ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIX (3) SECTIO EE 2011

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The bacteriological state of mink's kittening houses in perinatal period

Stan bakteriologiczny środowiska domków wykotowych norek
w okresie okołoporodowym

Summary. Reducing the number of young minks falls is a resultant of many factors among which a high standard of hygiene maintenance in the premises plays an important role, as indicated by differences in morbidity and mortality of young minks, depending on the farm. The aim of this study was to determine the microbiological status of the mink houses environment in the perinatal period. Studying the microbiological properties of litter collected in two treatment periods, significant differences in the number of mesophilic bacteria were found. The presence of pathogenic and potentially pathogenic bacilli on the body surface and in the intestines of young minks, from the mothers of both weigh groups were observed. Own research, which showed abundant growth of pure bacterial cultures from both the litter and dead bodies of young minks, may indicate a significant effect of bacterial factors on the health and survival of young minks in the perinatal period of these animals.

Key words: minks, glucose, body condition

INTRODUCTION

Young minks survival from birth to weaning is a very important factor of the mink farms productivity estimation. The diseases in the early stages of rearing are the major cause of economic losses due to falls, the cost of treatment and convalescence weaker development [Schneider and Hunter 1993a, b]. Reducing the number of young minks falls is a resultant of many factors among which a high standard of hygiene maintenance

in the premises plays an important role, as indicated by differences in morbidity and mortality of young minks, depending on the farm. The causes of nurslings falls analysis is difficult, because of both infectious (viral and bacterial) and non-infectious factors overlap. Microorganisms can enter the newborn's body during delivery, from the female reproductive tract, by lactogenic path or contaminated environment [Śmielewska-Łoś and Klimentowski 1996].

The aim of this study was to determine the microbiological status of the minks houses environment in the perinatal period.

MATERIAL AND METHODS

Directly after preparing food and after 20 days of its storage, microbiological examination and the number of peroxide value in mcq/kg of fat were determined. Samples of feed collected twice in February and March at an air temperature of 3 to 5°C and in April/May with average air temperatures 16–18^{o}C. Test sample consisted of 26 sample units. The study was conducted according to ZHW Gdansk procedures.

The study comprised straw litter from the kittening houses of pastel variety mink's cages in the perinatal period. During mating, experimental females were divided into two weight groups of 15 units. The first group included minks, with 1025–1172 g body weight (score 1–2), the second 1200–1410 g (score 4–5). The partition was based on the Body Condition Scoring System (BCS) [Hynes *et al*. 2004].

Collected litter samples also contained biological material from the cages in which the falls of young minks occurred. Total of 15 young minks were subjected to bacteriological analysis, including 10 from mothers of group (score 4–5) and 5 born in the nests of the group (score 1–2) mothers.

Bacteriological analysis of the litter was performed twice, a few days before mink's birth (mid April) and during the numerous young mink's falls (early May). The study involved a quantitative analysis of mesophilic bacteria, as well as the presence of microbial taxa of the *Enterobacteriaceae* family, and were carried out according to Polish Standards [ISO21528-2, PN-Z-19000-1]. The study material was collected in sterile containers, cooled in the refrigerator and transported to the laboratory. Litter cultures were performed on substrate selectively differentiating ENDO-Les, XLD and selectively-insulating BGA, SS.

In order to isolate the pathogenic microorganisms that may cause young mink's falls, bacteriological examination (direct prints method) of body surface, internal organs (heart, lungs, liver, intestine) of dead young minks was performed, according to the [Malicki and Binek 2004] methodology. The test material was cultured on the blood agar and Mc' Conkey's base. In order to bacteria identification, microscopic slides stained with Gram method were made. In case of Gram-positive cocci the catalase and coagulase test was performed. The *Enterobacteriaceae* family adherence was determined using oxidase test. For the species *Enterobacteriaceae* family determination, a special Kligler and Christensen base with urea and peptone water from tryptophan, forming the so-called biochemical chain used to identify bacteria with this family, were exploit.

RESULTS AND DISCUSSION

Food bacteriological examination showed *Salmonella* bacilli contamination. From the 10 food samples tested during the spring, 6 were positive and were respectively: *S. enteritidis* – 1, *S. Dublin* – 2*, S. typhimurium* – 3 samples. In the summer period, the number of positive samples was 8 and constituted of the following serotypes: *S. enteritidis* – 2, *S. dublin* – 2, *S. typhimurium* – 3 and *S. agona* – 1 sample. A large share of poultry in mink's nutrition poses a potential risk of contamination of food by different salmonella serotypes . The epizootic threat against the salmonellosis, associated with long-term extraction of the farms, ensue from carrier-state of these bacilli by carnivorous animals. Kopczewski *et al*. [1988] reported that animals with latent asymptomatic infections with Salmonella are practically non-recognizable carriers and sowers of the bacilli among other animals and humans, which affects the environment contamination through excretions. Latent carrier-state is practically undetectable, even in the sanitary-veterinary examinations, after the slaughter. An additional element which increases the possibility of Salmonella bacilli infection, is a traditional method of poor feed disposal and relatively too low processing temperatures.

Most common pathogen isolated in all feed samples were *E. coli.* In one case, the average large growth of Proteus genus microorganisms and single fungal and mold colonies were found. All isolated *E. coli* bacilli were not eneropathogenic, and researches indicated a satisfactory sanitary condition of feed. Fat oxidation was not accelerated, and the peroxide value remained constant at > 10.

The materials used in the carnivorous furry animals feeding, such as: animal's production side-products and confiscations from slaughterhouses or carrion, often characterized by a low sanitary state, may contain pathogenic or conditionally pathogenic bacteria and viruses, and even invasive forms of protozoa. Often fostered by the constant presence of rodents in the farm environment. In Poland, in the absence of feed kitchens, breeders prepare food by themselves, and only few of them agree ration according to the standards of nutrition, but even then feed is not subjected to microbiological and chemical analysis. At times, an extreme irregularity in the required proportion of energy from nutrients and low exogenous amino acids and certain vitamins concentration. Vitamin deficiencies contribute to a direct reduction in breeding performance, by inhibition of growth, skin quality and reproduction results deterioration, as well as in an indirect way, which is associated with reduction in the reactivity of the body's immune system. Low herd immunity increases the risk of infection, even with conditionally pathogenic microorganisms, and influences their serious and more massive character. Among the different ways of germs penetration, in the carnivorous furry animals alimentary infection dominates, and the feed is the most common source of infection [Śmielewska-Łoś and Klimentowski 1996].

Studying the microbiological properties of litter collected in the two testing periods, significant differences in the number of mesophilic bacteria was found. In kittening houses of (score 1–2) mothers group, it was shaped at 1.5×10^5 during the preceding mink's birth period, while during the ypung mink's falls, number of bacteria increased to 0.9×10^6. In litter collected from the cages of (score 4–5) mothers group, total number of mesophilic bacteria was 2.6×10^6 in the first period and increased to 2.9×10^7 during the young mink's falls.

Table 1. The results of bacteriological tests of the young mink's both weight groups internal organs
Tabela 1. Wyniki badań bakteriologicznych narządów wewnętrznych norcząt z obu grup wagowych

Examined organ Badany narząd	Types of isolated microorganisms Rodzaj wyizolowanych drobnoustrojów						
	E. coli	*Proteus* spp.	*Strept.* spp.	*Enero-bacter* spp.	*Staph.* spp. *CNS*	*Staph. aureus*	*Citro-bacter* spp.
Body surf. Pow. ciała	+ + +	+	+	—	+ + +	+ +	—
Heart Serce	—	—	—	—	—	—	—
Lungs Płuca	—	—	—	—	+	—	—
Liver Wątroba	+ +	—	—	—	—	+	—
Intestine Jelito	+ + +	—	—	+ +	+ + +	+ + +	+

Explanations: *Staphyl.* – *Staphylococcus, Strept.* – *Streptococcus,* +++ – abundant growth in cultures of all tested animals – obfity wzrost w posiewach wszystkich badanych zwierząt, ++ – average, abundant growth in cultures of all tested animals – średnio obfity wzrost w posiewach wszystkich badanych zwierząt, + – single colonies in cultures of all tested animals – pojedyncze kolonie w posiewach wszystkich badanych zwierząt

Table 2. The positive bacteriological results number of feed samples (n = 20)
Tabela 2. Liczba dodatnich wyników bakteriologicznych próbek karmy (n = 20)

Months Miesiąc	Types of isolated microorganisms Rodzaj wyizolowanego drobnoustroju					
	Salmonella enteritidis	*Salmonella dublin*	*Salmonella typhi*	*Salmonella agora*	*E. coli*	*Proteus*
III–IV	1	2	3	-	10	1
V–VI	2	2	3	1	10	-

Table 3. The number of mesophilic bacteria in the mink's kittening houses litter (n = 30)
Tabela 3. Liczba bakterii mezofilnych w ściółce domków wykotowych norek (n = 30)

Months Miesiąc	Weight group Grupy wagowe	
	score (1–2)	score (4–5)
III–IV	1.5×10^5	2.6×10^6
V–VI	0.9×10^6	2.9×10^7

Table 4. Indicators of mink's females breeding of both weight groups (n = 30)
Tabela 4. Wskaźniki rozrodu samic norek obu grup wagowych (n = 30)

Description Wyszczególnienie	Score (1–2)	Score (4–5)
Length of pregnancy (days) Długość ciąży (dni)	49.8	47.2
The average litter size (pcs.) Średnia wielkość miotu (szt.)	7.30	7.00
Alive – Żywe ($\overline{x}$)	7.00	6.50
Dead – Martwe ($\overline{x}$)	0.30A	0.50A
Losses of young's up to 7 days (%) Straty szczeniąt do 7 dnia (%)	1.9	5.5
Young mink's losses 7–14 day (%) Straty szczeniąt 7–14 dzień (%)	0.5	1.2

$\overline{x}$ – average values – wartości średnie
A,B – statistically significant at $p \leq 0.01$ – istotne statystyczne przy $p \leq 0{,}01$

In litter collected from the cages of both females weight groups, before mink's birth, single colonies of bacteria from the *Enterobacteriace* family was isolated, among which the dominant species was *Escherichia coli, Klebsiella* spp*., Entrobacter* spp*.* In addition, in litter from two cage's vestibules, belonging to a (score 4–5) mothers group, the *Salmonella* spp*.* was found, whose presence can be attributed to the sanitary condition of food.

During mink's birth, in litter from the cages, rapid growth of bacteria from the *Enterobacteriacae* family was found, and in all collected samples of litter *Escherichia coli* was isolated. In addition, most samples reported the presence of *Klebsiella* spp*.* and *Citrobacter* spp*. Salmonella* spp*.* was found in the litter from four cages of both groups (score 1–2) and (score 4–5) females.

Inoculated from the Mc'Conkey's base bacilli growth, recorded on Kligler and Christensen's base was the confirmation of their belonging to the *Enterobacteriaceae* family. As a result of Gram staining, the advantage of Gram-negative bacilli was obtained. Among these species, a lactose-positive bacilli such as *Escherischia coli* dominated by an abundant growth, a little less abundant growth of slower and less lactose fermenting taxa such as *Citrobacter* spp*., Enterobacter* spp., and a single lactose-negative colonies of *Salmonella* spp*.* Studies by Clausen and Dietz [2004], concerning the nest's bacteriological environment in which, the problem of the young minks falls occurred, showed the *Staphylococcus* spp*.,* including *Staphylococcus aureus* and no-hemolytic streptococci presence.

In the case of young minks, the falls relate mostly to the 1-5 weeks old animals, and their common causes are diarrhoeas against bacterial infections [Järplid and Majerland 1998]. Hynes *et al.* [2004] researches, found a high degree of correlation, between body condition and body weight versus the health and survival of young minks.

Martino and Villar [1990] stated that the individual physiological characteristics of young minks and inadequate farm management, may predispose to high mortality of nurselings. Confirmation of this thesis is the fact, that the authors have noted highest

young mink's mortality in the first week of their life (61.9%), wherein the most important cause of death was systemic mink's infection, where the main infectious agents were *E. coli., Proteus vulgaris, Corynebacterium pyogenes, Streptococcus zooepidemicus* i *Staphylococcus aureus.*

Bacteriological test results from the body and internal organs were collected in Table 1. From colonies of staphylococci and streptococci grown on blood agar as well as the bacilli on the McConkey's base, additional microscopic slides were performed and stained by Gram method. As a result Gram-positive cocci, and among them catalase-positive *Staphylococcus* spp. and catalase-negative *Streptococcus* spp. were obtained. Single colonies of streptococci isolated from the body surface of several young minks from (score 4–5) mothers. On the bloody base streptococci caused β hemolysis. Catalase-positive cocci were also tested for coagulase production. On the bloody base Gram-positive, catalase-positive, and coagulase-negative staphylococci (CNS) have grown, belonging probably to the *Staphylococcus epidermidis*. On the blood agar coagulase-positive *Staphylococcus aureus* was identified, surrounded with additional β hemolysis. Staphylococci partition to pathogenic and nonpathogenic was also based on the difference in their growth on the Chapman base. It has been shown that *Staphylococcus epidermidis* occurred essentially on the surface of the young mink's body. The presence of *Staphylococcus aureus* was observed primarily on the surface of the body, liver and intestines of dead young minks. Profuse coagulase-positive and coagulase-negative staphylococci growth was observed in young's from both female weight groups. Studies by many authors [Martino and Villar 1990, Hunter and Prescott 1991, Schneider and Hunter 1993a, Vulfson *et al.* 2002, Vulfson *et al*. 2003] carried out on Scandinavian farms, confirmed the obtained results. They also isolated a high abundance of *Staphylococcus epidermidis, S. aureus, S. intermedius, Escherischia coli* bacteria, on the body and internal organs (liver, lungs) of dead few days old minks.

CONCLUSIONS

Maintaining an adequate level of hygiene standards of premises spaces affects the limitation of morbidity and increases young minks survival. Results of bacteriological examinations of the body surface and internal organs of minks, reflect the potential impact of increased number of environmental bacteria on the number of falls.

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Streszczenie. Ograniczenie liczby upadków młodych norek jest wypadkową wielu czynników wśród których istotną rolę odgrywa utrzymanie wysokiego standardu higieny w pomieszczeniach pawilonowych, na co wskazują różnice w zachorowalności i śmiertelności młodych w zależności od fermy. Celem badań było określenie stanu mikrobiologicznego środowiska domków wykotowych w okresie okołoporodowym. Badając właściwości mikrobiologiczne ściółki pobranej w dwóch badanych okresach, stwierdzono znaczące różnice w ilości bakterii mezofilnych. Obecność chorobotwórczych i potencjalnie chorobotwórczych pałeczek stwierdzono na powierzchni ciała i w jelitach szczeniąt pochodzących z obu grup wagowych matek. Badania własne, w których wykazano obfity wzrost czystych hodowli bakteryjnych pochodzących zarówno ze ściółki, jak i ciał padłych norcząt, mogą świadczyć o istotnym wpływie czynników bakteryjnych na zdrowie i przeżywalność norcząt w okresie okołoporodowym.

Słowa kluczowe: norki, glukoza, kondycja ciała