mmol/l	8,85	0,55	8,66	0,62
Erythrocytes	10^{12}/l	7,94	0,96	7,36	1,12
Leucocytes	10^{9}/l	10,75	4,16	7,98	2,25
Total protein	g/l	69,80	9,50	71,50	9,70
Urea	mmol/l	9,10*	1,80	5,13	1,02
Glucose	mmol/l	5,74	1,52	7,04	2,66
Sodium (Na)	mmol/l	133,70	18,71	130,76	15,85
Kalium (K)	mmol/l	7,97	1,71	7,27	0,57
Calcium (Ca)	mmol/l	2,97	0,59	3,05	0,46
Magnesium (Mg)	mmol/l	1,22	0,25	1,02	0,15
Phosphorus inorganik (P)	mmol/l	2,97	0,70	3,75	1,05
The significanse of defferences between the groups: * at P< 0,01					

Quantification and confirmation of trichothecenes by gas chromatography-mass spectrometry-selected ion monitoring

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Summary

A rapid method for the simultaneous determination of deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3-Ac-DON), diacetoxyscirpenol (DAS), fusarenon-X (FUS-X), neosolaniol (NEOS), T-2 toxin (T-2) and HT-2 toxin (HT-2) has been developed. Cereal samples are extracted with acetonitrile-water (84:16), and cleaned up using Romer MycoSep™ #227 Column. Confirmation of identity is made by gas chromatography-mass spectrometry-selected ion monitoring of two ion characteristics of trimethylsilyl derivatives of the mycotoxins. Confirmation of identity as well as quantification can be achieved at level of 86-172 µg/kg depending on the mycotoxin. The limit of detection of the method is 40-86 µg/kg. Recoveries of the toxin spiked into the barley at level 172 µg/kg ranged from 67 to 117%.

Keywords: Trichothecene, mycotoxin, cereals, gas chromatography/mass spectrometry

Introduction

Fusarium mycotoxins, trichothecenes, frequently detected in food and feed, are very hazardous substances. A number of analytical methods have been developed for the detection of mycotoxins. These methods include thin layer chromatography (TLC, Betina 1985), high performance liquid chromatography (HPLC, Kuronen 1989) and gas chromatography mass spectrometry (GC-MS, Rosen 1984, Black 1987). TLC has been used as a rapid screening method while sensitivity of HPLC is limited because most mycotoxins have weak or only end absorption in the UV range. One commonly used quantification procedure is gas chromatography of derivatized trichothecenes with mass spectrometric detection. GC-MS is necessary for multiple trichothecene determination and is in use in several laboratories worldwide.

Most clean-up procedures include purification of the extract on a florisil or silica gel column and different evaporation steps. Samples are often derivatized with n-trimethylsilyl imidazole (TMSI, Rizzo 1986). This paper describes a rapid and sensitive method for quantification of trichothecenes by GC-MS in cereals using Romer MycoSep™ #227 Column.

Apparatus

Table centrifuge, flask shaker and vortex mixer, gas chromatograph (5890 Hewlett Packard), automatic sampler (7673A Hewlett Packard), mass selective detector (5971 Hewlett Packard), control and integration systems (ChemStation Hewlett Packard), and dry bath (Termolyne Dri Bath).

Reagents

Extraction solvent was created by mixing 840 ml HPLC grade acetonitrile with 160 ml water purified with Millipore Milli-Q system. The stock standard solution was made by diluting 1mg 3-acetyldeoxynivalenol (Sigma no A-6166) in 10 ml ethyl acetate to obtain 100 ng/µl. It was mixed with Romer Labs trichothecene standard (#S3410) containing 100 ng/µl of each Type A (T-2, HT-2, neosolaniol and diasetoxyscirpenol) and Type B (deoxynivalenol, fusarenon-X and nivalenol) as a dry film to obtain a concentration of 100 ng/µl of each. The working standard was made by diluting the stock standard with ethyl acetate to obtain 10 ng/µl in ethyl acetate and stored in the freezer until use. TMSI (Pierce no 88623) n-hexan and ethyl acetate were of analytical grade.

Extraction

25 g of the ground sample was weighted into a 250 ml bottle. 100 ml Acetonitrile/Water 84/16 was added. The mixture was shaken vigorously in a shaker for two hours and then quickly filtered through a folded filter-paper.

Purification and evaporation

7.5 ml extract was poured into a test tube. The Romer MycoSep™ #227 Column was pushed into the test tube and 4.0 ml of the purified extract was transferred into a derivatization tube and evaporated to dryness under a stream of nitrogen in a dry bath at +40°C. The sample is now ready for derivatization.

Derivatization and dilution for gas chromathograph

100 µl of TMSI was added into the derivatization tube, and the tube was capped and shaken for two minutes in a multi-tube-vortexer. The tube was heated for one hour at +60°C and cooled. The derivatized sample was diluted with 250 µl n-hexane and the solution was washed twice with 1 ml water. If the separation of phases after washing was not complete, the samples were centrifugated and the upper layer was collected. The sample was then injected into gas chromatograph.

Gas Chromatography and Mass Spectrometric Determination

Carrier gas was helium at the flow rate of 0.6 ml/min. The column was HP-1, 25 m x 0.2mm x 0.33 µm. Injection volume was 1 µl. Splitless injection was used.The temperature of the injection port was + 250°C. The temperature program was as follows: Initial temperature was + 60 °C for one minute, then raised quickly up to +220°C for one minute. Then the temperature was gradually raised +1 °C/min up to +275 °C and sustained there for one minute, and finally raised +20°C/min up to the final temperature of +285°C.

Mass selective detector was on electron impact mode. Electron energy was 70 eV. Ion source temperature was +190°C. Transfer line heater was + 300°C. Solvent delay was 16 min, and EM

absolute resulting voltage was 2500. The quantification was made by selecting ion monitoring mode (SIM). Two ions were measured and main ions is underlined. DON m/z 235, 422, DAS m/z 378 379, 3-Ac-DON 377 467, FUS-X 251 480, NIV 289 482, NEOS 252 436, T-2 350 436, HT-2 347 466. The calibration curve was fitted quadratically, forced through the origin. Calibration curve was made from standards between 5.0-0.078 µg/ml.

Results

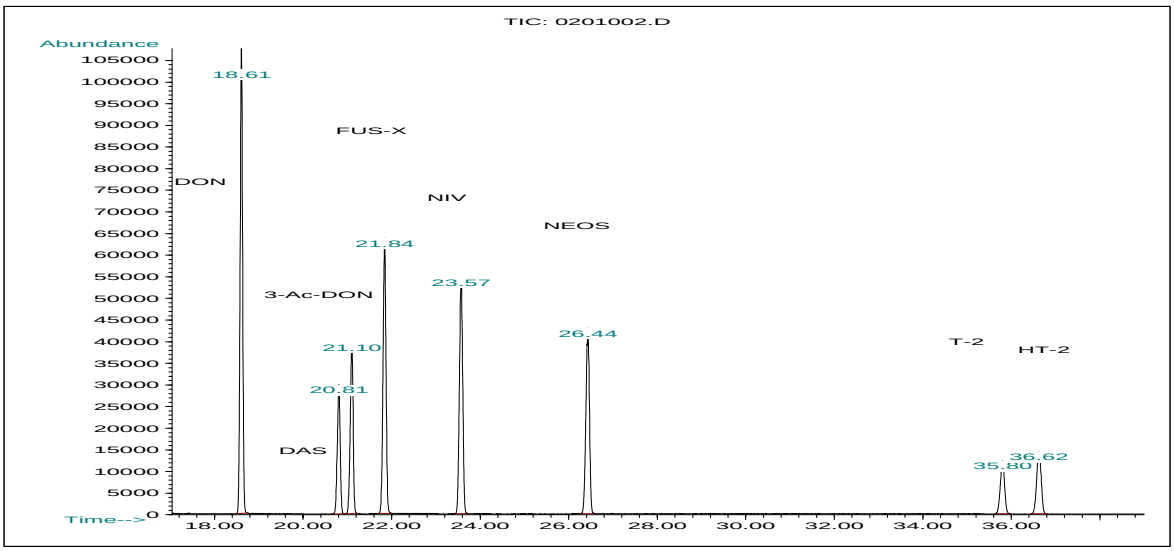
In our experience, this method is quite easy and requires considerably less time and solvent for purification than many other methods. Romer (1986) has used 50 ml elution solvent to obtain optimum recovery. There is no need to connect the column to a vacuum system, or to use a rotary evaporator; the sample is purified by pushing the column through the sample extract manually and then the clear sample is pipetted straight into the derivatization tube and evaporated under nitrogen. The recovery experiment was made by adding a certain amount of trichothecenes to blank barley extract purified with MycoSep Column. The results are represented in TABLE I. As shown in the total ion chromatogram (Figure 1), the mycotoxins are well separated.

TABLE I RECOVERY OF TRICHOTHECENES ADDED TO BARLEY EXTRACT

	1250 µg/kg n=4		625 µg/kg n=4		312.5 µg/kg n=8		172 µg/kg n=5		86 µg/kg n=3		
	rec.%	cv%	rec.%	cv%	rec.%	cv%	rec.%	cv%	rec.%	cv%	
DON	**87**	5	**86**	4	**83**	10	**85**	8	**97**	17	
DAS	**100**	5	**99**	4	**93**	8	**102**	10	**117**	0	
3-Ac-DON	**84**	5	**83**	4	**77**	9	**76**	16	**87**	0	
FUS-X	**92**	5	**90**	5	**81**	11	**79**	17	**87**	0	
NIV	**59**	4	**60**	5	**57**	12	**67**	12	**78**	22	**X**
NEOS	**107**	5	**108**	6	**106**	10	**117**	15	**126**	13	
T-2	**100**	4	**106**	8	**111**	13	**114**	11	**165**	20	
HT-2	**98**	3	**97**	7	**98**	10	**102**	10	**126**	13	

Table1. rec.=average of recovery, CV%=coefficient of variation%, n=number of analyses
x= single ion detected

Figure 1. Total ion chromatogam of the trichothecenes



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A study on the epidemiological aspects of fascioliasis in buffaloes in Lahore District.

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Summary

The study was designed to find the prevalence and intensity of Liver fluke infection in buffaloes in Lahore District. The study is based on the data collected from flour Veterinary Hospitals in Lahore Districts from Ist April, 1995 to 31st July, 1995. Based on history and clinical, signs 229 (10.48%) buffaloes were found positive for fascioliasis. In order to determine the intensity of f'ascioliasis in infected animals, quantitative faecal examination was done (and it ranged from 32.6 to 45.6 EPG indicating that the infection was recorded by examining forty livers along with their bile ducts collected from slaughtered buffaloes and found 16(40%) liver fluke infection. Identification of liver flukes from the infected flukes revealed that 8 (50%) had mixed infection of fasciola hepatica and fasciola gigantics, 5(31.25%) had fasciola gigentica infection while 3(18.75%) had fasciola hepatica infection.

Key Words: Buffaloes, Fascioliasis, faecal samples, Liver samples. Treatment, Epidemiology and Eggs rater gram of feces.

INTRODUCTION

In Pakistan the climatic factors and poor husbandry practices offer optimal conditions for the growth and multiplication of parasites. Fascioliasis is one of the major parasitic problem in livestock of Lahore district. Fasciola hepatica and Fasciola gigantic cause fascioliasis in buffalo, cattle, sheep and goat**.** Emaciation, loss of hair and wool, retarded growth, low fertility and decreased milk yield are main signs of fascioliasis. The parasites pass through liver parenchymn and damage the tissues, which leads to the cirrhosis in chronic cases. The aim of the present study was to identify the foci of fasciola infection and to help in improving the health and productivity of the buffalos. It will also help in planning strategies by the veterinarians for the control and treatment fascioliasis.

MATERIALS AND METHODS

Faecal samples collected directly from the rectum of each animal examined were subjected to the following examinations:

i) Fresh Smear Examination (Urquhart et. al. I987).
ii) Sedimentation Method (Ganti. 1985).
iii) Egg Counting. Technique.
Egg counting was done by quantitative zinc sulphate flotation, method as described by Davies (l984).
iv) Examination of Liver and Bile Ducts.

The liver samples and bile ducts were incised to examine the presence of parasites. The isolated trematodes were put in the glass bottles containing physiological saline solution.

The liver flukes were fixed and then transferred into 70% alcohol. They were stained by placing in paracarmine (stain) for one day and after washing in 70 % alcohol were placed in acid alcohol and then dehydrated in a series of alcohol as follows: 3 changes of 70% alcohol remaining 15 minutes in each. 95% alcohol for one hour and 3 changes of absolute alcohol for 15 minutes each. After clearing within xylol they were mounted in canada balsam.

RESULTS AND DISCUSSION

Veterinary Hospitals record revealed the prevalence of fascioliasis in buffaloes. It was found that 229 out of 2184 (10.48%) buffaloes were treated for fascioliasis during a period of four months. The results are justified with the findings of Buriro et al., 1984, Masud and Majid, 1984 and Tembely et al. 1988. Overall incidence of fascioliasis was 37.5% as 75 animals out of 200 were found infected. The prevalence from April 1995 to July 1995 was found to he 26% 36% 42% and 46% respectively. The results are justified with the reference of Griffith et al. 1986 who examined 1711 bovine faecal samples from 113 fanns in dairy areas of Colombia for the presence of trematodal eggs and found the Fasciola hepatica eggs in the faeces from 60 % of the farms. Average EPG (Eggs per gram) of faeces per month per animal were found to be 34.7, 32.6, 40.5 and 45.6 respectively. These findings are in accordance with the findings of Gonzalez (1989) who recovered the Fasciola hepatica eggs in 10% of the total 1301 cattle at monthly intervals. The average 51.6 $\pm$ 4.5 EPG in the faeces throughout the year. The results of this study are in line with those of Razali el al. (1983) who reported a higher prevalence of fasciola infection in adult cattle than young calves. Forty livers along with their bile ducts of the buffaloes revealed the prevalence of 40% hepatic flukes.

Table Showing Fascioliasis in Treated Buffaloes in four Veterinary Hospitals of District Lahore

Name of Hospital	Total Buffaloes Examined	Cases Treated for Fascioliasis			Fasciola Infection %age
		Adult*	Young**	Total	
R.A..Bazar	580	18	19	37	6,38
Shamkey Bhattian	460	29	14	43	9,35
Rakh Candra	534	37	19	56	10,49
Herbanspura	610	90	3	93	15,25
Total	2184	174	55	229	10,48

Adult = 2 years and above * Young = 2 years.

CONCLUSION

Fascioliasis is one of the major parasitic problems in buffalo irrespective of their sex and age. It was observed that annually, there is a great economical loss due to the infection of liver fluke in buffalo in Lahore district. It needs to adopt appropriate measures for the treatment and control of this infection to avoid the losses.

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Comparative efficacy of cloprostenol, estradiol and gentamycine for the treatment of endometritis in buffaloes.

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Summary

This study was designed to compare the efficacy of cloprostenol, estradiol and gentamycine for the treatment of endometritis in buffaloes. For this purpose eighty buffaloes of endometritis 20 in each group were subjected to different treatments while their visit to the college hospital for treatment purposes. The recovery rate and conception rate in cloprostenol treated group was 65 % and 58.33 % respectively. Similarly in estradiol treated animals the recovery rate was 75% and conception rate was 64.28%. In a group of animals treated with gentamycine, the recovery and conception rate was 45% and 42.85% respectively. In control group D where no treatment was given, only 35.71% animals were conceived after insemination. It is concluded that estradiol and cloprostenol results were significantly better as compared to treated with gentamycine and Control group. The cost of treatment with estradiol is lesser than cloprostenol and gentamycine.

Key words: Buffoloes, endometritis, cloprostenol, estradiol, gentamycine, conception rate, estrus.

INTRODUCTION

Among several reproductive disorders of buffalo and cow, endometritis is one of the most common problem. Endometritis adversely affects the calving interval and consequently decreases reproductive efficiency of buffaloes. The incidence of Ist degree endometritis has been reported 56.2 percent (Samad et al. 1984) in the buffaloes in and around Faisalabad.

The use of cloprostenol in cattle causes regression of the corpus luteum and reducing plasma progesterone to basal levels thus removing the negative feed back effect and resulting in occurrence of LH surge and the ultimate expression of oestrus. Under the influence of estrogen, cervix dilates allowing the evacuation of the purulent uterine contents (Rowsen et al. 1953) and return to normal in most cases. On the other hand estrogen will promote development of the blood supply to uterine mucosa (Hansel and Asdell, 1951, Hechter et al. 1941). The mechanism of the heneficial effect is thought to be exposure of the genital tract to estrogens which increases the natural defence i.e. uterine phagocytosis. This project has been designed to test the efficacy of cloprostenol, estradiol and gentamycine for the treatment of endometritis in buffaloes.

MATERIALS AND METHODS

A total of eighty buffaloes were examined from six different hospitals in and around Lahore. The criteria of Settergren (1980) was used to identify the endometritis animals and were included in the study, which three types of endometritis i.e. First, Second and Third degree endometritis were described (Morrow, 1980). The following drugs were used for the treatment of different groups. Each group consisted of 20 animals. The eighty buffaloes selected for this study were randomly allotted for each of the following treatments. The animals in all the groups were

examined on the first estrus following the specific treatment and if in heat were inseminated with frozen buffaloe semen.

In Group A, buffaloes were initially examined on the day of oestrus and thereafter 7 days i.e. day eighth of the cycle (early diestrus), rectal palpation was done to ascertain the presence of corpus luteum on one of the ovaries. Prostaglandin F2α (Estrumate 2 ml, ICI) was administered intramuscularly while observing the routine aseptic measures.

In Group B after initial examination at the day of oestrus (estrous = day 1), the animals were given intrauterine infusions of estradiol (diethylstilbestrol). As diethylstilbestrol is an oily preparation every attempt was made to reduce the particle size in the mixture by vigorous stirring.

In Group C animals were given intrauterine infusion of gentamycine.

In group D after the initially examination the buffaloes were inseminated with frozen semen without giving any treatment. And this group served as control for this study.

All animals including in this study were rectally palpated for the presence of pregnancy two months following the day of insemination.

RESULTS AND DISCUSSION

In this study cloprostenol treated animals the recovery rate and conception rate was 65% and 58.33% respectively (Table 1).

Table 1: Comparison between treatment of cloprostenol, estradiol and gentamycine in endometritis in buffaloes

Treatment group & Drug used N=80	No. of recovered animals (%)	No. of Pregnant animals
A. Cloprostenol n=20	13 (65)	7 (58.33%)
B. Estradiol n=20	15 (75)	9 (64.28%)
C. Gentamycine n=20	9 (45)	3 (42. 85 %)
D. Control n=20	5 (35)	5 (35.71%)

The results of present day indicate that recovery and conception rates observed with estradiol were 75% and 64.28%, respectively. Similarly conception rate shown in control groups was 35.71%. The results suggest that the curative and conception rate observed was significantly higher as compared to control. The results of present study indicate that recovery and conception rates observed with gentamycine were 45 % and 42.85% respectively. Similarly conception rate shown in control group was 35.31%. The results suggest that the curative and conception rate in group C were significantly higher compared to control group.

The results of this study are in agreement with other studies conducted in cows (Fazeli et al. 1980). They compared estradiol and cloprostenol for the treatment of endometritis in cows and observed curative rate of 94.9% and 75% with estradiol and cloprostenol respectively. Based on these curative and conception rates. Estradiol was recommended as drug of choice. However, a non-significant difference was observed between both treatments. Estradiol was preferred for endometritis in buffaloes. This comparative study for the efficacy of cloprostenol, estradiol and gentamycine for the treatment of endometritis in buffaloes is conducted for the first thne in Pakistan. The results of present study strongly suggest that the use of estradiol is more effective as compare to cloprostenol and gentamycine for the treatment of endometritis in buffaloes. Therefore, based on the findings of present study the author recommends stilboestrol as a drug of choice for the treatment of endometritis in buffaloes. In general the cost of treatment with

estradiol is lower compared with cloprostenol and gentamycine. On the other hand a certain degree of inconvenience remains with Stilboestrol therapy as intrauterine infusion demands special skill.

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Toxicological investigations of kidneys in spontaneous cases of nephropathy in pigs and necessary hygiene control

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Summary

Toxicological investigations were carried out in kidney samples (a total 96) coming from regularly slaughtered pigs in Bulgaria and exhibiting kidney lesions from the type of "enlarged mottled or pale kidneys" at slaughtering time during the meat inspections in 1993 - 1994. It was established that 83.98% of investigated kidney samples were positive for ochratoxin A (OA). The mean concentration of OA fluctuated from 1.3 ± 1.25 to 6.95 ± 7.10 and 7.17 ± 2.83 ng/g for various batchs of pigs coming from various farms. The highest percentage of macroscopical changes from the type of "enlarged mottled or pale kidneys", as well the highest percentage of OA-contaminated kidneys, were established during the spring-summer period. It is offered a cheep and effective procedure for preventing and decreasing the contamination of the production from animal origin with nephrotoxic mycotoxin OA by changing the feed source for about a week or prolongation of hungry diet before slaughtering.

Key words: ochratoxin A (OA), mycotoxic porcine nephropathy, hygiene control

Introduction

Spontaneous mycotoxic nephropathy is a chronic renal disorder caused by various nephrotoxic mycotoxins (predominantly OA) which are encountered in various suspected pig's feeds stored in poor conditions and exceeded humidity. Because of the scarce clinical signs (polyuria, polydipsia, growth depression) these nephropathies are detected almost exclusively during the slaughtering meat inspection, where the indication of the disease are kidney damages from the type of "enlarged pale or mottled kidneys".

Recently, similar nephropathies were quite usual at the slaughter time and in 1987, 1990, 1993 they reached the largest dissemination (Stoev 1994). This imposes the necessity of investigations with the aim of elucidating the aetiological nature of these nephropathies in pigs, their frequency and duration in certain farms and especially hygienic evaluation of meat, obtained from such pigs.

Materials and Methods

The present studies were carried out during the period 1993 - 1994 and included kidneys from 96 regularly slaughtered pigs exhibiting lesions from the type of "enlarged pale or mottled kidneys" identified during the meat inspection. The pigs originated from large-scale pig farms, as well from smaller fattering farms from different regions of the country. Kidney samples (50 g from each separated case) were frozen at -20° C until toxicological examination, performed by HPLC technique. The samples represented about 20% of the changed kidneys from each affected pig farm.

Results and Discussion

The frequency and percentage of the observed nephropathies in different batchs of slaughtered pigs varied significantly - from 1-2 % up to 50-60%. The duration of these nephropathies in various farms was different too and ranged from 1-2 up to 5-6 months, but sometimes the nephropathies were observed during the all year. Always, those nephropathies were widely spread during the spring-summer period.

Surveys for residues of OA in kidneys from various spontaneous cases of porcine nephropathy in Bulgaria during certain periods in 1993 and 1994 revealed that 83.98% of all investigated kidney samples were positive for OA. The average contamination levels varied from 1.32 ± 1.25 to 7.17 ± 2.83 for different farms (Table 1).

The percentage of contaminated kidney samples was very high compared to that in other countries with similar problems - 35% frequency of residues of OA in such kidneys in Denmark (Krogh 1977) and 25% in similar cases in Sweden (Rutqvist 1977). On the other hand the established levels of OA in kidneys in most of the cases were low and didn't exceed the maximum tolerance for OA in kidney tissues of pigs slaughtered for meat from 10 ng/g established in Denmark (Elling, 1983).

Table 1. Residues of ochratoxin A (OA) in kidneys from various farms with mycotoxic nephropathy

origin of samples	year	percentage of positive	mean values of OTA (ng/g)	number of samples
mean	**1993**	**64.29%**	**1.32 ± 1.25***	**14**
Nojarevo	**1994**	**100%**	**7.17 ± 2.83***	**6**
Sitovo	**1994**	**80%**	**3.42 ± 4.50***	**10**
N. Cerna	**1994**	**85.7%**	**6.95 ± 7.10***	**14**
Cernogor	**1994**	**90.9%**	**5.23 ± 4.80***	**22**
Dulovo	**1994**	**63.3%**	**1.50 ± 2.39***	**30**

*** - SEM (standard error of the mean)**

Although the examined

kidneys in our cases were condemned at the slaughterhouses because of the observed damages, the corresponding carcasses passed the meat inspection and entered commercial channels. Since the OA is relatively heat stable, ordinary cooking does not eliminate the substance. Therefore, a limited occurence of OA might anticipated in prepared food.

Some previous investigations revealed that it is need at least 1-2 months for developing characteristic renal lesions in pigs exposed to natural or experimental OA contaminated barley. On the other hand the renal lesions induced at an early age did not disappear when the pigs were fed on OA-free diet (Elling 1983). This suggests that it is not very convinient to investigate all "enlarged pale or mottled kidneys" for OA at the slaughterhouses, as it is made in Denmark, because in spite of the toxicological investigations of the such kidneys OA-contaminated pork may enter the food chain and thus represents a potential public health hazard.

Because of the assumption that mycotoxins and especially OA are involved in aetiology of Balkan endemic nephropathy (Krogh 1972; Stoev and Petkova-Bocharova 1994) it is need to prevent the exposure of humans to this very hazardous toxin from pork. The better procedure for that would be a toxicological analysis of a few blood samples from the suspected of mycotoxic porcine nephropathy farms 1-2 weeks before slaughter time and change the feed source if it is necessary. On the other hand it may prolong the hungry diet before slaughtering up to 24-30 hours. Because of the short half-life of OA in pig's serum (72 - 120 hours) (NNT 1991) its concentration in blood quickly decreases after changing OA-contaminated feed by OA-free diet. In this way the loss of condemnation of swine production would be prevented and it would be realized the better procedure for preventing the exposure of humans to OA from pork.

Conclusions

The high percentage of contaminated kidney samples compared to that in other countries with mycotoxic porcine nephroparhy suggests that OA probably is the main aetiological agent of the observed nephropathy.

The low levels of residues of OA in kidneys samples suggest that it is very possible to realize some synergistic effects between OA and some other toxins, which enhance its toxicity or various other interference, which needs to be proved.

Recommendation

Because of the high percentage of contaminated kidney samples and comparatively low contamination levels of OA in the same kidneys, the better procedures for preventing the exposure of humans to OA from pork are proposed:

1) to analyse toxicologically a few blood samples from the suspected of mycotoxic porcine nephropathy farms 1-2 weeks before slaughter and change the feed source if need.
2) to prolong the hungry diet before slaughtering to 24-30 hours in the case when the pigs are transported to slaughterhouses, if the changes from the type of "mottled or pale kidneys" at slaughter time are evident.

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Influence of ingreased doses of heavy metals in rations on the reproductive abilities of rams

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Summary

Experiments were carried out in an artificial ecosystem of the "mesocosm" type whit three groups consisting of 8 rams Zigai breed (one control and two experimental) fed on standard fodder mixture for 90 days and containing lead and cadmium 50 and 100 fold above the Highest Permissible Values (HPV).

The above results indicated that independently of the probable compensatory ability of rams to eliminate the unfavorable effect of heavy metals on the biological properties of their semen liquid, after a centrain threshold this ability descreased which was the reason for the established differences.

Key words: lead, cadmium, rams, mesocosm, semen liquid

Introduction

The influence of lead and cadmium on plants, animals and humans is a topic of numerous studies. As a basis of the present analysis the following publications of the World Health Organization were used: Health hazards of the human environment /1989/, Lead-Environmental Aspects /1989/, Cadmium /1992/.

In the review WHO Cadmium /1992/ is included information on the toxic and metatoxic influence on the liver, kidneys and other parenchym organs as well as calcium metabolism and chaemopoesis of test animals. The unfavorable influence of cadmium on the sexual system of test animals.

When Pb quantity in the rations of male animals was increased bioaccumulation in the kidneys, the liver and the testises was observed. According to IARC /1989/ Pb was accumulated in the seminal vesicles and the prostate gland test male animals /mice/. The proven by this author reduced fertility of the population was due to the disturbed fertility of sperm caused by changes in their morphophunctional condition.

These and other publications do not provide a final answer to a very important question concerning the exploitation of anthropocenoses: the influence of subtoxic concentrations on the reproduction capacity of farming populations.

Material and methods

Three groups of 8 rams Zigai breed /one control group and two test groups/ fed for 90 days on standard fodder rations and rations containing lead and cadmium 50 and 100 fold the Highest Permissible Concentrations /HPCs/ for both metals, respectively.

The lead and cadmium content in the studied samples /fodder and semen/ was determined using AAS"Varian-40 Zeeman" according to the standard methods of Jorchem (1993) on 1,45 and 90 day from the beginning of the experiment.

The morphological studies of the spermatogenous epithelium were performed according to the Bulgarian State Standard (BSS) 15378-81. The unconditioned reflexes of the animals after treatment with heavy metals were studied according the method of Bratanov, modified by Nestorova et al. (1991).

Results and discussion

The results from the study of the microclimatic factors (temperature, humidity, air velocity, gas composition) indicated that during the experimental period the studied abiotic factors in the ecotecnical system for ram breeding were within the limits of the technological requirements.

The results concerning lead and cadmium concentrations in the semen liquid are presented in Table 1.

Table 1. Average values of Pb and Cd in the semen liquid of rams (mg/kg)

Groups	**n**	**Cadmium**	**Lead**
		beginning	
Control	8	0.277 ±0.025	0.776 ±0.052
I group	8	0.256 ±0.020	0.639 ±0.045
II group	8	0.263 ±0.015	0.785 ±0.042
		45 days	
Control	8	0.240 ±0.016	0.850 ±0.060
Igroup	8	0.293 ±0.022	0.930 ±0.065
II group	8	0.310 ±0.025	1.035 ±0.070
		90 days	
Control	8	0.249 ±0.015	0.765 ±0.030
I group	8	0.179 ±0.012	0.822 ±0.032
II group	8	0.187 ±0.014	1.034 ±0.070

It could be seen from the Table that on the 45 day the quantity of these elements was increased and reached highest values in the second group where concentrations were 100 fold the HPC. On the 90 day their quantity decreased as a result of metalothionein synthesis in the organism, this tendency being more prominent with cadmium. Independently of the lower values of the studied chemical elements some changes in the sexual activity of rams were observed. Till the 45 day no changes in the sexual activity was registered in the test groups though semen liquids contained higher concentrations of lead and cadmium. After the 90 day a decrease in the sexual activity of rams in the experimental groups was observed. This difference was most pronounced in ram from the II group compared to I group and the control. No other significant differences were observed among the groups.

The results concerning the biological parameters of the semen liquid of the brood animals were of special interest. The results indicated that till the 45 day from the beginning of the experiment no differences were observed in all studied parameters - volume of ejaculate, spermatozoid concentration with straight forward movement (SSFM), thermoresistence and changes in spermatozoid configuration (pathologic spermatosoids). After this period certain changes were observed in some of the above parameters which were more pronounced in the II

experimental group. On the 90 day we established a decrease of SSFM with 2.3% in the second group compared to I group and 4.1% compared to the control. Parallel with this we established certain changes in the configuration of spermatozoids and acrosome, respectively displayed in swelling and rapture of ejaculates from the second test group compared to the control and the first test group with 5.3% and 3.2%, respectively. For the rest of the studied indices we did not discover any significant differences between the control and test groups.

Conclusions

The results obtained concerning the studies of the biological qualities of the semen liquids give us grounds to suppose that independently of the probable compensatory ability of rams to eliminate the unfavorable effect of higher concentrations of heavy metals in the rations (metalothionein synthesis) there exists a certain threshold after which this capacity is reduced which in fact is the reason for the established differences.

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Application of Hemax in feeding of growing polar foxes

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Summary

The studies were carried out on 60 polar foxes of Norwegian born at the beginning of June. The animals were divided into 2 groups: control and experimental. The only factor differentiating the foxes division was the kind of iron supplement being given to them. To the experimental groups fodder HEMAX", containing iron glutaminian, was added. Every two weeks the mass of the body of all the foxes was measured. By the end of the experiment they marked, the number of red cells, Hb, Htk, iron total, TIBC and degree of transferring saturation, largeness and structure of the body, and on this ground they qualified skins into different sizes.

Key words: foxes, iron diet, haematological coefficients, skin

The iron deficit in fur animals can be brought about, among others, by too small concentration of this component in food, too big impact of certain kinds of fodder (fish fluor, powder milk, powder whey), inflammation of the alimentary canal, as a result of a high microbiological denaturation of the fodder, and others (Työppönen at all 1987). The iron deficiency in foxes and minks causes anaemia, what brands at reduced size of the blood corpuscles and reduced haemoglobin contents (Brandt, Hejborn 1987). The lack of iron influences as well poor appetite, slow height rate, smaller number of litter, high mortality of whelps, discoloration and poor quality of fur with worse structure and hair density (Hansen at all 1987). The fundamental aim of the studies was to define the influence of HEMAX supplement in food upon salubriousness and production indices at foxes.

Materials and Methods

Studies was carried out on a private farm near Bydgoszcz in the period betwen August to October 1996. They used 60 both sexes polar foxes of Norwegian origin, born at the beginning of June. The animals were divided into 2 groups: control and experimental, each consisting of 30 individuals. During the experiment, all the foxes were given the same kind of food. The only factor differentiating the foxes division, was the kind of iron supplement being given to them. The control group was receiving food with iron supplement, which we can conventionally determine as the ,,X". To the experimental group's fodder ,,HEMAX", containing iron glutaminian was added.(4)

Moreover, every two weeks the mass of the body of all the foxes was measured. By the end of the experiment blood samples from 10 individuals of each group were taken in which they marked the number of red cells, Hb, Htk, iron total, TIBC and degree of transferring saturation. Before the slaughter they looked at the foxes in the aspects of and structure of the body.

After the slaughter they measured the length of all the dried skins and on this ground they qualified them into particular sizes: ,,000", ,,00", ,,0" and ,,1".

Results

During the experiments all the animals were in a very good condition and ate all their food rations. The level of iron in the fodder was 293 mg/kg in the control group, yet in the experimental, ,,Hemax" supplement amounted to 278,7 mg/kg . The table 1 shows the results and dynamics of recorded changes of the body mass of the animals . In the first stage of the experiment they noted higher body mass at the foxes from the control group. In the 24th and 26 th

week they confirmed slightly higher body mass at the foxes fed with fodder containing 0,1 % of,,HEMAX".

Table I. Body mass haematological indices of blood and survival cast estimation of Norwegian polar foxes fed with iron supplement ,,X" and HEMAX".

	SPECIFICATION	GROUP	
		CONTROL	EXPERIMENTAL
Body mass (g)	The age of the foxes (in g):		
	- 10 week	2773,3	2508,3
	- 18 week	6325,0	6143,3
	- 22 week	7610,0	7606,7
	- 26 week	7303,3	7820,0
Haematological indices of food	Number of red cells (TIL)	10,65	10,82
	Haemoglobin (g/1)	181,91	183,62
	Hematocrit (1/1)	0,49	0,50
	Total iron (μmol/1)	39,95	42,54
	TIBC (μmol/1)	148,73	178,68
	Degree	33,03	24,51
Survival cast estimations points)	Largeness and structure of the body	4,13	3,40
	Hue type	3,00	3,00
	Hue	5,80	5,93
	Hair covering density	4,67	5,67*
	Structure covering density	5,23	5,67
	General appearance	2,30	2,77
	Sum of points	25,13	26,44

As a result of the blood examinations, carried out before the slaughter they didn't state any significant differences between the control and experimental group in respect the level of chosen haematologic indices (tab. 1). Survival cast estimation showed slightly bigger sizes of the foxes from the control group comparing with the animals from the experimental one. The estimated largeness didn't have any influence upon the length ofthe obtained skins. In the control group, which was fed with the iron preparation ,,X" they didot notice longer skins.

The amount of skins was described like this: ,,00" - 15 articles, ,,0" - 14 artices and ,,1" - 1 article. In the experimental foxes, which were given fodder with ,,HEMAX" supplement in the whole period of the experiments, they gained 2 pieces of skin in sizes ,,000", 22 pieces of skin in sizes ,,00" and 6 pieces of skin in sizes ,,0". In particular, the hair covering density in the experimental foxes was statistically higher than the density in the control group.

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The influence of ferric chelate formulations on the growth and development of piglets.

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Summary

Experiments were carried out on 20 pigs, race wbp, divided into two groups. In the control group they applied 3 times the injection of iron dextren, yet the piglets from the experimental group, since birth until breeding, were given to drink 3% solution of iron chelate called ,,FeMax". Better health effects and gained rate of body mass were observed in the experimental piglets. Better homeostasy of the organisms confirmed as well studies of Htk, Hb, number of red and white cells, total iron, TIBC, UIBC and degree of transfering saturation.

Key words: breeding piglets, iron, hematological and biochemical coefficients.

Anaemia at piglets in the initial period of life has a physicological character, since the piglets are born with only little amount of iron (about 30 mg/kg), which decreases quickly and must be then supplemented (Malinowska 1987). The protection against losses connected with anaemia is a profilactic application in the first days of life of iron preparations that can be used in the injection or oral form (Tramstad, Egeli 1985). The aim of the work was the comparsion of the effects of breeding and the haematological and biochemical indices of blood in dependence on the kind of applied iron preparation.

Material and methodics

The experiments were carried out in The Experimental Department of Zootechnic Institute in Melno on 20 pig litters, race wbp, which were divided into two groups: control and experimental. In the control group they applied 3 times the injection of iron dextrane, yet the piglets from the experimental group, since birth until breeding, were given to drink 3% a solution of iron chelate called ,,Fe-Max". The effects of breeding were evaluated on the basis of morbidity, mortality and the rate of body mass gain. The blood for haematological and biochemical studies was taken out from the ear vain in the 3rd, 14 th, 28 th and 45 th day of life of the piglets and marked: Htk, Hb, number of red and white cells, level of iron, TIBC, UIBC and degree of transfering saturation. The results were statistically analysed.

Results

From the table 1, it is visible that the applied preparation, containing aminoacidal solution of iron chelate (,,Fe-Max"), profitably influenced the breeding effects in the comparsion to injectional form of iron chelate. The morbidity in the first case amounted to 9 pigs (7,83%) and nortality - to 2 pigs (1,7%), yet in the second case - 26 pigs (23,0%) and 7 pigs (6,2%). The average body mass gain until weaning amounted, after ,,Fe-Max" application, amounted to 225 g/pig/d, yet after Fe- dextran application - to 174 g/pig/d.

The **table 2** shows the results of haematological and biochemical blood studies at piglets in the period from birth until breeding. What has been seen is that both haematological indices and iron economy has been presented much more profitably in the experimental group. Despite significant changes occuring in a young organism, analysed blood parametres at piglets being

given to drink 3% solution of iron chelate, showed a physiological stability. According to Wisiński at all 1995, very intensive development of sucklings in the first days of life is connected with the neccesity of producing about 20 ml of blood per 24 hours, what means the gain of over 100 mld of erytrocits, reguiring a supply of 10-15 mg of iron. Apart of the iron in ,,Fe-Max", which in a chelate form makes an ideal supplementation of this microelement in the first and most important part of life in piglets (Grela, Kilijanek 1994), the other important component is HCL, causing the decrease of the stomach acidity. HCL, in an organism, is secreted in a free form, although until the 60th day of life in piglets its secretion is limited. A substantial component of this preparation is also glucose, which is a source of easy accessible energy, what has an influence, in the first period of life, on iron economy.

Having analysed seperate blood indices at piglets, they shown a higher value of blood indices at piglets drinking 3% of,,Fe-Max" solution, in comparsion to the animals which were given the injection of dextran iron.

Tab. 1. Breeding indices of piglets in experiment period.

SPECIFICATION	GROUP	
	CONTROL	EXPERI MENTAL
Number of litters	10	10
Amount of litters (items)	8-15	8-16
Average number of piglets in a litter (items)	11,3	11,5
General number of piglets (items)	113	115
Weight of litters after weaning (kg)	10,82	10,61
Number of piglets after weaning (items)	106	113
Number of piglets in a litter after weaning (items)	10,6	11,3
Weight of 1 piglet after weaning (35th day)	7,75	8,56
Average body mass gain after weaning (g)	174	225
Weight of 1 piglet 2 weeks after weaning (kg)	11,39	12,94
Mortality of piglets (items),(%)	7 (6,2%)	2 (1,7%)
Morbidity of piglets (items),(%)	26 (23,0%)	9 (7,83%)

Tab. 2. Average results of blood haemotological studies in the breeding period.

	GROUP	BREEDING DAYS			
		3	14	28	45
Hematokryt 1/l	C	0,258	0,308	0,341	0,328
	E	0,259	0,299	0,347	0,353
Hemoglobin μmol/l	C	6,49	6,23	7,89	7,31
	E	6,59	7,68	9,38	9,02
Number of red cells x 10^{12} /l	C	3,65	4,14	4,59	5,31
	E	3,83	4,45	5,55	7,17
Number of white cells x 10^{9} /l	C	6,69	7,74	9,19	8,33
	E	5,56	6,22	6,60	6,27
Iron, μmol/l	C	16,57	61,87	43,86	29,57
	E	16,41	62,77	45,22	35,54
TIBC, μmol/l	C	103,0	98,3	101,1	114,7
	E	103,0	99,5	107,4	124,1
UIBC, μmol/l	C	88,7	36,4	57,2	85,2
	E	86,6	36,7	62,2	88,6
Degree of transferin saturation, in %	C	16,11	63,02	43,41	25,81
	E	15,95	63,20	42,17	28,67

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HEAVY METALS CONTAMINATION OF ROE DEER IN SOME REGIONS OF POLAND

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Summary

The experiment was carried out in north-east (agricultural) and south-west (industrial) part of Poland. The research material comprised samples of stomach content, liver, kidneys and muscles of 45 roe deers. It was found that the concentration of cadmium in internal organs of roe deer origin from agricultural region is bigger than in industrial region. The concentration of lead in muscles and mercury in kidneys of roe deer origin from industrial region is higher than in agricultural region. It was also found many corelations between concentration of heavy metals in stomach content, internal organs and roe deer muscles. On the ground of our research we determined that in roe deer organism cadmium is cumulated on highest level.

Key Words: lead, mercury, cadmium, roe deer, cumulation, elimination

Introduction

The concentration of heavy metals in organisms of game depends on the actual level of environmental pollution and is a result of intensivity of paralell cumulation and elimination reactions.

There are some regions in Poland which are relatively not polluted, where influences of industrial polutions are small. That region is north-east part of Poland. In opposite south-west region of Poland is on intensive influence of industrial emissions origin from energetics, foundry etc. The source of this pollutions is our home-industry, but some of it comes from transborder emissions from Germany, and Czech Republik.

In our research we tried to find how big is concentration of lead, mercury and cadmium in feed, internal organs and muscles of roe deer origin from both regions of Poland: north-east and south-west.

Material and methods

The experiment was carried out in north-east (agricultural region) and south-west

(industrial region) of Poland in seven hunting areas. Experimental areas had individual different levels of environmental pollution. The investigation material comprised samples of stomach content, liver, kidneys and muscles of 45 roe deer hunted in years 1992-1993. The age of roe deer war determine by tith - dental model, and exchange from milk-teeth to solid teeth, and attrition processes (Lochman 1987).

Among researching roe deer three groups of age was specified:
1) young before first year (8 animals) 2) middle - between second and fourth year of age (11 animals) 3) older - after fifth year (26 animals).

The estimation of concentration of cadmium and lead was done, after dry mineralisation with method of atomic spectrometry, using Atomic Absorption Spectrometer Spectr AA-Varian. The estimation of mercury concentration was provide after mineralisation in acid in closed circulation using LDC Milton Roy-Mercury Monitor. The concentration of examinated heavy metals was shown in mg per kg of fresh weight of samples. Results were rated statistically using multiway analysis of variance and corelation method.

Results and discussion

The results of our research shows that the concentration of cadmium in liver and kidneys of roe deer origin from agricultural region are higher (respectively 0,206 mg/kg fresh weight, and 1,113 mg/kg fresh weight), than it was noted in thats internal organs origin from industrial region (respectively 0,151 mg/kg fresh weight, and 0,825 mg/kg fresh weight) though any significant statistical differences was found. We suspect that this situation is in majority the consequence of soil acidity (Kabata-Pendias, Pendias 1993) lower than 7 in north-east region of Poland what is reason of higher supply of cadmiun in that region. The concentration of lead in liver and muscles of roe deer is higher in industrial region (0,610 mg/kg fresh weight, 0,151 mg/kg fresh weight) than in agricultural region (respectively 0,298 mg/kg fresh weight, and 0,108 mg/kg fresh weight). The highest concentration of mercury was found only in case of kidneys (0,021 mg/kg fresh weight) from roe deer sarn origin from industrial region compare to agricultural region of Poland (0,016 mg/kg fresh weight).

Only the cadmium concentration in roe deer kidneys origin from agricultural region and lead concentration from roe deer origin from both regions are insignificant higher levels than hygienic norms customary in EU.

The mercury contamination of internal organs of roe deer are on a very low level, but in both regions was found significant statistical differences between the level of concentration of mercury in liver, kidneys and muscles. The lowest concentration of mercury was found in

muscles (agr. 0,007 mg/kg, ind. 0,006 mg/kg) and the highest in kidneys of roe deer (agr. 0,016 mg/kg, ind. 0,021 mg/kg).

Statistical analysis showed that the cadmium concentration in the stomach content have positive corelations with level this element in kidneys, what confirm huge activity in this organ. Besides the cadmium level in stomach content have an significant inverted proportion influence on concentration of lead and mercury in stomach content. By the way the level of cadmium contamination have significant positive corelations with level this element in liver. In case of lead analysis, significant positive corelations was found between level of this element in liver and the significant inverted proportion influence between mercury concentration in stomach content and concentration of lead in this organ. Also was found significant positive corelations between mercury level in stomach content and concentration of this element in roe deer kidneys.

Fig 1-3 shows percentage contamination of liver, kidneys and muscles by heavy metals. The level of stomach content contamination by heavy metals was taken to this analysis as 100%. In roe deer liver, kidneys and muscles the cumulation of cadmium is on the highest level. In the lowest percentage lead is cumulated in internal organs and muscles of roe deer. The cumulation of mercury is on a highest level in kidneys of roe deer.

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Fig.1 The percentage concentration of cadmium in liver, kidneys and muscles compare to it's concentration in stomach content (as 100 %)

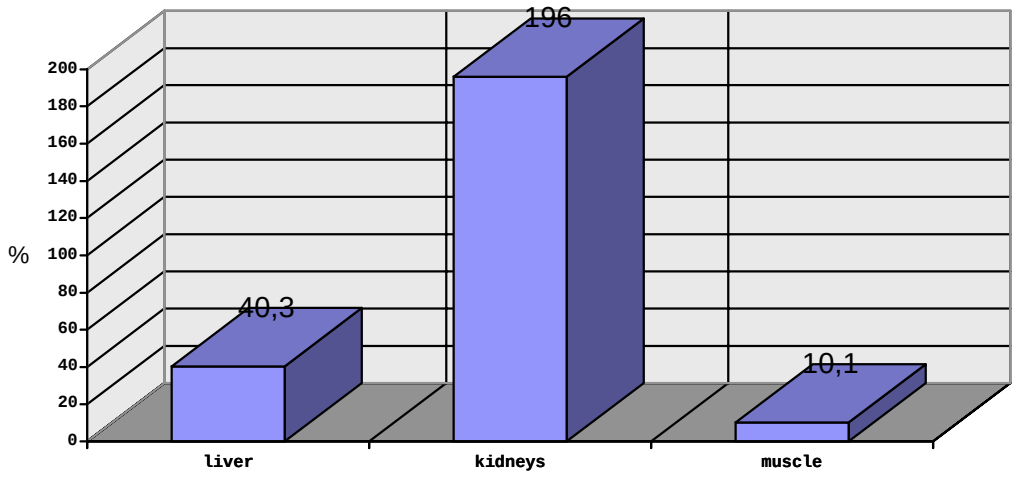


Fig.2 The percentage concentration of lead in liver, kidneys and muscles compare to it's concentration in stomach content (as 100 %)

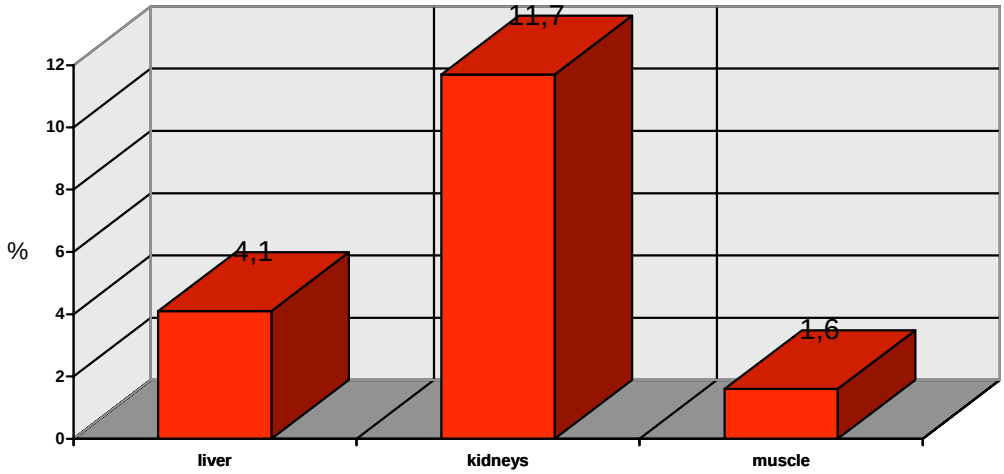
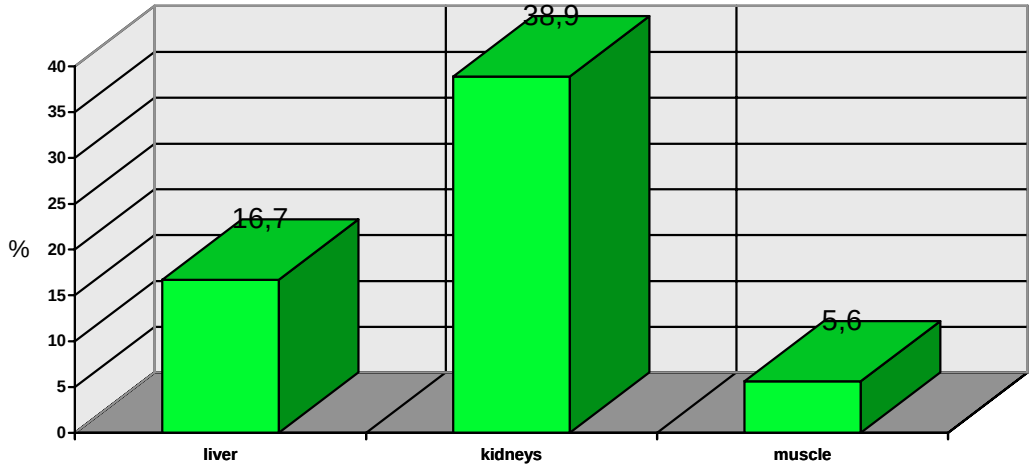


Fig.3 The percentage concentration of mercury in liver, kidneys and muscles compare to it's concentration in stomach content (as 100 %)



Estimation correlation of protein, total leukocyte count and differential leukocyte count in blood and milk of sub-clinically mastitic buffaloes

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Summary

Estimated average of total protein, total leukocytic count, neutrophils, lymphocytes, eosinophils and basophils in blood were 6.84 gm/100 ml, 4036 cells/ml, 48.17%, 48.32%, 2.34%, 0.69% and 0.48 respectively.

Estimated average of total whey protein, total leukocytic count, neutrophils, lymphocytes, monocytes, eosinophils and basophils in milk were 1.02 gm/100 ml, 4665000 cells/ml, 70.42%, 24.19%, 3.45%, 1.30% and 0.37% respectively.

Key words: Total protein, TLC, Lymphocytes, Eosinophils, Basophils, Blood, Milk.

Introduction

The diagnosis of clinically apparent mastitis is quite easy land may not need any laboratory assistance except when isolation of the causative organism and demonstration of its sensitivity against antibiotics is required. The diagnosis of subclinical mastitis is of tremendous importance if the disease is to be controlled in a herd.

A study was conducted to estimate the prevalence of subclinical mastitis in buffaloes brought to out door hospital, C.V.S., Lahore. Milk and blood of the positive animals was examined for Estimation and Correlation of Protein, Total Leukocyte Count (TLC) and Differential Leukocyte Count (DLC) in Blood and Milk of Subclinically Mastitic Buffaloes.

Identification of Mastitic Animals

A total of one hundred sub-clinically mastitic animals out of 450 were detected by using the Whiteside Test (Hussein et al., 1984).

Haematological Studies

1. Total Leukocyte count (Thousand per mm^3) (Benjamin, 1967).
2. Differential Leukocyte count (Percent of total leukocyte count) (Schalm, 1965).
3. Total Blood Protein (Biuret Method) (Coles, 1980).

MILK STUDIES

Total Leukocyte Count

A Reichert' binocular microscope equipped with a mechanical stage and fitted with a 100 times magnification oil immersion lens and a 10 times magnification ocular pieces, was used for cell counting. With this optical combination each field cover an area of $2x10^{-6}$ cm^2 (Beck et al., 1996).

Differential Leukocyte Count

The slides used for total leukocyte count also were used for differential counting of leukocytes. A total of 300 cells per slide were counted using a hand counter (Marbel Blood Counter Company, III. U.S.A.)

Determination of Protein Contents in Milk Whey

Total proteins in milk whey were determined by the Biuret Reaction described by Cornall et al. (1949).

Results and Discussion

Total blood protein was estimated during the study at an average of 6.84±0.07 gm/l00ml which is in accordance with the work of Knight (1983). Milk whey protein analysis revealed its average of 1.02 ± 0.01 gm/100ml which shows an increase of total milk whey protein. This work is in accordance with the work done by Casado et al. (1988) and Carroll (1963).

Blood total leukocyte count in mastitic positive samples was estimated 4036 cells/mm^3 which shows decrease in the total blood leukocyte count which shows decrease in the total blood leukocyte count which was also reported by another scientist worker Knight (1983).

Total leukocyte count in milk was estimated at an average of 4665000 cell/ml. This result shows a marked increase of total leukocyte count in milk which has also been reported by Jafri (1981).

Differential leukocyte count in blood gave a percentage of 48.17, 48.32, 2.34, 0.69 and 0.48 for neutrophils, lymphocytes, monocytes, eosinophils and basophils respectively, which shows an increase of neutrophils and decrease of lymphocytes and monocytes. Whereas no significant difference was seen for eosinophils and basophils. this study is also in accordance with the study of Knight (1983) and Schalm (1977).

Differential leukocyte count of milk gave an average of neutrophils, lymphocytes, monocytes, eosinophils and basophils as 70.42%, 24.19%, 3.45%, 1.30%, and 0.37% respectively, which shows an increase of neutrophils and decrease of lymphocytes. This study is also in accordance with the work of Jafri (1981) and Saad and Ostensson (1990).

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THE ECOLOGICAL WAY OF STRESS PREVENTION, GROWTH & DEVELOPMENT STIMULATION OF BIRDS

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Summary

Rational schemes and doses of usage of succinate at different stages of bird ontogeny have been worked out. It should be used in solutions, aerosol, or given with food or water for increasing hatch ability and productive qualities of birds. The treatment of hatching eggs with water solution of succinate had positive influence on embryo genesis and viability of chicks. Hatch ability raises by 4-5 percent. Usage of succinicacid for breeding of chicks and layers reduce mortality by 1,5 -2,5 times, feed consumption per 1 kg of the body weight by 6 -8% and increases laying by 3-5%. This substance stimulates hemopoiesis and aminotransferase and succinatdihydrogenase activity. Research results have been tested on 700 thousand layers and meat breed hens, with 8-fold of economic effect.

Key words: succinate, embryos, stress, aminotransferase, succinatdihydrogenase.

Introduction

It is necessary to work out ecologically safe preparations for all stages of agricultural production, for example succinic acid. It is known that it has adaptogenic action and demonstrates antihypoxic, antioxidative effect, normalizes energy metabolism, general physiological state of an organism, intensifies biosynthesis under pathological and extreme conditions.

Succinate participation in corticosteroid biosynthesis makes it universal antistress preparation and that is why, succinate is widely used in medicine and plant growing. /1,2,3,4,5,6/ This is due to the contradictions in literature data, on the effectively of the application of different regimes of the injection of succinate. Up till now the long-term use of succinate on embryos, chicks and layers of the parent stock at different stages of the technological cycle has been sufficiently studied. The possibility of the use of succinate solutions for aerosol treatment of embryos and chicks is not studied at all.

Proceeding from the above-mentioned the aim of the experiment is the working out of effective methods of the application of succinate for increasing the resistance and the productivity of the parent stock of hens at different stages of embryonic and postembryonic development.

Material and methods

The experiments were conducted together with the association "Vnedrenie" at the Poultry factory in the Moscow region on embryos, chicks and hens of the parent stock of the white leghorn breed "BELARUS -9" and "SMENA" cross7 The following 3 tasks were set up:

Number 1. To establish the optimal regime, way and dosing of succinate and study its effect on growth, development and viability of embryos and chicks.

Number 2. To study the effect of different doses of succinate on resistance, productivity, quality of the eggs of the parent stock.

Number 3. To establish the economic effectivity of the application of succinate in poultry.

The complex of zoo veterinary, biochemical and economic indicates was studied. In search for optimal doses and regimes for injection of succinate 6 experimental and one control group of chicks, 300 head in each group were formed, which were given isopprotein and isocaloric rations. 2 feeding regimes with succinate were studied: The first: ten days regularly with 10-day intervals; and the second: within 10 days after hatching, then only 5 days before and after stress, technological and veterinary stresses 3 doses of 10,20.30 mg/kg of birds weight were used.

Results and discussion

For the periods of breeding mortality rate in experimental groups reduced in control by 1,5 to 2,5 times. By experimental data, the minimal death rate was established in groups which were given sucinate under stress conditions in doses of 20 mg/kg. The maximum body weight was established in the group where succinate was used according to the second regime with the dose of 30 mg/kg. Thus, the second feeding regime with the dose of succinate of 20 to 30 mg/kg turned out to be optimal.

The testing of data of the first experiment on large stock /19.000 hens/, according to the death rate within the breeding period never exceeded 2,1% whereas in the control group it was 2,5 times higher /5,2%/. Live weight of the chicks reached 643±11,Og in contrast to 610±9,0 in the control group. The difference is statistically significant. Food conversion lkg of body weight in the experimental house decreased up to 4 kg against 4,8 kg in the control one.

In further experiments studies were made on the effectivity of the application of succinate solutions, used for aerosol treatment of embryos. The investigations showed that in this case the sanitary conditions of air improved the resistance of embryos. The treatment of hatching eggs with water solution of succinate had positive influence on embryo genesis and viability of chocks. Hatch ability raises by 4-5 percent.

In the experiments on hens and cocks of the parent stock succinate was at all stages of the post-embryonic development in stress situations by the second regime. Investigations showed that with the use of succinate the losses of hens and cocks within the period of exploitation were lowered by 1,5-2,2 times. The productivity of hens grew by 3 to 6%. Commercial and incubation qualities of eggs were not lower. Research results have been tested on 700 thousands layers and meat-breeds, with 8-fold of economic effect.

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DO MILK UREA AND PROTEINS REFLECT THE N-SOURCE OF FOOD IN CATTLE?

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Intrduction

Carbaminde or urea (H_2NCONH_2) is one of the end-products of the mammalian metabolism. The nitrogen content of the food carbamide. Carbamide is produced in the carbamide cycle but in case of certain species (e.g. fish) the end-product of the decomposition of purine-type compounds is also carbamide. In plants and micoorganisms carbamide decomposes to carbon-dioxide and ammonium on the effect of urease enzyme.

In nitrogen secretion of animals carbamide is a urine product of protein metabolism. Carbamide is produced from CO_2 and NH_4^+ in a complicated cycle in the presence of ATP the first intermediate is carbamyl-phosphate, that binds to ornithin forming citrallin. This reacts with L-aspartic acid producing argino-succin(ic) acid, from which fumaric acid separates out resulting in arginine.

The arginine decomposes to ornithin and carbamide on the effect of arginase enzyme. The essence of the carbamide cycle is that toxic ammonia turns into non-toxic carbamide.

Energetically carbamide-production is luxurious, which goes on in the organism so that the ammonium does not accumulate in high concentration. The urease, urea-aminohydrolase enzyme catalyzes the irreversible hydrolysis of carbamide into CO_2 and ammonia. The enzyme has absolute substrate specificity and it is essential because it makes it possible to use urea as a source of nitrogen. The urease was the first of enzymes to be chrystallized (Sunner 1926). There has been an increase in cattle-diseases causing a fall in production which can bee derived from metabolic processes after growing appliance of modern breedin and feeding technology especially in stocks with high milkproduction bred on farms with huge numbers of animals.

The rising of milk production, the mistakes in feeding and breeding technologies cause disfunction in animal homeostasy. We can draw conlusions regarding the reasons of the metabolic disfunction from studying the enviroment and by methods of laboratory diagnostics based on specific physiological and feeding features. These data provide us with information on changes in the enviroment (breeding hygiene, feeding).

Materials & Methods

In our experiment the hygienic circumstances of two farms with different numbers of animals were compared (farm A: 10-15 milker cows, farm B: 300 milker cows). Our aive was to study whether milk, the most important product of the cattle farms is suitable for judging the hygienic circumstances of the animal breeding farms. Millk samples were taken 3 times of each cow and their carbamide content was measured by enzymatic colorimetry.

Preparation of milk samples

After taking the samples, they were chilled for some hours (to +4C) mixed with chloroform and shaken for 3 minutes. Then they wre centrifuged for 5 minutes (5000 g) and their carbamide content was measured according to Berthelot's method: the urease decomposes carbamide specifically and the ammonia produced reacts with sodium hypochlorite and phenol in alkaline medium and farms blue indophenol. One (light absorbance) of the product is in proportion with the carbamide content of sample.

Compution

Carbamide (mmol/l= Abs sample/ Abs standard x standard concentration). The milk samples were examined by a Humalyzer 815 automatic photometer. The protein content of the same samples was also measured by Biuret method after a similar prepatation process.

Results

Carbamide-nitrogen determination

The samples taken at farm A continued 4,2 mmol/l carbamide-nitrogen for the first time , then after two weeks the samples taken from the same cows contained almost half of it, 2,3 mmol/l carbamide-nitrogen. The results of the thirol sampling show similar results with first one: 3.9 mmol/l carbamide-nitrogen. The carbamide-nitrogen content of the sample taken to the large scale farm show a gradual increase from about 4.0 mmol/l for the first time though 4.4 mmol/l (second sampling) up to almost double as much as in the first sampling: 7.9 mmol/l.

Determination of protein content of milk

The protein content of the milk in both farms was almost identical 25 g/l, independently of the sampling, time and feeding.

Conclutions

A certain period of lactation was studied in two milker farms with different keeping technology. The aim of the study was to gain data about the effects of environmental changes by methods of on-the-spot environmental testing & laboratory diagnostics determining the carbamide-nitrogen content of the milk. Futher on conclutions about the level of the animal feeding technology could be drawn from the changes of the carbamide-nitrogen content of the milk and the methods were sought to determine the polluting impact of the two different types of animal breedin farms.

According to our results the energy and crude protein supplies influence the carbamide-nitrogen content in the milk. The surplus of crude protein increases the average level of carbamide. In case of balanced or negative crude protein supply the carbamide-nitrogen concentration in milk is 3.0 mmol/l.

We would like to continue our studies alng the same lines explaned by the paralell analysis of the milk samples and blood sers of the milker cows.