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Study of microbial species composition in the production environment of livestock facilities

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ABSTRACT

Introduction. Livestock facilities serve as a reservoir for microorganisms of various families and genera, including both opportunistic and pathogenic microorganisms. Continuous microbiological monitoring of the production environment in livestock facilities, along with the detection and identification of microorganisms, allow for the microflora control in these facilities, thereby preventing the risks of infectious diseases and ensuring timely implementation of appropriate veterinary, sanitary, and zoohygienic measures.

Objective. Study of microbial species composition in the production environment of livestock facilities including contamination level and classification of the isolated mircoorganisms by families and disinfectant-resistant groups.

Materials and methods. Swabs from the surfaces in the production facilities for cattle (namely, dairy cow facility, calf facility, calving area, and milking hall) on the cattle farm located in the Omsk Oblast were taken for study of microbial species composition. The microorganisms were classified using MMT E24 и MMT S multi-biochemical microtests and selective nutrient medium.

Results. Tests showed that the microflora circulating in cattle facilities included both pathogenic and opportunistic microorganisms of the following species: *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Klebsiella aerogenes, Citrobacter freundii, Morganella morganii, Hafnia alvei, Klebsiella ozaenae, Enterococcus faecalis, Bacillus cereus, Staphylococcus sciuri, Staphylococcus capitis, Staphylococcus simulans, Staphylococcus intermedius* and *Staphylococcus lentus.*

Conclusion. The recovered microorganisms belonged to the families *Enterobacteriaceae, Bacillaceae* and *Staphylococcaceae* and to the following disinfectant-resistant groups: low-resistant, moderately-resistant and highly-resistant. The highest microbial load was detected on floor, walls and stall dividers in the facility for dairy cows and in milking hall, the detected microorganisms demonstrated high species diversity. The lowest microbial load was detected in calving area and calf facility.

Keywords: microorganisms, microbiological load, production environment

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Изучение видового состава микроорганизмов производственной среды животноводческих помещений

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РЕЗЮМЕ

Введение. Производственные объекты животноводческих комплексов являются резервуаром микроорганизмов различных семейств и родов, среди которых есть как условно-патогенные, так и патогенные представители. Постоянный микробиологический мониторинг производственной среды животноводческих помещений, индикация и идентификация микроорганизмов дает возможность контролировать микрофлору данных помещений, тем самым предотвращать риски возникновения инфекционных заболеваний и своевременно проводить качественные ветеринарно-санитарные и зоогигиенические мероприятия.

Цель исследования. Изучение видового состава микроорганизмов производственной среды животноводческих помещений, уровня контаминации и классификация выделенной микрофлоры по семействам и группам устойчивости к дезинфицирующим препаратам.

Материалы и методы. Для изучения видового состава микрофлоры были взяты смывы с поверхностей в производственных помещениях для содержания крупного рогатого скота (коровник – дойное стадо, телятник, родильное отделение и доильный зал), расположенных в животноводческом хозяйстве Омской области. Идентификацию микроорганизмов проводили с использованием биохимических мультимикротестов ММТ Е24 и ММТ С и селективной питательной среды.

Результаты. В результате проведенных исследований установлено, что микрофлору, циркулирующую в помещениях для содержания крупного рогатого скота, составляют как патогенные, так и условно-патогенные микроорганизмы, которые представлены следующими видами: *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Klebsiella aerogenes, Citrobacter freundii, Morganella morganii, Hafnia alvei, Klebsiella ozaenae, Enterococcus faecalis, Bacillus cereus, Staphylococcus sciuri, Staphylococcus capitis, Staphylococcus simulans, Staphylococcus intermedius* и *Staphylococcus lentus.*

Заключение. Выделенные микроорганизмы представлены семействами *Enterobacteriaceae, Bacillaceae* и *Staphylococcaceae* и принадлежат к следующим группам устойчивости к дезинфектантам: малоустойчивые, устойчивые и особо устойчивые. Наиболее высокая микробиологическая нагрузка наблюдалась на таких объектах, как пол, стены и ограждения в стойлах, расположенных в коровнике (дойное стадо) и доильном зале, микрофлора характеризовалась большим видовым разнообразием микроорганизмов, низкий уровень микробной диссеминации установлен в помещениях родильного отделения и телятника.

Ключевые слова: микроорганизмы, микробиологическая нагрузка, производственная среда

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INTRODUCTION

Modern large-scale animal farming is characterized by a high concentration of cattle in specialized livestock establishments. The animal farming industrialization and its transfer to a large-scale mass production imply a profound qualitative restructuring of all technological processes. Under such intensive farming conditions, biological agents accumulate at various production facilities of livestock establishments. This leads to the emergence of mass dysbiosis in animals and, as a result, to increased number of infectious diseases [1, 2, 3].

The production facilities of livestock establishments serve a reservoir of microorganisms of various families and genera, including both opportunistic and pathogenic microorganisms. Under prolonged exposure to high humidity, various microflora contaminates the building structures of livestock establishments, thereby increasing the risk of infectious diseases [4, 5, 6, 7].

Infectious diseases of farmed animals are responsible for significant losses to the livestock industry. Poor veterinary and sanitary practices on farms of various levels is the one of the main causes for infectious disease occurrence. All these often provoke the infectious gastrointestinal, respiratory and other pathologies caused by both pathogenic and opportunistic microflora (cocci, proteus, klebsiella, etc.), which virulence increases when the animal resistance weakens due to adverse factors related to feeding, care and housing condition violations [8, 9, 10].

Veterinarians have to take into account the whole range of animal habitat factors that have changed significantly due to technological progress in order to create an optimal environment. However, under modern conditions, veterinarian's attention is focused on the animal, its health and performance, as well as on protection of the environment from various contaminants associated with the large-scale livestock establishment activities. The strict observance of veterinary containment and security measures plays a crucial role in the livestock establishments. The high density of facilities and animals concentrated in a limited area requires strict measures to protect establishments from the introduction of infectious diseases [11, 12, 13].

A poorly maintained production environment is a major obstacle to effective infectious disease control. This risk extends beyond highly dangerous pathogens to include opportunistic microbes, which can turn pathogenic under suitable conditions and cause significant damage. A significant number of microorganisms are shed by animals during the physiological acts: coughing, sneezing, defecation, urination. The production environment of livestock facilities, where pathogenic and opportunistic microorganisms are shed, is typically not their natural habitat. There are often no favourable living conditions here: nutrients, optimal temperature and pH of the environment. However, in facilities containing large quantities of organic matter, such microorganisms can maintain their viability but also pathogenicity for long periods. They are detected on the surfaces of livestock buildings, vehicles, in manure, animal-origin raw materials, and many other objects. The level of production facility contamination depends mainly on the presence of infectious diseases in animals. Diseased animals constantly shed pathogens into the production environment. Pathogens become to further spread from inadequately decontaminated surfaces within the facility. One of the persistent causes of microbial contamination in the production environment is carrier animals. These animals pose even greater risk of pathogenic microflora spreading and the disease maintenance within the establishment than apparently diseased animals, since the latter can be isolated until their recovery [14, 15, 16, 17].

Animals shedding pathogenic and opportunistic microorganisms with faeces and airborne droplets are the main source of livestock production facility contamination, and the more intensely the environment is contaminated with secretions, the higher the probability of contamination

of objects with the relevant pathogens. For many microorganism species, the intestine is a biotope, that is, their only habitat. Consequently, the detection of intestinal microflora in the tested material (water, feed, samples from livestock facility surfaces, etc.) serves as a direct indicator of faecal contamination of the object and possible presence of pathogens of intestinal infections (salmonellosis, yersiniosis, etc.) [18, 19, 20].

Many microorganisms circulating in livestock facilities naturally possess resistance mechanisms rooted in their cellular structure and metabolism. These include a multilayer cell wall, biofilm formation, enzymatic breakdown or active xenobiotic efflux pumps. Bacterial spores possess a unique cell membrane that enables them to withstand biocide concentrations thousands of times higher than those effective against vegetative cells. Spore dense coating membrane prevents the penetration of biocide into the cell and neutralizes the effect of those that do breach its barrier. The coating membrane accounts for up to 50% of the spore dry mass. All these features provide spores with the resistance to environmental factors, including biocides. Mycobacteria are also highly resistant to many biocides, resistant to acids, alkalis, chlorhexidine, quaternary ammonium compounds, heavy metals and dyes. Mycobacteria are able to form biofilms (for example, in water supply systems), which are more difficult to remove than enterobacterium biofilms [21, 22, 23].

Biofilm formation is one of the manifestations of bacterial survival strategy, conferring resistance to adverse factors, including biocides. Biofilm is a microbial community, often multispecies, embedded within a self-produced extracellular polymeric matrix (glycocalyx) that acts as a protective barrier against external factors. Increased resistance to biocides has been found in the following biofilm-growing species of microorganisms: *Pseudomonas*, *Burkholderia cepacia*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Legionella pneumophila*, *Salmonella typhimurium*, and *Yersinia enterocolitica* [24, 25, 26].

Numerous highly effective broad-spectrum antibiotics are widely applied in veterinary practice. They are highly effective when used for respiratory and gastrointestinal infection prevention and treatment. However, prolonged and uncontrolled use of antibiotics leads to the emergence of a significant number of resistant microorganism strains [27, 28].

The significance and innovation of this work lie in comprehensive analysis of the microbial species composition and levels of production environment contamination in livestock facilities as well as classification of the isolated microorganisms by families and disinfectant-resistant groups that enables implementation of proper and prompt veterinary-sanitary measures, such as cleaning and disinfection, for prevention of infectious disease risks.

The study was aimed at examination of microorganism species composition in the production environment of livestock facilities, and contamination level and classification of the isolated microflora by families and disinfectant-resistant groups.

MATERIALS AND METHODS

Tested materials: 184 samples taken before cleaning and disinfection from various surfaces in 4 facilities of the livestock establishment located in the Omsk Oblast.

General zoo-technical characteristics of the establishment. The establishment is surrounded by 500-meter sanitary-protective zone and located apart of other livestock/agricultural establishments and facilities including backyards. The main economic activity types are legumes cultivation and dairy cattle farming, breeding, raw milk production. The establishment has the status of a breeding farm for breeding Red Steppe dairy cattle. The dairy cow facility is a reconstructed standard tie-stall cattle facility for 200 animals. There is a "Parallel" automated milking parlour (commissioned in 2020) for 24 animals, S = 420 m^2 in the establishment. The parlour is divided into three parts: a milking hall and cow facilities on milking hall both sides.

The establishment is free from acute infectious diseases. Diagnostic tests for infectious diseases such as tuberculosis, brucellosis, leucosis, hypodermatosis, chlamydia, leptospirosis are carried out according to the plan of anti-epizootic preventive measures. Animals are vaccinated against anthrax, blackleg, brucellosis, pasteurellosis, enterococcal infection, colibacillosis, salmonellosis, klebsiellosis and proteus infection, ringworm and treated against hypodermatosis.

Annual plan comprising organizational, zootechnical, and veterinary measures for leucosis prevention is developed. Key strategies include the isolated rearing of replacement heifers and selection of heifers based on their family history.

Regular disinfection of all livestock facilities is carried out. The establishment is fully fenced, there is a sanitation checkpoint at its entrance, and there are disinfection barriers at the entrances to the cow facilities and calf facilities.

Tested facilities. Dairy cow facility (48 samples from 6 sites): stall floor (rubber covering), stall walls, the walls at the entrance, wooden window frames, stall fences, wooden door to the cow facility. Calf facility (48 samples from 6 sites): the floor inside the cages (straw), stall walls, walls at the entrance, plastic window frames, fences of cages for calves, door to the calf facility. Calving facility (48 samples from 6 sites): stall floor (rubber covering), stall walls, the walls at the entrance, wooden window frames, partitions in the stalls, door to the calving facility. "Parallel" milking parlour (40 samples from 5 sites): milking hall floor (rubber covering), tiled walls, plastic window frames, milking machines, fences of the milking plant.

The samples were taken at relative humidity of 81% in the cow facility, 72% in the calf facility, and 74% in calving facility; indoor temperature was (24 ± 2) °C; the calving facility was equipped with automated ventilation system.

Samples were taken by swabbing the surfaces of various objects according to the methodological guidelines MG 4.2.0220-20[1]. Sterile swab was moistened by dipping it into the Amies transport medium immediately before swabbing. A document including data required for unambiguous identification of the object, sampling place, reason and conditions, date and time, conditions and time periods of sample delivery to the diagnostic laboratory was drawn up.

Biochemical multimicrotests: MMT E24 and MMT C (NPO Immunotex, Russia), were used for *identification of microorganisms* belonging to the *Enterobacteriaceae* and *Staphylococcaceae* family, respectively. These

[1] MG 4.2.0220-20 Methods of sanitary and bacteriological testing of environmental objects for microbial contamination: methodological guidelines (approved by the Federal Service for Supervision of Consumer Rights Protection and Human Welfare on 4 December 2020). https://docs.cntd.ru/document/573595605?ysclid=mguk1xg4sw975021985 (in Russ.)

Table 1
Results of study of species composition of the microorganisms circulating in the cattle facility (dairy herd), *n* = 93

Microorganisms	Tested surfaces					
	Floor (rubber)	Stall walls	Walls at the entrance	Windows (wooden)	Stall fences	Door to the cow facility
Positive samples, %						
Enterobacteriaceae family microorganisms						
E. coli	100.0	87.5	0.0	0.0	62.5	0.0
P. mirabilis	75.0	87.5	0.0	0.0	0.0	0.0
K. aerogenes	75.0	0.0	0.0	62.5	62.5	0.0
C. freundii	62.5	75.0	62.5	0.0	0.0	0.0
M. morganii	62.5	75.0	0.0	0.0	0.0	0.0
E. faecalis	100.0	62.5	62.5	0.0	62.5	0.0
Bacillaceae family microorganisms						
B. cereus	0.0	75.0	0.0	0.0	0.0	0.0
Staphylococcaceae family microorganisms						
S. capitis	0.0	0.0	0.0	0.0	62.5	0.0
S. sciuri	0.0	75.0	0.0	37.5	0.0	62.5
S. simulans	0.0	0.0	0.0	0.0	62.5	0.0

multimicrotests are designed to determine the biochemical activity of enterobacteria and staphylococci during bacteriological analysis and their species identification and are based on the determination of these microorganisms' enzyme systems reacting to the corresponding substrates. *Bacillaceae* family microorganisms were identified using Donovan's selective nutrient medium containing lithium chloride (selective agent). The tests were carried out at the Diagnostic Research and Biotechnology Laboratory of the Omsk Agrarian Scientific Center.

The test results were statistically processed using the Microsoft Excel software.

RESULTS AND DISCUSSION

Test results showed that the microflora in dairy cow facility rooms includes various microorganisms. Thus, stall floors, walls and stall fences are particularly prone to significant microbial contamination. For 48 swab samples tested, the following types of microorganisms were detected in 122 cases, *n* = 93 (Table 1): swabs from the floor – *E. coli* and *E. faecalis* (100.0% of swabs), *Proteus mirabilis* and *Klebsiella aerogenes* (75.0% of swabs), *Citrobacter freundii* and *Morganella morganii* (62.5% of swabs); swabs from the walls – *E. coli* and *P. mirabilis* (87.5% of swabs), *C. freundii*, *M. morganii*, *Bacillus cereus*, and *Staphylococcus sciuri* (75.0% of swabs), *E. faecalis* (62.5% of swabs); swabs from stall fences – *E. coli*, *K. aerogenes*, *E. faecalis*, *Staphylococcus capitis* and *Staphylococcus simulans* (62.5% of swabs).

Tests revealed low microbial contamination of window frame surfaces: *K. aerogenes* (62.5% of swabs) and *S. sciuri* (37.5% of swabs); door to the cow facility: *S. sciuri* (62.5% of swabs); as well as in samples taken from the walls at the entrance: *C. freundii* and *E. faecalis* (62.5% of swabs).

For 48 swab samples collected in calf facility the following heavily contaminating microorganisms were isolated in 54 cases (*n* = 49): in swabs from floor (*Hafnia alvei* – 100.0%, *C. freundii* – 75.0% and *E. faecalis* – 75.0% of swabs), fences of cages for calves (*E. faecalis* – 87.5% and *C. freundii* – 37.5% of swabs), stall walls (*C. freundii* – 62.5% of swabs). *Staphylococcus lentus* was detected in 75.0% of swab samples from window frames, walls at the entrance and door to the calf facility (Table 2).

For 48 swab samples collected in calving facility, microorganisms were detected in 52 cases (*n* = 46). A high microbial load was detected in the samples collected from floor surface (*Klebsiella ozaenae* – 87.5%, *H. alvei* – 87.5%, *P. mirabilis* – 75.0% of swabs) and from stall walls (*Staphylococcus intermedius* – 87.5%, *H. alvei* – 62.5% and *P. mirabilis* – 62.5% of swabs).

Table 2
Results of study of species composition of the microorganisms circulating in the calf facility (up to 6 months), *n* = 49

Microorganisms	Tested surfaces					
	Floor (straw)	Stall walls	Walls at the entrance	Windows (plastic)	Cage partitions	The door to the calf facility
Positive samples, %						
Enterobacteriaceae family microorganisms						
H. alvei	100.0	0.0	0.0	0.0	0.0	0.0
C. freundii	75.0	62.5	0.0	0.0	37.5	0.0
E. faecalis	75.0	0.0	0.0	0.0	87.5	0.0
Staphylococcaceae family microorganisms						
S. lentus	0.0	0.0	75.0	75.0	0.0	75.0

Table 3
Results of study of species composition of the microorganisms circulating in the calving facility, *n* = 46

Microorganisms	Tested surfaces					
	Floor (rubber)	Stall walls	Walls at the entrance	Windows (wooden)	Partitions	Door to the calving facility
	Positive samples, %					
Enterobacteriaceae family microorganisms						
K. ozaenae	87.5	0.0	0.0	0.0	25.0	0.0
H. alvei	87.5	62.5	0.0	0.0	0.0	0.0
P. mirabilis	75.0	62.5	37.5	0.0	0.0	0.0
Staphylococcaceae family microorganisms						
S. intermedius	0.0	87.5	0.0	87.5	0.0	37.5

A low microbial load was detected in swab samples taken from window frames and doors in the calving facility – *S. intermedius* (87.5% and 37.5% of swabs, respectively). *K. ozaenae* was detected in 25.0% of swabs collected from fences, *P. mirabilis* was detected in 37.5% of swabs taken from the walls at the entrance (Table 3).

For 40 samples collected in the milking hall, microflora characterized by a wide variety of microorganisms was detected, in 84 cases (*n* = 69). *E. coli*, *Proteus vulgaris* and *S. simulans* were detected in 87.5% of swab samples from floor, *H. alvei*, *C. freundii*, *M. morganii*, and *E. faecalis* were detected in 75.0% of swab samples; *E. coli* (62.5%), *H. alvei*, *M. morganii*, *E. faecalis*, and *S. intermedius* were detected in 37.5% of swab samples from the milking plant fences (Table 4).

The microbial contamination of walls, windows and milking machines was low. *S. sciuri* was detected in 75.0% and *C. freundii* was detected in 62.5% of swab samples taken from wall surfaces; *S. simulans* and *M. morganii* were detected in 62.5% and 37.5% of swab samples taken from window surfaces, respectively, and *S. sciuri* was detected in 37.5% of swab samples collected from milking machines.

The isolated microorganisms belong to the following disinfectant-resistant groups: low resistant group: *E. coli*, *P. mirabilis*, *P. vulgaris*, *K. aerogenes*, *C. freundii*, *M. morganii*, *H. alvei*, *K. ozaenae* and *E. faecalis*; moderately resistant group: *S. capitis*, *S. simulans*, *S. intermedius*, *S. sciuri* and *S. lentus*; highly resistant group: *B. cereus*.

CONCLUSION

The study results allow us to conclude that the microflora in the cattle facilities included both pathogenic and opportunistic microorganisms belonging to the *Enterobacteriaceae*, *Bacillaceae* and *Staphylococcaceae* families. The members of the first of them were: *E. coli* (causative agent of colibacillosis in young livestock animals), *P. mirabilis* (causes purulent-inflammatory processes in wounds), *P. vulgaris* (causes feed-borne toxic infections, purulent-

Table 4
Results of study of species composition of the microorganisms circulating in the milking hall, *n* = 69

Microorganisms	Tested surfaces				
	Floor (rubber)	Walls (glossy tiles)	Windows (plastic)	Milking machines (inner surface)	Fences of the milking plant (duralumin)
	Positive samples, %				
Enterobacteriaceae family microorganisms					
E. coli	87.5	0.0	0.0	0.0	62.5
P. vulgaris	87.5	0.0	0.0	0.0	0.0
H. alvei	75.0	0.0	0.0	0.0	37.5
C. freundii	75.0	62.5	0.0	0.0	0.0
M. morganii	75.0	0.0	37.5	0.0	37.5
E. faecalis	75.0	0.0	0.0	0.0	37.5
Staphylococcaceae family microorganisms					
S. intermedius	0.0	0.0	0.0	0.0	37.5
S. sciuri	0.0	75.0	0.0	37.5	0.0
S. simulans	87.5	0.0	62.5	0.0	0.0

inflammatory processes in wounds, enteritis, peritonitis and sepsis), *K. aerogenes* (causative agent of opportunistic infections), *C. freundii* (causative agent of infectious urinary, respiratory and circulatory diseases), *M. morganii* (urinary tract infections), *H. alvei* (urinary tract infections, pneumonia, sepsis), *K. ozaenae* (respiratory tract infections), *E. faecalis* (urinary tract infections, endocarditis, and gastrointestinal infection). *B. cereus* belonging to *Bacillaceae* family and causing gastrointestinal infections was detected in samples collected in production facilities. The following pathogenic microorganisms belonging to *Staphylococcaceae* family were detected: *S. sciuri* (responsible for urinary and circulatory infections, endocarditis), *S. capitis* (causative agent of infectious meningitis, osteomyelitis, endocarditis), *S. simulans* (bacteraemia, endocarditis), *S. intermedius* (causative agent of mastitis, skin infections), *S. lentus* (responsible for abscess, sepsis).

The data on the microbial load in the production environment of livestock facilities allowed us to identify the places of highest bacterial contamination. The highest microbial load was detected on floor, walls and stall partitions in dairy cow facility as well as floor and milking machine fences located in milking hall. The detected microorganisms demonstrated high species diversity. The lowest microbial load was detected in calving facility and calf facility where small number of animals are kept.

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