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**Hygiene and sanitation evaluation of dairy cows barn
with regard to environmental protection**

Ocena sanitarno-higieniczna obory krów mlecznych w aspekcie ochrony
środowiska

Summary. The study aimed at the evaluation of hygiene and sanitation conditions of a dairy cows barn with regard to environmental protection. The research material comprised swab samples collected from the cows and environment. The biological aerosol was measured in the barn air by the sedimentary method. The microbial population isolated most frequently on the cow body proved to be Gram negative bacteria, while coagulase-negative staphylococci were most commonly identified at the milking equipment components. Importantly, the percentage of *Staphylococcus aureus* was the highest out of all microorganisms isolated in the barn. The barn air showed high levels of mesophilic bacteria and mold fungi counts.

Key words: bioaerosol, cow barn, microorganism

INTRODUCTION

The problem of animal production in relation to the global and national efforts towards the environmental protection has occupied the top place among the public concerns. According to the European Union legal regulations, animal breeding conditions have to meet a number of requirements concerning both, reduction of hazardous substances emitted to the atmosphere and animal welfare standards. The biological hazards are equally important issues, often underestimated, in the public health concern due to human occupational exposure conditions in these production sectors [Rozporządzenie... 2005].

Farm buildings are ecological niches producing specific microclimate for microorganisms to live and proliferate at. Air contaminated with dusts, gaseous substances and microbes is released from farm buildings directly to the air creating, serious environmental burden. Thus, extra risks to human health occur caused by toxic air pollutants. It was found that farm buildings harbor dozens of bacteria species, several hundreds of species of moulds, lots of bryophyte and lichen communities, algae, mites [Dutkiewicz *et al.*, 2002, Zyska 1999].

The present research objective was the sanitary-hygienic evaluation of dairy cow barns in relation to the environment protection.

MATERIAL AND METHODS

The studies were carried out in a dairy farm located in the Lublin Province. During the research period, the barn housed 40 cows of black and white variety with varied share of HF breed genes (Polish Holstein-Friesian breed black and white variety). The animals were managed at the tie-stall barn system in the main hall with the central feed passage, the manure passages at both sides and the stall automated milking system applied.

The research material comprised swabs collected from the cows and the surroundings. The swabs were taken from the mammary gland, oral cavity, cow back and abdomen, milking cups, external milk lines, udder cloths and milk pipe tubes. The study involved 6 randomly chosen cows. Besides, the research material included swabs collected from the stalls, walls, stall flooring, passages: feed and manure, drinking bowls. The swabbings taken with disposable sterile swabs were inoculated directly on blood agar, McConkey's and Sabouraud's media. After the 24 and 48h incubation period, the qualitative and quantitative evaluation of the grown colonies was performed. The colonies were identified using the microscopic methods, Gram staining procedure, biochemical tests (the test for catalase, coagulase presence).

Measurement of air microbial contamination was made by the sedimentation method in two replications. The Petri's dishes with the agar medium and the Sabouraud medium were placed in three measurement sites in the barn. After the 15 minute exposure time, the dishes were transported to the laboratory and incubated in compliance with the guidelines in the PN-89/Z-04111/02 and PN-89/Z-04111/03. A concurrent analysis of the basic microclimatic parameters such as, temperature, moisture and air motion was conducted. The results were analyzed and summarized in the tables.

RESULTS AND DISCUSSION

The recent years have been marked with increasing interest in the studies on the indoor air microflora in buildings. It is general knowledge that this status along with the sanitary conditions translate directly into health of farm animals housed over there as well as quality of final animal product obtained [Korbas 2007, Tymczyna and Bartek 2007].

The results of analyses on microorganisms detected at cow body and milking devices are presented in Table 1 and 2. The highest percentage of positive samples (75%) was established for Gram negative rods, whose presence was detected in the animal oral cavity, at the back and abdomen. Staphylococci from group CNS (coagulase negative staphylococci) and staphylococcus aureus were identified in half of the samples taken from the animal bodies. Streptococci and moulds accounted for 25% of positive samples. The presence of anascogenic yeasts was not recorded. The lowest microbial load was detected in the abdominal part of animal body and cow udders. The CNS staphylococci and Gram negative rods were identified solely on the abdomen, whereas on the mammary gland in addition, streptococci were found.

Table 1. Analysis of microorganisms recovered from cows body
 Tabela 1. Analiza mikroorganizmów bytujących na ciele krów

Sampling site (animal) Miejsce pobrania (zwierzę)	Streptococci Paciorkowce	Staphylococci (CNS) Gronkowce (CNS)	<i>S. aureus</i>	G-rods Pałeczki G-	Yeasts Drożdże	Molds Pleśnie
Udder Wymię	+	+	-	-	-	-
Oral cavity Jama ustna	-	-	+	+	-	-
Abdomen Brzuch	-	-	+	+	-	+
Back Grzbiet	-	+	-	+	-	-
Percentage of positive samples Odsetek prób dodatnich	25%	50%	50%	75%	0%	25%

Table 2. Analysis of microorganisms recovered from milking devices
 Tabela 2. Analiza mikroorganizmów bytujących na urządzeniach udojowych

Milking devices Urządzenia udojowe	Streptococci Paciorkowce	Staphylococci (CNS) Gronkowce (CNS)	<i>S. aureus</i>	G-rods Pałeczki G-	Yeasts Drożdże	Molds Pleśnie
External milk tubes Przewody mleczne zewnętrzne	-	+	-	-	-	-
Hangers, washer Stojaki, płuczki	-	+	-	+	+	-
Milk pipeline (tubes) Rurociąg mleczny (przewody)	-	+	-	-	-	+
Milking cup Kubek udojowy	-	-	-	-	-	-
External tank Zbiornik zewnętrzny	-	-	-	-	-	-
Percentage of positive samples Odsetek prób dodatnich	0%	60%	0%	20%	20%	20%

Table 3. Analysis of microorganisms recovered from the cow barn
 Tabela 3. Analiza mikroorganizmów bytujących w oborze

Object Obiekt	Streptococci Paciorkowce	Staphylococci (CNS) Gronkowce (CNS)	<i>S. aureus</i>	G-rods Pałeczki G-	Yeasts Drożdże	Molds Pleśnie
Stall Przegroda	-	-	-	-	-	-
Wall /ceiling Ściana /sufit	-	+	+	-	-	+
Wall /window Ściana /okno	-	+	+	-	-	-
Floor stalls Podłoga stanowiska	-	+	-	+	-	+
Floor Podłoga	-	+	+	-	+	-
Floor – feeding passage Podłoga – korytarz paszowy	-	-	+	-	-	-
Manure passage Korytarz gnojowy	-	-	-	+	-	-
Wall Ściana	-	+	+	-	-	-
Wall/doors Ściana/drzwi	-	-	-	-	-	-
Stall/pole Przegroda/shup	-	+	+	-	-	+
Drinker Poidło	-	-	+	-	-	-
Percentage of positive samples Odsetek prób dodatnich	0%	54%	63%	18%	9%	27%

The dairy equipment units, where no microorganisms were isolated included a milking cup and the external tank (Tab. 2). The highest percentage of coagulase negative staphylococci (60%) was identified at the external milking lines, stands, washers and milk pipes. There were also positive samples indicating the presence of, among others, Gram negative rods, yeasts (stands, washers) and moulds (milk pipe). It is worth mentioning that the swabs collected from the for milking devices did not detect staphylococcus aureus which is a major human and animal pathogen.

The genus Streptococci contains numerous clinically significant species that are important animal pathogens and the predisposing factors are large breeding objects with relatively small production halls, crowded conditions or poor sanitation [Grajewski 2006, Kamińska 1998, Tymczyna and Bartęcki 2007].

Table 4. Mean concentration of mesophilic bacteria and mold fungi in cow barn air
Tabela 4. Średnie stężenie bakterii mezofilnych i grzybów pleśniowych w powietrzu obory

Exposure point/measurement Punkt ekspozycji/pomiaru	Mean concentration of mesophilic aerobic bacteria Średnie stężenie bakterii tlenowych mezofilnych	Mean concentration of molds Średnie stężenie grzybów pleśniowych
Corner of I building Róg I budynku	1.4×10^5	8.5×10^3
Building inside Środek budynku	8.8×10^4	1.1×10^4
Corner of II building Róg II budynku	5.6×10^4	1.1×10^4
Mean concentraion in object (CFU/m ³) Średnie stężenie w całym obiekcie (jkt/m ³)	9.5×10^4	10.2×10^3

In the pool of microorganisms identified in the cow barn, the highest percentage was made by the samples examined for staphylococcus presence (63%) (Tab. 3). Coagulase negative staphylococci were isolated from the ceiling, windows, stall flooring, flooring, walls and the pile. The samples positive to coagulase negative staphylococci accounted for 54%. A slight proportion of anascogenic yeasts (9%) was identified in the flooring, whereas no streptococci presence was recorded in the flooring samples. However, high number of samples taken from the barn proved positive to moulds (27%). The manure passage and stall flooring were the places where Gram negative forms were identified to make up 18% of positive samples. An alarming fact is that swabs taken from the drinking bowls have displayed the presence of staphylococcus aureus which is a potential pathogen not only for animals but animal care workers as well.

The sanitary-hygienic evaluation of the barn indicated high microbial contamination rate of the object. In the barn, mean indoor temperature averaged 18.2°C, relative humidity 65% and air motion ~0.13m/s which complied with the generally accepted standards in zoohygiene. The results of barn air analyses are summarized in Table 4. Total count of mesophilic bacteria and mold fungi was very high. Many authors report that microbial load in the farm building air is dependent on animal health status [Kamińska 1998, Krukowski 2006, Tymczyna and Bartek 2007].

Mean concentration of mesophilic bacteria reached 9.5×10^4 cfu/m³ and moulds – 10.2×10^3 cfu/m³. The highest bacteria load was noted in the corner I – 1.4×10^5 cfu/m³ and fungi concentration in the centre and the corner II – 1.1×10^4 cfu/m³ each. Total mesophilic bacteria counts substantially exceeded the admissible limits according to the norm [PN-89/Z-04111/02].

Similar studies were conducted by Kamińska [1998] who evaluated the air microflora in the sheep house. The air comprised numbers of bacterial and fungal isolates. The mean bacterial concentration in the sheep house air ranged between 4.2×10^4 and 2.1×10^5 cfu/m³, while mold fungal concentration averaged 4.4×10^3 cfu/m³. Analyzing

the present research results, it should be stated that the air of the studied barn proved to be more contaminated than that of the sheep house.

Chmielowiec-Korzeniowska [2008] reports that the major reservoir for microorganisms in the farm buildings are animals, their feces, secretions, peeling off epidermis and finally, feedstuffs. Although Krukowski [2006] emphasizes that it is litter that harbors fungi but generally, fungal pathogens favor humid old straw and sawdust which provide extremely conducive conditions for their growth and proliferation. Microorganisms induce unfavorable changes in farm building air quality due to overall metabolic processes resulting in release of endotoxins, enterotoxins, exotoxins, enzymes and mycotoxins. These substances being mixed up with plant residues, decayed organic plant and animal matter, soil, feces may be transported over long distances by air movements. The direct results of inhaled airborne agents or any contact with them may cause different types of allergic responses. Airborne particles may also get into animal organism indirectly, i.e. with feed [Pałczyński 2007].

The sanitary – epidemiological evaluation has indicated that the presence of potential pathogenic bacteria, especially fungi and their metabolites may pose life and health hazard to the human population and breeding animals.

CONCLUSIONS

1. Efficient elimination of potential pathogenic microorganisms occurring in the animal housing area necessitates the joint action through the imposed sanitary measures.
2. Bioaerosol concentration must be monitored in the dairy barn as it represents a potential source of occupational and environmental hazards as well as the risk for the immediate residential zone.

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Streszczenie. Celem badań była ocena sanitarno-higieniczna obory krów mlecznych w aspekcie ochrony środowiska. Materiał badawczy stanowiły wymazy pobrane od krów oraz z ich środowiska. Pomiar aerosolu biologicznego w powietrzu obory przeprowadzono metodą sedymentacyjną. Na ciele krów wyizolowano najczęściej pałeczek Gram-ujemnych. Z elementów urządzeń udojowych izolowano głównie gronkowce koagulazoujemne. Natomiast największy odsetek ze wszystkich mikroorganizmów izolowanych z obory stanowiły gronkowce złociste. W powietrzu obory wykazano wysoką koncentrację ogólnej ilości bakterii mezofilnych i grzybów pleśniowych.

Slowa kluczowe: aerozol biologiczny, obora, mikroorganizm