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PREVALENCE OF GASTROINTESTINAL TRACT PATHOLOGY AND HELICOBACTERIOSIS IN HORSES

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Abstract

This article presents information on the prevalence of helicobacteriosis in horses of different age groups. It has been found that gastrointestinal tract (GIT) pathology is widely prevalent in horses, ranging from 50% to 80%. The main condi-tions among GIT pathologies in horses are gastrophylosis, erosions, ulcers, and helicobacteriosis. Helicobacteriosis is registered in 66.7% to 100% of the investigated adult population and in 20% to 66.7% of the young horses. Stomach examinations using endoscopy and video gastroscopy provide a clear picture of the mucous membrane's condition in different parts of the stomach and allow for the collection of biomaterials for additional research. The percentage of animals affected by helicobacteriosis increases with age. Animals suffering from helicobacteriosis experience significantly reduced productivity, delayed growth and development in young horses, leading to premature culling.

It is incorrect to only test suspicious animals with indications for helicobacteriosis, as not all carriers of Helicobacter pylori show symptoms of the disease.

The use of the drug Domosedan at a dose of 0.5 μg per kilogram of body weight, or Combistress at a rate of 0.5 cm^3 per 100 kg of body weight, effectively calmed the animals, fully relaxed the GIT, and facilitated successful endoscopy and video gastroscopy examinations.

Key words: gastrointestinal tract pathology; helicobacteriosis; horses; *H. pylori*; video gastroscopy.

Basic position and Introduction

Equestrianism in Kazakhstan is developing at a moderate pace. However, there are several factors hindering the growth of this industry, primarily internal non-infectious pathologies that are not timely diagnosed, resulting in the absence of measures to address them.

Practitioners have observed a widespread occurrence of gastrointestinal tract (GIT) disorders in horses of unknown etiology. These disorders occur throughout the year, manifesting as weight loss due to poor appetite, inability to digest consumed feed, and the presence of painful symptoms and colic. All of this causes significant economic damage to equestrian establishments. Despite the research conducted by domestic and foreign scientists on GIT disorders in horses, this problem remains unresolved. Therefore, a detailed

study of the causes of this pathology in equestrian establishments is essential.

The connection between *H. pylori* infection and chronic gastritis, gastric and duodenal ulcers, and malignant gastric tumors has been scientifically proven in human medicine [1].

In clinical veterinary practice, gastric diseases in horses are a common and widespread problem. Gastric diseases are often accompanied by erosive and ulcerative changes in the mucous membranes, which can vary depending on the severity and type of gastric wall involvement [2, 3].

In modern veterinary practice, devices and equipment for diagnosing various animal pathologies are increasingly being used. Timely and accurate diagnosis allows for the selection of optimal treatment regimens and reduces treatment costs. Moreover, modern diagnostic devices are considered environmentally safe and do not harm the examined animals. One such device is the endoscope (ES) and video gastroscopy (VGS).

The authors note that endoscopy is a non-invasive method for examining the condition of the lumens and mucous membranes of the GIT, upper respiratory tract, urinary organs, and other body cavities [4, 5]. In addition to monitoring the condition of the examined organ, endoscopy allows for the collection of pathological material through biopsy for histomorphological, bacteriological, and other analyses. In some cases, endoscopy can be used for foreign body extraction or the administra-tion of medications into the organ's lumen [6].

Currently, *H. pylori* is one of the most extensively studied microorganisms globally due to its significance and social impact on diseases in which it plays a leading role. Two decades of studying *H. pylori* epidemiology have shown its widespread prevalence, with peptic ulcer disease being one of the most common gastro-intestinal disorders in animal populations [7, 8].

Transmission of the microorganism usually occurs from one animal to another. Domestic cats and rhesus macaques have been proven to be reservoirs of *H. pylori* infection, and the most common modes of transmission are oral-oral and fecaloral [9, 10].

According to several scientists, the prevalence of gastric ulcers in horses ranges from 60% to 90% in the adult population and from 25% to 51% in young horses. Helicobacteriosis significantly reduces the productivity of affected animals, hinders the growth and development of young horses, and is a major cause of premature culling of horses [11].

Histological examination of gastric tissues is known to be an effective, informative, and highly accurate diagnostic method. It requires obtaining gastric tissue samples through biopsy and subjecting them to microscopic examination [12].

Based on the above, our objective was to determine the prevalence of helicobacteriosis among different age groups of horses in the southeastern region of Kazakhstan under various management and husbandry conditions.

Materials and Methods

The research subjects included horses of different age groups, breeds, and physiological conditions. Samples for research included stomach mucus, biopsies, and blood serum. Some animals were kept in pasture conditions with access to water, while others were kept in stables.

The study material consisted of 32 horses from "Sunkar" Stud Farm (Thoroughbred English riding breed), 22 horses from "Kokbastau" Stud Farm (Arabian breed) in Zhambyl district, 26 horses from "Akhaevo" Stud Farm (Thoroughbred English riding breed) in Karasai district, 22 horses from "Akhal-Teke bishi" Stud Farm (Akhal-Teke breed), 65 horses from "Sarsibek" Stud Farm (American Standardbred breed) in Talgar district of Almaty region, 195 horses from "Azamat 2" collective farm in Beskaragai district (Mugalzharsk breed) of Abai region, and 30 horses from "Aqylbai" Eshkeldy district of Zhetysu region (Thoroughbred English riding breed). These farms had registered animals with frequent episodes of colic and signs of gastrointestinal tract diseases.

Out of the 392 horses, 273 suspicious and diseased animals (69.6%) were selected for the study on the prevalence of gastrointestinal tract diseases. This included 19 stallions, 91 mares, 34 colts born in 2019, 36 colts born in 2020, 46 filly born in 2019, and 47 filly born in 2020.

The research was conducted using the standard methodology for animal medical examinations. Special investigations were carried out using a SureVisionTM VLS-150 D endoscope (Digital Video System, USA, Figure 1) and an AGVE-68 HAL video gastroscopy (VGS) with VIS-68 video processor (China, Figure 2).

Figure 1 - Endoscope VLS-150 D (USA)

Endoscopy and VGS were performed to study the prevalence of gastrointestinal tract pathology (GIT), along with histological examinations and blood tests using the Helicobacter pylori test (Figure 3). Anamnestic data was collected for all age groups of animals

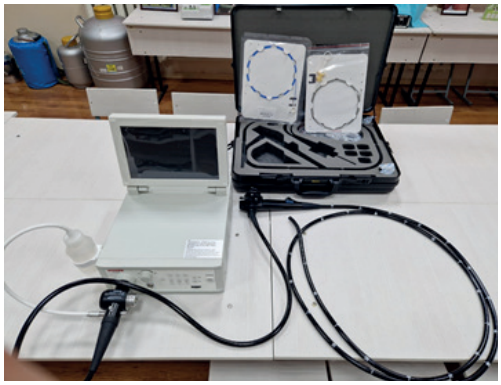

Figure 2 - Video gastroscope AGVE-68HAL (China)

Horses for conducting endoscopy (ES) and video gastroscopy (HCV) studies were selected based on their medical history and clinical signs such as emaciation, colic, loss of appetite, and the presence of unpleasant breath odor and abnormal feces. To prevent complications and ruptures of internal organs, the horses were kept on a fasting diet for 12 hours. Prior to ES and HCV, horses from the Almaty and Zhetysu regions were administered premedication through intravenous injection of the drug Domosedan at a dose of 0.5 μg per kilogram of body weight. In the Abay region, Combistress (from Belgium) was used at a dosage of 0.5 cm3 per 100 kilograms of live animal weight. Subsequently, the animals were placed in a warm location without drafts and provided with soft bedding.

A B

Figure 3 - A - Positive result of the Helpeel test;
B - Negative result of the Helpeel test

During the examination of animals with gastric ulcers and erosive lesions, biopsies (Figure 4) were taken for further investigation. To diagnose helicobacteriosis, blood samples were collected from the jugular vein of horses, and laboratory tests were conducted within 2-4 hours. For early diagnosis of helicobacteriosis in horses and obtaining rapid results, the Helpeel test was used. It is designed for onestep, fast, and qualitative "in vitro" determination of antibodies against helicobacteriosis in whole blood.

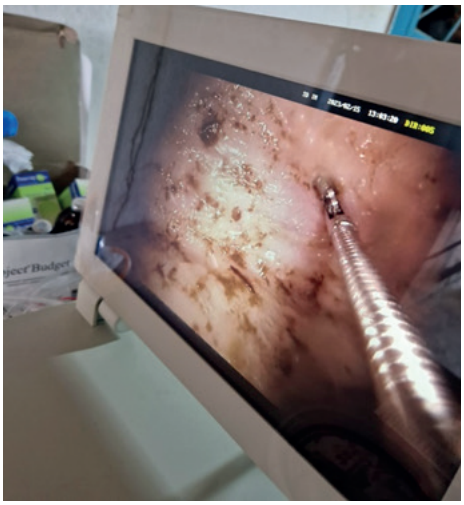

Figure 4 - Stomach Biopsy

The components of the kit and the specimens under study were kept at room temperature (+18-25°C) for 5-10 minutes before analysis. Then, the strip package was opened, and using a pipette, 2 drops (~80 µl) of venous blood were added to the sample tube, followed by 1 drop (~40 µl) of the diluent reagent. Afterward, the strip was vertically dipped into the sample tube, following the direction of the arrow. The results were visually evaluated after 10 minutes and within 20 minutes.

In the Abay region, out of 195 horses, 108 underwent VGS, which involved obtaining mucus and biopsies from different parts of the stomach. These samples were placed in a sterile 1.5 cm3 tube for subsequent histological and microbiological analysis. In the Almaty and Zhetysu regions, ES was performed on 143 horses.

The statistical analysis of data was conducted using "Microsoft Excel" software on a personal computer, calculating mean values (M), standard errors (m), and the significance of the compared parameters (P).

Results

This study is the result of interrelated clinical and laboratory investigations on the clinical and laboratory diagnosis of erosions and gastric ulcers associated with H. pylori in horses.

As known, helicobacteriosis is an infectious disease transmitted through the fecaloral route, with a strong affinity for the gastric epithelium. Therefore, this pathogen plays an important role in the pathology of the digestive system in horses. Consequently, conducting scientific research involves monitoring the prevalence of helicobacteriosis in horses. Moreover, the diagnosis of this pathology requires special studies. For instance, obtaining gastric biopsies from horses with helicobacteriosis is only possible through endoscopy for confirmatory histological analysis.

During the monitoring studies on helicobacteriosis in horses, we collected data on the prevalence of gastrointestinal tract pathologies among the horse population. The results of the anamnestic investigations indicated a wide distribution of gastrointestinal tract pathologies among horses. The conducted clinical studies confirmed the initial assumptions regarding the prevalence of gastrointestinal tract pathologies in horses (Table 1).

In the Abay region of the Beskaragay district, out of the total horse population (195 animals), 104 were selected for examination across different age and sex groups. Specifically, for clinical and specialized research, samples were taken from 6 stallions, 40 mares, and 58 young horses born in 2019 and 2020. Blood samples were collected from all selected animals from the jugular vein for rapid diagnosis using the Helpil test.

Performing ES and VGS studies required pre-anesthesia to ensure relaxation of the esophagus, thereby reducing the risk of injury. Horses in the Almaty and Zhetysu regions were pre-medicated with Domosedan at a dose of 0.5 µg per kilogram of body weight, while horses in the Abay region were administered Combistress at a dose of 0.5 cm3 per 100 kg of body weight. It should be noted that both drugs effectively calmed the horses, fully relaxed the gastrointestinal tract, and ensured successful research (Figure 5). Therefore, we recommend the wider application of these drugs

for ES and VGS studies.

During the insertion of the ES and VGS probes, the trachea was maintained in a non-sleep state, necessitating stimulation of the swallowing reflex to facilitate the passage of the probe through the pharynx into the esophagus and then into the horse's.

Stomach. Therefore, we performed tracheal insufflation with sterile physiological solution, which induced the swallowing reflex and allowed for easy.

In healthy horses, the mucous membrane of the esophagus appears pale pink and shiny. In pathological conditions, various morphological changes are observed, including hyperemia (increased blood flow), epithelial desquamation (shedding of the epithelial layer), hemorrhages, and others (Figure 6, 7).

In a normal state, the mucous membrane of the stomach is grayish in color, while the glandular region appears dark pink, with folded and shiny surfaces. No blood vessels are visible beneath the mucous membrane. In pathological conditions, there may be hyperemia of blood vessels, grayish deposits of erosive nature on the mucous membrane, inflamed areas, and various types of ulcers.

Thus, conducting endoscopic examinations under visual control has allowed us to identify pathological changes in the esophagus and stomach of horses. We consider ES and VGS examinations to be valuable diagnostic procedures for visualizing the nasal cavity, trachea, pharynx, esophagus, and horse's stomach. The introduction of innovative devices into veterinary practice will enable precise diagnosis of gastrointestinal pathologies and expand the arsenal of diagnostic and therapeutic procedures.

During the process of conducting ES and VGS examinations in the stomachs of the majority of the studied horses, regardless of age, gender, and physiological condition, we observed erosive and ulcerative changes primarily in the pyloric re-gion of the gastric mucosa. Our research confirms the data from literary sources that H. pylori causes chronic active gastritis in infected animals, which can lead to peptic ulcers and gastritis [13].

During ES and HCV studies, gastric contents and biopsy specimens were collected from the affected areas of the stomach. All collected samples were further subjected to research within two hours.

Of the selected population of horses of the Mugalzhar breed, gastrointestinal pathologies were detected in 60.0 ± 1.10% of stallionsproducers, in 76.7 ± 2.77% of mares and replacement young animals born in 2019-2020 - in 53.8-68.4%.

The results of laboratory diagnostics on Helpil-test testified to a significant susceptibility helicobacteriosis of horses. Moreover, from the number of patients with pathologies of the gastrointestinal tract, helicobacteriosis was confirmed in 66.7 ± 0.82% of stallions and 69.7 ± 0.82% of mares, and in replacement young animals - in 41.7 to 60.0%.

Table 1 - Incidence of gastrointestinal tract diseases and manifestation of helicobacteriosis in horses of different breeds depending on conditions of use and maintenance

Age and gender groups	Total number of animals	Number of heads examined	Gastrointestinal tract diseases		Horses affected by helicobacteriosis		
					From the number of animals with gastrointestinal tract pathologies		Throughout the herd
			quantity	%	quantity	%	%
Mugalzhar breed, Abay region							
Stallions	7	5	3	60.0±1.10	3	66.7±0.82	60.0±1.10*
Mares	70	43	33	76.7±2.77*	23	69.7±2.64	53.5±3.27
Colts born in 2019	15	8	5	62.5±1.37**	3	60.0±1.10**	37.5±1.37**
Colts born in 2020	25	13	7	53.8±1.80	3	42.9±1.31	23.1±1.52
Fillies born in 2019	38	19	13	68.4±2.03**	7	53.8±1.80**	36.8±2.10**
Fillies born in 2020	40	20	12	60.0±2.19	5	41.7±1.71	25.0±1.94*
Thoroughbred English riding breed, Almaty and Zhetysu regions							

Stallions	7	5	4	80.0±0.89	4	100*	80.0±0.89*
Mares	29	16	13	81.3±1.56	10	76.9±1.52	62.5±1.94
Colts born in 2019	12	7	5	71.4±1.20**	4	80.0±0.89**	57.1±1.31**
Colts born in 2020	14	6	4	66.7±1.15	3	75.0±0.87	50.0±1.22
Fillies born in 2019	14	7	5	57.1±1.31**	3	50.0±1.00**	28.6±1.20**
Fillies born in 2020	12	6	4	50.0±1.22	2	33.3±0.82	16.7±1.15
Arabian breed, Almaty region.							
Stallions	2	2	1	50.0±0.71	1	100*	50.0±0.71
Mares	5	5	4	80.0±0.89	3	75.0±0.87	60.0±1.10*
Colts born in 2019	4	4	3	75.0±0.87	2	66.7±0.82	50.0±1.00
Colts born in 2020	3	3	2	66.7±0.82	2	100.0**	66.7±0.82**
Fillies born in 2019	4	4	3	75.0±0.87	2	66.7±0.82**	50.0±1.00**
Fillies born in 2020	4	4	2	50.0±1.00	1	50.0±0.71	25.0±0.87
Akhal-Teke breed, Almaty region							
Stallions	2	2	1	50.0±0.71	1	100*	50.0±0.71*
Mares	7	7	4	57.1±1.31*	3	75.0±0.87	42.9±1.31
Colts born in 2019	4	4	3	75.0±0.87**	2	66.7±0.82	50.0±1.00
Colts born in 2020	3	3	2	66.7±0.82	2	100.0**	66.7±0.82**
Fillies born in 2019	3	3	2	66.7±0.82**	1	50.0±0.71	33.3±0.82
Fillies born in 2020	3	3	1	33.3±0.82	-	-	-
American Standardbred breed, Almaty region							
Stallions	3	3	2	66.7±0.82	2	100.0*	66.7±0.82*
Mares	23	15	12	80.0±1.55*	9	75.0±1.50	60.0±1.90
Colts born in 2019	8	7	5	71.4±1.20**	3	60.0±1.10**	42.9±1.31**
Colts born in 2020	9	8	5	62.5±1.37	2	40.0±1.10	25.0±1.22
Fillies born in 2019	11	9	6	66.7±1.41**	3	50.0±1.22**	33.3±1.41**
Fillies born in 2020	11	10	5	50.0±1.58	2	40.0±1.10	20.0±1.26

Note: * - significance of disease prevalence in stallions and mares;
** - significance of disease prevalence between colts born in 2019 and 2020

Figure 5 – Insertion of the endoscope probe into the stomach

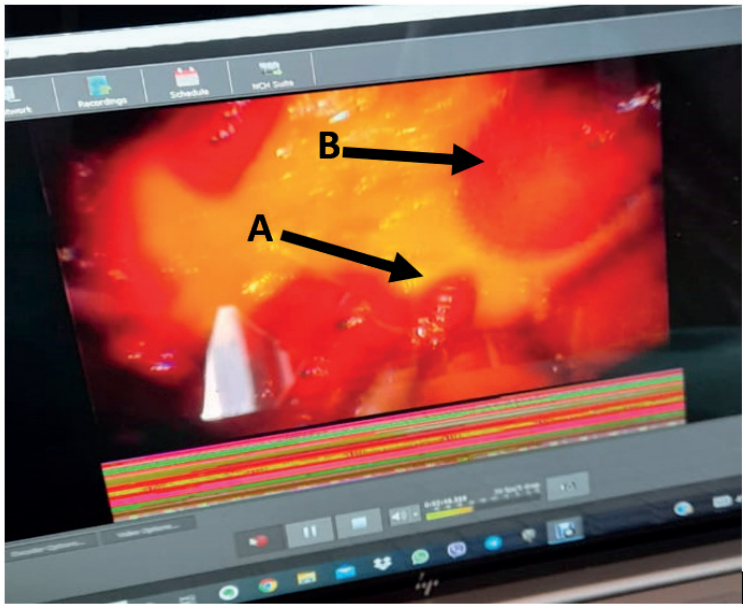

Figure 7 - Neoplasms in the gastric wall

Our research supports the opinions Murray M.J. et. al. about the significant prevalence of this pathology among the adult population of horses and young animals [14].

Helicobacteriosis was confirmed in 40.0±1.10 percentage of stallions and 53.5±3.27 percentage of mares from the number of the studied livestock. Moreover, the differences in indicators between the sexes in adult animals were significant P≤0.001.

The incidence among young animals was 23.1-37.5%. At the same time, the differences in the incidence rates between the age groups of replacement young animals were also significant (P≤0.001). Consequently, the susceptibility to helicobacteriosis significantly increased with age.

From the pathology of the gastrointestinal tract, gastrophylles, erosions and ulcerative lesions of the gastric mucosa were recorded. The greatest changes were observed in the pyloric part of the stomach. The conditions for keeping these animals were around the clock grazing, watering - in the wild without additional feeding.

The dependence of the susceptibility of horses to pathologies of the gastrointestinal tract, including helicobacteriosis, on the conditions of operation and maintenance has been established. Thus, studies conducted in a number of farms in Al-maty, Zhetysu regions indicate that this pathology is widespread in horses of the English thoroughbredriding breed. These horses were in training and participated in racetrack trials. At night, the horses were kept in stalls, and during the daytime - in levada or were in training. Horses were fed according to the approved diet and schedule. Thus, in adult horses of the English Thoroughbred riding breed, gastroin-testinal pathologies were observed in 80.0-81.3%, and in replacement young ani-mals - 50.0-71.4%. At the same time, in 76.9-100.0% of adult animals with pathologies of the gastrointestinal tract, the Helpil-test for Helicobacter pylori was positive and in replacement young animals 33.3-80.0%. Of the number of examined horses of this breed, helicobacteriosis was confirmed in 62.5-80% of adults and in 16.7-57.1% of replacement young animals. Apparently, stress and sports loads dur-ing training and competition, to a certain extent, were reflected in the morphofunc-tional state of the gastrointestinal tract, and the whole organism as a whole.

In the adult stock and replacement young stock of horses of the Arabian breed, there was also a widespread pathology of the gastrointestinal tract, respectively 50-80% and 50-75%. A large percentage of helicobacteria in horses with gas-trointestinal pathologies of 75-100% and 50-100% has also been established. The specified number of horses were also used in sports - a smooth race. The difference between the incidence in sex and age groups was significant. A similar pattern of gastrointestinal diseases and helicobacteriosis was observed in Akhal-Teke and American Standardbred horses from the entire studied population. These findings were confirmed by the results of bacteriological studies. The incidence rates differed significantly among adult and young horses.

During gastroscopic examinations of 124 horses from Almaty region, small erosions and mucosal hyperemia were detected in the stomachs

of 68 horses (54.8%), no changes were observed in 16 horses (12.9%), and hyperemia was present in 36 horses (29.0%). A similar picture was observed during gastroscopic examinations of horses from the Zhetysu region. Specifically, 16 horses showed erosions and mucosal hyperemia (53.3%), hyperemia was present in 10 horses (33.3%), and no changes were observed in 4 horses (13.3%). In the Abay region, small erosions and mucosal hyperemia were detected in the stomachs of 45 horses (41.7%), no changes were observed in 33 horses (30%), and hyperemia was present in 30 horses (27.8%).

Thus, helicobacteriosis is an infectious disease transmitted through contaminated water and feed. The causative agent, H. pylori, has a strong affinity for the gastric epithelium, playing an important role in the pathology of the digestive system in horses. Therefore, special attention should be given to animals with frequent colic episodes, decreased appetite, and poor body condition during the diagnosis of helicobacteriosis.

Foreign scientific data indicate a high prevalence of ulcers among horses and foals [15, 16].

In some cases, infected carriers of Helicobacter pylori do not show any symptoms of the disease. Therefore, it is erroneous to only investigate suspicious animals with indications for helicobacteriosis.

Thus, the results of our research indicate that gastrointestinal pathologies are widely spread among horses. Helicobacteriosis in horses occupies a prominent place among gastrointestinal pathologies, necessitating further study and the development of treatment and prevention measures.

Discussion

The conditions of horse husbandry to some extent influenced the morphofunctional state of the gastrointestinal tract (GIT). From the above data, it can be seen that pasture-based management (in the case of Mugalzhar breed horses) resulted in significantly fewer GIT pathologies compared to stable-based management (in the case of horses of other breeds). There was also a significantly lower prevalence of helicobacteriosis.

Thus, both training and husbandry conditions have a negative impact on the prevalence of gastrointestinal tract (GIT) pathologies, both in adult horses and in young stock. In our research, we frequently diagnosed gastric erosions and ulcers in horses of sport breeds and those involved in competitive activities such as racing, dressage, show jumping, etc. Our findings support the data from various authors indicating that the prevalence of GIT pathologies, including helicobacteriosis, can reach up to 80% during intensive training periods. This is likely associated with stress factors related to transporting the animals to competition venues, intensified training, changes in diet, as well as individual characteristics of the animal's nervous system.

The obtained data indicate that in horses used for training and competitive events and housed in a stable environment, *H. pylori* is significantly more prevalent than in horses of productive direction that are kept on pasture round the clock.

Conclusion

Gastrointestinal tract (GIT) pathologies in horses have a wide prevalence ranging from 50% to 80%. The main pathologies in the equine GIT include gastrophilosis, erosions, ulcers, and Helicobacteriosis. Helicobacteriosis is registered in 66.7% to 100% of the examined adult population and in 20% to 66.7% of the young horses.

Endoscopic and gastroscopic examinations provide a clear picture of the condition of the mucous membrane in different parts of the stomach and enable the collection of biomaterials for further investigations. The percentage of Helicobacteriosis increases with age in animals.

It is incorrect to only test suspicious animals or those with symptoms for Helicobacteriosis since not all carriers of Helicobacter pylori show signs of the disease.

During endoscopic and gastroscopic examinations, it is recommended to use Domosedan at a dose of 0.5 mcg per kilogram of body weight or Combistress at a rate of 0.5 cm3 per 100 kg of body weight. These medications effectively calm the animals and fully relax the GIT, ensuring successful examination procedures.

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BOVINE SARCOCYSTOSIS IN KOSTANAY REGION

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Abstract

This article presents the results of the prevalence of sarcocystis invasion of cattle in the Kostanay region. The selection of cattle muscles and morphological studies were carried out from January to April 2023. In total, 100 carcasses, in-cluding 75 carcasses of bulls aged 2-3 years and 25 carcasses from cows aged 7-9 years, were examined at the slaughterhouses of the city of Kostanay, where cattle for slaughter come from different districts of the Kostanay region. During visual veterinary and sanitary examination of carcasses, macrocysts were not found. Microscopy of 300 samples of muscle tissue was carried out to determine the contamination of carcasses. During the research, it was found that sarcocysts were found in the muscles of steers and cows. The localization sites, shape, size of sarcocysts and their intensity were studied. According to morphological features the cysts correspond to the description of Sarcocystis bovicanis (S.cruzi).

Key words: cattle; extensiveness; intensity; microscopy; muscles; sarcocystosis.

Basic position and Introduction

Protozoal diseases caused by parasites belonging to the type of protozoa are widespread in many countries of the world and represent a serious danger to modern society. Sarcocystis occupies an important place among protozoan diseases, due to its wide spread, multiplicity of hosts, constant growth of morbidity and significant social and economic damage caused by it. According to Lithuanian scientists, sarcocystosis is one of the most widespread parasites of domestic cattle, as well as of many wild mammals, birds and humans. The high prevalence of infection in cattle reaches 44.9-98.1%, in sheep 100%, and in pigs 30.1-50.0% and horses 34.7-63.9% [1].

According to G.S. Sivkov, S.A. Ryabov, A.A. Listishenko (2005), V.I. Abakumov, O.V. (2008), N.M. Polyanskaya, P.A. Svintsova, O.V. Serdobintseva (2002), J.K. Latif, V.S. Delemi, Mohammed (1999) domestic ruminants are especially strongly affected and the invasion by sarcocysts in the regions ranges from 0.2 to 98% [2-7].

Sarcocystis is a chronic disease of animals and wild birds characterized by affection of skeletal muscle tissue, including muscles of tongue, pharynx, esophagus and internal organs. Severe damage results in muscle degeneration, emaciation, tissue hydraemia and often fatal outcomes. To date, sarcocysts have been found in the muscles of over 150 animal species. The causative organisms are parasites belonging to the genus Sarcocystis [8, 9, 10].

Currently, there is no information on the spread of sarcocystosis among farm animals in the Kostanay region, in this regard, the purpose of our research was to study the prevalence of bovine sarcocystosis in Kostanay region, to identify indicators of extensiveness (EI) and intensity (II), as well as determine the morphological features of muscle cysts.

Materials and Methods

To study spread of sarcocystis infestation in cattle at the slaughter points of Kostanay city where cattle are sent for slaughter from different districts of the region in the period from January to April 2023 were examined 100 carcasses, including 75 carcasses from steers aged 2-3 years, belonging to farms of Karasu, Uzunkol and Naurzum regions and 25 carcasses from cows aged 7-9 years from Denisovsky region. Visual veterinary and sanitary examination of the carcasses, macrocysts were not found. Further research was conducted at the Research Institute of Applied Biotechnology of A.Baitursynov Kostanay Regional University.

The intensity of invasion was determined by microscopic studies using compressor MI 4.2.2747-10 [11].

The material for the research was 300 samples of muscle tissue from the neck, diaphragm legs and skeletal muscles obtained from 100 cattle. Muscle parts were applied to a synthetic mesh. The muscle samples were impregnated with 0.2% aqueous methylene blue solution for 20-30 minutes. After staining, muscle pieces along with the synthetic mesh were placed on filter paper for drainage. This process is performed to remove excess dye. The samples were held on the filter paper for only a few seconds. The stained muscle pieces were immersed in a 1.5% acetic acid solution for 15-20 minutes to lighten the samples. Light agitation was performed to avoid sticking of the samples. Next, the samples were placed again on filter paper for drainage and then transferred to a glass compressor consisting of two glass plates tightened with screws.

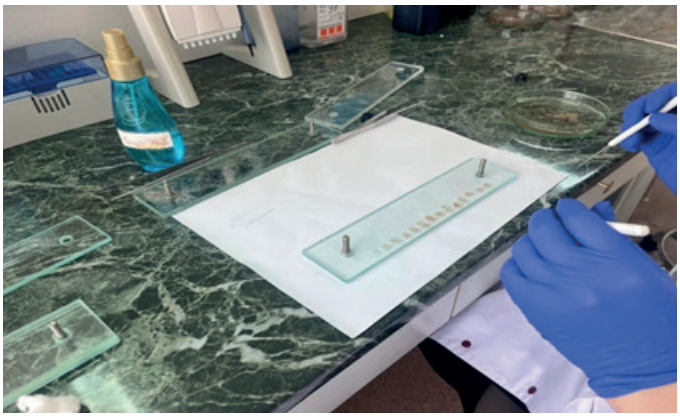

Figure 1 - Fresh muscle preparations placed in a glass compressorium

Muscle tissue samples stained with methylene blue were examined using a light microscope at a magnification of ×100 to ×400, where the number of sarcocysts in each slice was counted, and the intensity of invasion was estimated by counting sarcocysts in 28 slices of muscle tissue. The total parasite count was used to determine the intensity of invasion. The criteria for assessing the infestation intensity are shown in Table 1.

Table 1 - Criteria for assessing the intensity of sarcocystic invasion

The intensity of invasion	Number of cysts of Sarcocystis spp.	
	in the microscope field of view	in 28 slices of compressorium
weak	1-3	under 50
medium	under18	51-200
strong	over 18	over 201

Sarcocyst invasion was conventionally classified as strong (over 200 sarcocysts in 28 sections), medium (51-200 sarcocysts), and weak (up to 50 sarcocysts) [12-15]. The size characteristics of sarcocysts were statistically processed using Statistica StatSoft 10 software.

Cytometric measurements were performed using an OPTIKA PRIVIEW microscope software calibrated with an ocular ruler. The confidence coefficient was determined by conventional methods using the Microsoft Office Excel 2007 software package.

Results

Kostanay region is located in the north of Kazakhstan and borders five re-gions of the Republic of Kazakhstan (Aktobe, Ulytau, Karaganda, Akmola and North Kazakhstan) and three regions of the Russian Federation (Orenburg, Chelyabinsk and Kurgan). The territory is characterized by relatively flat terrain and "extreme" continental climate. Winters are long, frosty, with strong winds and snowstorms, and summers are hot and dry. The annual rainfall is 350-500mm in the north of the region and 240-280mm in the south. The region includes 16 dis-tricts and 4 cities of regional subordination. In order to carry out the research the territory of the region was conditionally divided into zones: northern, southern, western and eastern. The northern zone, a zone of temperate moisture (forest-steppe), united four northern districts; the southern zone, a zone of insufficient moisture (steppe) - four districts and the city of Arkalyk. The remaining eight dis-tricts are assigned to the western and eastern zones.

From January to April 2023, 300 samples of muscle tissue were examined. Animals came from four zones of the region. Infection of slaughtered cattle with sarcocysts of different ages and zones is presented in Table 2.

Table 2 - Extent of cattle infestation

Region	Species of animals	Age	No. of surveyed	No. of infected	% of infection
North	steers	2-3 y.o.	25	24	96
South	steers	2-3 y.o.	25	25	100
Western	cows	7-9 y.o	25	25	100
Eastern	steers	2-3 y.o.	25	25	100

The data in Table 2 show that in the northern region out of 25 examined carcasses of two-three-year-old steers, sarcocysts were found in 24 carcasses. Infestation rate in the northern zone is 96%. In the Southern, Eastern and Western zones the rate of infestation of steers and cows aged 7-9 years is 100%.

Carcasses of 2-3-year old steers of the northern, southern and eastern zones (Table 3) were highly infested with Sarcocystis spp.

Table 3- Number of Sarcocystis spp. cysts in cattle muscle by zone

Region	Species of animals, Age	Place of sampling	Sarcocystis spp. cysts detected (%) in muscle		
				medium	strong
North	steers 2-3 y.o.	Neck	88±4,89	8±0,44	-
		Diaphragm	17±0,94	9±0,50	-
		Skeletal	8±0,44	3±0,17	-
South	steers 2-3 y.o.	Neck	11±0,61	13±0,72	1±0,06
		Diaphragm	9±0,50	4±0,22	-
		Skeletal	5±0,28	-	-
Western	cows 7-9 y.o	Neck	72±4,00	28±1,56	-
		Diaphragm	48±2,67	36±2,00	8±0,44
		Skeletal	21±1,17	15±0,83	-
Eastern	steers 2-3 y.o.	Neck	12,5±0,70	48±2,67	-
		Diaphragm	48±2,67	10±0,56	4±0,22
		Skeletal	5±0,28	-	-

Infestation of neck muscles with sarcocysts was observed in 24 carcasses, which is 96%, in 5 carcasses diaphragm muscles were affected, which corre-sponds to 20%, skeletal muscles were affected in 7 carcasses, which corresponds to 28% of the total number of carcasses examined.

Weak intensity of invasion in the skeletal muscles was detected in 3 districts of the studied zones, medium - of four.

Carcasses from the northern zone of the region

were almost all infested, on-ly one carcass was not infested with sarcocysts. Neck, diaphragm and skeletal muscles of 2-3-year-old steers from the northern zone were weakly and moderately infested, and in steers from the southern and eastern zones the skeletal muscles were free from Sarcocystis spp.

Weak degree of sarcocysts lesion was found in 0.45 % of the examined carcasses, medium - in 0.13 %, strong degree - in 0.05 %. As a whole, microscopic examination of the muscle samples from 75 carcasses of 2-3 years old steers showed positive results for 74 carcasses. The muscles of the neck and crura of the diaphragm were more affected; in the skeletal muscles the invasion was classified as weak. Of the 75 cases of Sarcocystis infestation in animals, weak invasion was 55%, moderate - 38%, strong - 6.7% of all detected cases of infestation. The highest number of Sarcocystis spp. cysts was found in the neck muscles and crura of the diaphragm, the lowest number in the skeletal muscles (Table 3).

The maximum number of Sarcocystis spp. cysts was found in diaphragm muscles, 2,090.18 specimens; less in neck and skeletal muscles, 1,323.73 speci-mens.

In neck, skeletal, and diaphragm crura muscles, the bulk of Sarcocystis spp. cysts had elongated, spindle-shaped, and oval shapes with pointed and rounded tips, of different lengths (Fig. 2-4). In the diaphragm muscle fibers, cysts were predominantly shaped with long, sharp ends (Figs. 3). Individual specimens of cysts in neck muscles and crura of diaphragm, as well as in skeletal muscles, had a spindle shape (Figs. 4) and there were no significant differences in size.

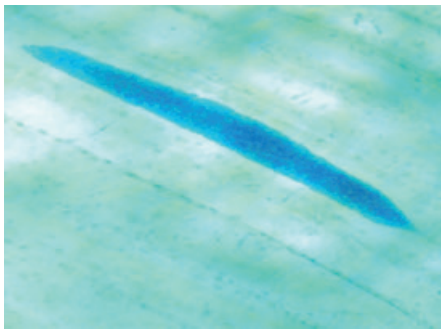

Figure 2 - Elongated-longitudinal shape of cysts in the neck muscles

Figure 3 - Spindle-shaped cysts in the diaphragm muscle

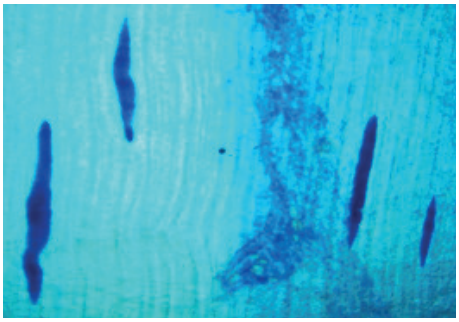

Figure 4 - Elongated cysts with

Discussion

During the visual veterinary and sanitary examination, pathological changes (exhaustion, hydroemia, discoloration, calcification of muscle tissue, degenerative changes) and parasite cysts were not detected in muscle samples. However, during microscopic studies, it was found that all samples of both necks, diaphragms and skeletal muscles contained tissue cysts of Sarcocystis spp.

When analyzing the data obtained, it was found that the highest EI was noted in all districts of the region. The extent of invasion in the northern zone is 96%. In the southern, eastern and western zones, the infection rate of bulls and cows aged 7-9 years is 100%. Carcasses of 2-3-year-old bull calves of the northern, southern and eastern zones turned out to be heavily infested with Sarcocystis spp.

A weak intensity of invasion in skeletal muscles was detected in 3 districts, the average – out of four. However, two heads of cows in the Denisovsky district and two- to three-year-old bulls in the Karasu district had a very strong intensity of invasion.

Conclusions

The received data testify to the wide spread of sarcocystosis invasion in cattle in Kostanay region. Extensity of invasion is 99%. The intensity of invasion is more weak, from 1 to 4-7 cysts in a slice, medium from 18 to 181 and strong from 207 to 279 specimens.

Morphometric data suggest the presence of invasion in cattle in our region by at least two representatives of the genus S. bovicanis and S. bovifelis, forming thin-walled and thick-walled cysts.

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STUDYING THE DIAGNOSTIC VALUE OF RECOMBINANT *CAMPYLOBACTER JEJUNI* ANTIGENS

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Abstract

This article describes the results of a study on the diagnostic value of *Campylobacter jejuni* recombinant antigens, *Campylobacter* Omp18 protein and major outer membrane protein (MOMP). In the present study, these proteins were used as antigens in enzyme immunoassays to detect specific antibodies in the serum of cattle. Commercial native protein antigens were used to compare the effectiveness with similar studies. In total, 95 blood serum samples from cattle from various farms in the northern region of the Republic of Kazakhstan were used. The greatest number of positive results was observed when using a commercial native antigen (52.6%); 47.4% of sera reacted positively with respect to the Omp18 antigen, and 38.9% with respect to the MOMP antigen. Statistical analysis revealed a high correlation between the native and Omp18 antigens and, to a lesser extent, with MOMP32. Because native antigens contain many different cellwall proteins, they are less specific. Therefore, for serological diagnosis of cattle using enzyme immunoassays, it is advisable to use the *C. jejuni* outer membrane recombinant antigen MOMP32, as it has valuable diagnostic properties.

Key words: Antigens; *Campylobacter jejuni;* campylobacteriosis; diagnostics; enzyme immunoassay; recombinant proteins; specific antibodies.

Basic position and Introduction

Campylobacteriosis is an infectious disease that affects animals of many species, caused by pathogenic microorganisms from the genus *Campylobacter*, and characterized by varying degrees of damage, severity of the course and polymorphism of manifestations. Campylobacteriosis has recently been reported as a food-borne disease in countries worldwide [1,2].

The antigenic structure of *Campylobacter* is complex, as evidenced by a large number of cross-reactions between type strains and freshly prepared cultures. The differences in the antigenic structure of *Campylobacter* are used for serological typing. Antigenically, *Campylobacter* are heterogeneous; they are clearly differentiated by agglutination and indirect haemagglutination reactions. The structure of campylobacteriosis-pathogen antigens is represented by three thermostable O-antigens, seven thermostable K-antigens, and thermolabile H-antigens. *C. jejuni* and *C. coli* are the most common *Campylobacter* pathogens in humans and animals and are serologically heterogeneous. Fifty-five serogroups based on thermostable antigens have been previously described [3].

In diarrhoea caused by *Campylobacter*, there is a classic immune response. First, it is directed to membrane proteins and the flagellar antigen. Using the ratio of immunoglobulins of individual classes in the body, one can judge the presence of campylobacteriosis and the approximate timing of the disease course. High IgG titres persist for 3 months or more, and IgA titres persist for 1 month

after illness [4].

At present, the existence of a "universal" *Campylobacter* antigen, that is, an antigen whereby antibodies would be detected in the serum of all patients in sufficiently high titres, remains unclear. Qian et al. suggested that for C. *jejuni,* this universal antigen is the p43 protein, whereas according to Blaser et al., it is the p44 protein [5,6].

To determine the role of various protein antigens in the immune response during campylobacteriosis, immunoblotting, which enables the simultaneous detection of antibodies against antigens with different molecular weights, is used. The intensity of the reaction and antibody titres increase significantly as the disease progresses and during the convalescence stage. Previously, immunoblotting revealed no cross-reactivity with 21 enterobacteria, staphylococci, and streptococci [7,8].

The most important surface antigens are the lipopolysaccharide and acid-soluble protein fractions. These antigens play a fundamental role in the serotyping of *C.fetus* and *C. jejuni,* and in the serological diagnosis of campylobacterio-sis. Antigenic differences among bacteria of different serotypes are associated with the carbohydrate composition of the internal lipopolysaccharides of *Campylobacter*. The chemical composition of *C. jejuni* lipopolysaccharides are similar to that of the antigens of other enterobacteria. In addition to the similarity in chemical structure, immunoblotting and other methods have established a signifi-cant immunological relationship between *Campylobacter* lipopolysaccharides and other intestinal infection pathogens, including *Salmonella, Yersinia, Brucella, Shigella* [9,10,11].

Therefore, the purpose of our study was to determine the diagnostic value of recombinant proteins of the outer membrane of *C. jejuni* with molecular weights of 18kDA and 32 kDa, and their potential use in the serological diagnosis of campylobacteriosis in animals.

Materials and Methods

Recombinant proteins of the outer membrane of *C. jejuni* (Omp18 and MOMP32) obtained from the laboratory of immunochemistry and immunobiotechnology of the National Center for Biotechnology, Astana, Kazakhstan [12], and a commercial native protein antigen C*ampylobacter jejuni* antigen (Code: NAT41600-100, Native Antigen, UK) were used as antigens.

To determine the molecular weight of the recombinant proteins, electrophoretic separation was performed according to the method described by Laemmli [13] on a polyacrylamide gel (11%) using a vertical electrophoresis apparatus (BioRad, USA). The activity of the recombinant proteins was determined by western blotting using positive control sera. Electrophoretic transfer of antigens from the gel to a nitrocellulose membrane (NCM) was performed using an immunoblotting device (Bio-Rad, Hercules, CA, USA) as previously described by Towbin et al. [14]. For immunochemical manifestation of specific antigens, NCM were incubated in a 1% solution of bovine serum albumin (BSA) overnight at 4°C. They were then washed three times with phosphate buffered saline (PBS) and PBS-Tween (Tw) and incubated for 1.5 h at 37°C in a solution of specific antibodies. The carrier was washed and incubated in the working dilution of peroxidase-labelled anti-species antibodies for 1 h at 37°C. The substrate was prepared before use:0.01 g 4-chloronaphthol (Sigma, USA) was dissolved in 2 ml of alcohol (90%), and mixed with 18 ml PBS (pH 7.2-7.4) and 0.01 ml of 3% hydrogen peroxide. After protein replicates appeared, the reaction was stopped by washing the membranes with distilled water.

Approximately 95 samples of blood sera were obtained from cattle from various farms in the Akmola, Karaganda and Kostanay regions.

Blood sera were analysed by enzymelinked immunosorbent assay (ELI-SA). The wells of a polystyrene plate (Thermo Fisher Scientific, USA) were sensitised separately with the protein antigens, Omp18 and MOMP32, and a commercial antigen at a concentration of 0.001 mg/ml. After sensitisation, the active sites of the solid phase were neutralised with 1% BSA solution. Dilutions of blood serum in PBS-Tw were prepared in two wells 1:100-1:200, incubated for 1 h, and labelled anti-bovine IgG antibodies (Sigma-Aldrich, USA) were added after washing. A reaction was considered positive if the optical density (OD) of the serum was two or more times higher than the average OD of the control sample at a dilution of 1:100. Blood serum from healthy animals was used as the negative control. To exclude unreliable results, the experiments were performed in triplicate.

Statistical analysis. Statistical analyses were performed using GraphPad Prism version

9.3.1. (GraphPad software). The statistical tests used as well as the p-values are indicated in the figure captions. A p-value of 0.05was considered statistically significant.

Results

To determine the activity of the recombinant proteins in comparison with native commercial antigens, electrophoretic separation was performed, followed by immunoblotting using polyclonal antibodies (pAbs) commercial native *C. jejuni* antigen. Western blot analysis showed that the pAbs reacted specifically with the recombinant proteins (Figure 1).

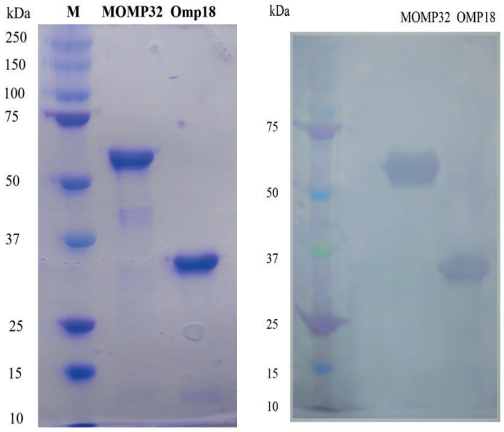

Figure 1 - Electrophoresis and western blot with recombinant antigens

As shown in Figure 1, the molecular weights of the recombinant C. jejuni OMPs were 36kDa and 64 kDa for OMP18 and MOMP32, respectively. The high molecular weights of the recombinant proteins were due to the presence of thioredoxin in the expression vector. These data were confirmed by Western blotting.

The immunoblotting results demonstrated that pAbs reacted with both recombinant proteins.

Recombinant proteins were used as antigens when establishing an indirect variant of an enzyme immunoassay with blood sera from cattle on farms in the Karaganda region. A native commercial antigen was used for comparison. The test results are presented in Table 1.

Table 1- Results of testing bovine blood sera using various antigens

OD multiplicity values OD of the test sample/ OD of the control sample	Commercial native antigen	MOMP32	OMP 18
	Number of samples	Number of samples	Number of samples
Total	95	95	95
0-0,999	37	45	32
1,000-1,999	8	13	18
2,000 and higher	50	37	45
Note: OD ratio is the ratio of the optical density of the reaction medium relative to the optical density of the negative control. Values of optical multiplicity from 2 and above show that the samples are positive			

As shown in Table 1, when using a commercial native antigen, 50 positive serum samples were detected, which represented 52.6% of the total number of samples tested. Specific antibodies to the recombinant MOMP32 antigen were reliably confirmed in 37 sera samples (38.9%), and an interaction with serum anti-bodies was recorded in 45 samples (47.4%) in the case of the OMP18 antigen.

To determine the diagnostic value of the recombinant antigens, the OD distribution of positively reacting blood serum samples with recombinant and native antigens was analysed. The results of this analysis are shown in Figures 2 and 3.

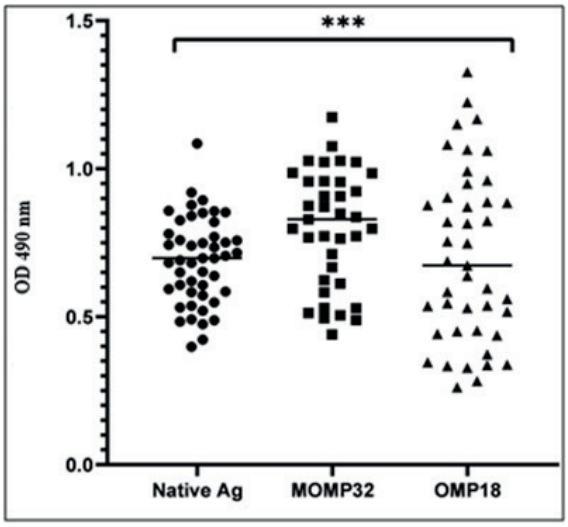

Figure 2 - Analysis of the distribution of optical parameters of positive sera with recombinant and commercial native antigens. Note: each character represents an optical parameter. Statistical analysis was performed with log-transformed data using the Student's t-test: ***p, 0.005.

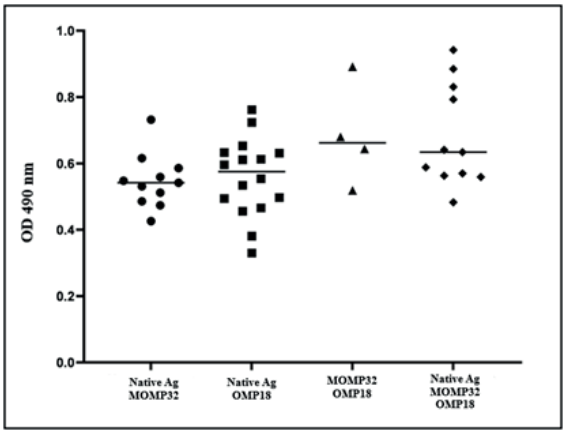

Figure 3 - Analysis of the distribution of coincidences with regards to optical parameters of positive sera in the reaction with recombinant and commercial native antigens

The analysis suggested that the most compact distribution of the optical parameters of bovine sera was observed for the commercial native antigen, with an average of 0.700 OD.The optical reaction parameters of sera with recombinant MOMP32 were also distributed compactly, but with higher OD values equal to an average of 0.800. The same sera containing the recombinant OMP18 antigen showed a wide range of optical reaction parameters ranging from 0.4 to 1.4 OD. This scattering resulted in a decrease in the mean OD values compared to the OD values of the commercial native and MOMP32 antigens. Despite the differences between the OD of OMP18, commercial native, and MOMP32 antigens, the results demonstrated the suitability and interchangeability of antigens for use as components of ELISA in the detection of campylobacteriosis.

This assumption was confirmed by analysing the distribution of optical reaction parameters that coincided with different antigens. Figure 3 shows that the maximum agreement between the results was recorded when using the commercial native antigen and the recombinant OMP18 antigen, which was noted when testing 16 blood serum samples (16.84%). When comparing positive results using the commercial native antigen and the recombinant MOMP32 antigen, a match was found in 10 cases (10.52%). When comparing the results of the interaction of antibodies in the sera with recombinant and native antigens, the coincidence increased slightly for up to 11 positive samples. Notably, the most insignificant coincidence accounted for the variant of the interaction of sera with the two recombinant antigens, which was established in only four cases.

Discussion

Campylobacteriosis is one of the main causes of food poisoning in humans and manifests as diseases of the gastrointestinal tract. It has been established that the main source of infection is the pathogen *C. jejuni,* which circulates in animals and inseminates livestock products during slaughter and butchering of carcasses.

At present, the standard diagnostic method for detecting *C. jejuni* is the bacteriological method; the limitation of this method lies in the complexity of isolating the culture, the duration of cultivation, and the need for special media. Of the modern diagnostic methods, PCR diagnostics can also be noted, but this method requires special conditions, the availability of qualified specialists, and expensive equipment.

Improvements in serological diagnostic methods, simple execution, and ef-fective performance will allow for the detection of specific antibodies that indicate the circulation of campylobacteriosis pathogens in the body of a given animal. One of these methods is the enzyme immunoassay; however, the specificity and sensitivity of the method depends on the quality of the antigen, which is the main component of the diagnosticum. The use of recombinant antigens is a promising approach for serodiagnosis [15,16].

As our studies have shown, recombinant antigens, as ELISA components in the diagnosis of campylobacteriosis, and native commercial antigens, have high specificity and sensitivity.

Conclusion

As a result of the study, the diagnostic value of previously obtained recombinant antigens of the outer membrane of *Campylobacter jejuni* was studied, and the possibility of their use in ELISA for the serological diagnosis of campylobacteriosis was investigated.

The effectiveness of the recombinant antigens was tested by western blotting and ELISA and compared with native commercial antigens. Western blot analysis showed that the recombinant MOMP32 (64 kDa) and Omp18 (36 kDa) antigens reacted with pAbs. ELISA of 95 bovine sera samples revealed 50 positive samples for the commercial native antigen, 37 samples for the recombinant MOMP32 antigen, and 45 samples for the Omp18 antigen. When analysing the results, it was found that the highest correlation was observed between native and Omp18 antigens, since matches were recorded with 16 samples. Antigen coincidence between native and recombinant MOMP32 was detected in 10 blood serum samples. It can be assumed that the difference in the number of positive results was dependent on the fact that many different cell wall proteins are present in native antigens; therefore, they are less specific.

Therefore, we recommend the use of the recombinant antigen MOMP32 from the outer membrane of *Campylobacter jejuni* in enzyme immunoassays for the serological diagnosis of campylobacteriosis in cattle.

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BIOLOGICAL CHARACTERISTICS OF RHODOCOCCUS EQUI ISOLATED IN THE REPUBLIC OF KAZAKHSTAN

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Abstract

Rhodococcus equi is characterized by pyogranulomatous bronchopneumonia, septic arthritis, osteomyelitis, ulcerative enterocolitis, mesenteric lymphadenopathy, neonatal diarrhea, and sudden death of young animals. The development of a clinical disease is associated with the immunocompetence of the body of foals. As a worldwide soil infection, it is responsible for approximately 3% of all foal deaths, with a mortality rate of approximately 50%.

The aim of our research was to study the morphological and biochemical properties of the putative *R. equi* strain isolated by us on the territories of our republic.

At about 3-4 months of age, foals are capable of mounting an immune response against *R. equi,* which is why vaccination is given at an early age. The horse industry worldwide urgently needs an effective vaccine to prevent *R. equi* disease in foals, and current scientific research is focused on developing a vaccine using a local strain.

We collected 27 pools manure and 27 pools soil samples from the Almaty, Zhambyl, Turkestan, and East Kazakhstan regions for bacteriological research. The samples were taken from exercise pens, areas within 100 meters of stables, passages, ditches, roads, and flower beds. We isolated a characteristic strain of *R. equi* and studied its cultural, morphological, tinctorial, and biochemical properties. Out of all the isolates, only the one from the Almaty region was a typical representative of the species *Rhodococcus equi*.

Key words: biochemical characteristic; blood agar; isolate; tryptone soy agar; ram erythrocyte; *Rhodococcus equi.*

Basic position and introduction

Rhodococcus equi is a soil aerobic actinomycete bacterium that infects animals and humans. Human infections are thought to be opportunistic and zoonotic in origin, and may be related to environmental exposure on farms [1-3]. Although clinical cases of *R. equi* are relatively rare in most animal species, foals are often sick, and the incidence is often high on horse farms in countries where horses are bred [4].

For more than 80 years, *R. equi* has been recognized as a lung pathogen in horses. Infection can spread from the lungs to other organs and joints when granulomatous lesions in the lungs rupture and infection of the intestinal mucosa causes diarrhea with ulcerative enteritis and R. equi mucosal invasion, which is often seen in chronic disease. Immune complex deposition can cause polysynovitis, which contributes to the development of uveitis, anemia, or thrombocytopenia in infected foals. Sometimes osteomyelitis and arthritis are also observed [5].

R. equi is a Gram-positive, aerobic, non-motile, non-spore-forming and metabolically diverse bacterium. Representatives of the genus

Rhodococcus (red pigmented cocci) belong to the phylogenetic group described as actinomycetes of the nocardia form. The infection causes subacute or chronic abscess or purulent bronchopneumonia, ulcerative lymphangitis, enteritis and is the cause of zoonotic infection in foals aged 1- 4 months [6, 7].

Although infections can occur in healthy adult horses, they are more common and severe in foals due to their weakened immune systems. It has been found that only a small fraction of all *R. equi* in soil can cause infection, and only *R. equi* carrying virulence plasmids can cause disease in foals [8].

It is known that in some strains of *R. equi*, the presence of a plasmid encoding a 15–17 kilodaltons protein, called protein A (Vap A), associated with virulence, is responsible for virulence [9]. In 85% of cases, the presence of the Vap A virulence plasmid has been associated with *R. equi* infection in foals over the past few decades [10]. Experiments have shown that the presence of the Vap A expression plasmid in *R. equi* can increase the percentage of macrophages killed in a standard trypan blue assay by 20-70% compared to an equivalent strain without the plasmid.

The present study was undertaken to study the biological characteristics of *R. equi*, isolated by us in the horse breeding farm of the Almaty region.

Materials and Methods

Collection and bacteriological examination of material from horse breeding farms (feces, soil) to isolate the culture of *R. equi.* From each farm, material was collected from three points:

1 - manure;

2 - "pen for exercise", within 100 meters from the stable;

3 - soil within 15 meters of the stable (passages, ditches, roads, flower beds, etc.).

Manure was collected from randomly selected horses. All samples were scraped from the ground with a small spoon and placed in individual sterile containers designed for collection of biomaterials with a screw cap in an individual package. They were stored at 4°C until further processing.

Samples collected from each of the three points in a given farm were combined to create a single pool. The number of samples are given in tables 1-3.

Table 1 - List of samples obtained from the territory of the Almaty region

No.	District name	Name of the village, farms	Number of samples		
			1	2	3
1	Karasai	v. Kairat	4	3	3
		v. Turar, IE "Dias"	3	3	3
2	Ili	v. Akshi	2	2	2
3	Zhambyl	v. Uzynagash	3	3	3
4	Talgar	v. Panfilovo, Bayserke-Agro LLP	6	4	4
		Nur p/a	6	5	3
5	Enbekshikazakh	Karazhota r/d., “Seisenbaev Zh.” p/a	8	4	4
		Karazhota r/d, “Maukenov N.” p/a	6	3	3
		Akshi r/d, “Kasenov Rahman” p/a	6	5	3
6	Kaskelen	“Aitumar” p/a	7	5	3
		"Gaziz" p/a	5	5	3
7	Kegen	Karkara r/d, “Kumteke” p/a	6	5	4
		Zhylysai r/d, “Bagasharov” p/a	8	5	3
8	Almaty city	Almaty Hippodrome	5	5	4
	Number of pools		14	14	14
Note: v - village; IE - individual entrepreneur; r/d - rural district; p/a - peasant agriculture					

Thus, materials were collected for bacteriological examination from all points in 14 pools from the horse breeding farms of the Almaty region.

The ranking of samples in the Zhambyl region is shown in Table 2.

Table 2 - List of samples received from the territory of the Zhambyl region

No.	District name	Name of the village, farms	Number of samples		
			1	2	3
1	Karasai	v. Kairat	4	3	3
		v. Turar, IE "Dias"	3	3	3
2	Shu	Baluan Sholak r/d, "Kalka" p/a	8	5	4
		Zhanakogam r/d, "Ospanov" p/a	7	5	3
3	Merke	Aktogan r/d, "Tuzelbay" p/a	6	5	3
		Aktogan r/d, “Yesen" p/a	8	5	3
		Zhambyl r/d, "Myrzakhan" p/a	7	5	3
	Number of pools		7	7	7
Note: v - village; IE - individual entrepreneur; r/d - rural district; p/a - peasant agriculture					

Thus, from the horse breeding farms of the Zhambyl region, materials were collected for bacteriological examination from all points in 7 pools.

Thus, materials were collected for bacteriological examination from all points in 6 pools from the horse breeding farms of the Turkestan region (table 3).

Table 3 - List of samples obtained from the territory of the Turkestan region

No.	District name	Name of the village, farms	Number of samples		
			1	2	3
1	Baidibek	v. Zhanatalap, IE "Turdykulov"	7	5	4
		Almaly r/d, IE "Akhataev"	5	5	3
2	Kazykurt	Sharbulak r/d, "Sapa" p/a	8	5	4
		Sharbulak r/d, LLP "Kayyp ata"	6	5	3
3	Tulkubas	Keltemashat r/d, "Uzyn Bulak" p/a	7	5	3
		Ryskulov r/d, "Ak bastau" p/a	8	5	4
	Number of pools		6	6	6
Note: v - village; IE - individual entrepreneur; r/d - rural district; p/a - peasant agriculture					

The total number of samples collected in all three areas was as follows:

1) manure - 27 pools;

2) "Pen for exercise," within 100 meters from the stable - 27 pools;

3) soil within 15 meters of the stable (passages, ditches, roads, flower beds, etc.) - 27 pools.

Additionally, 40 fecal samples from horse breeding farms belonging to the East Kazakhstan region were also subjected to bacteriological research.

Bacteriological research. For selective isolation of *R. equi*, trypton-soy agar medium with ram erythrocyte was used at concentrations of 5-10%.

To prepare the nutrient medium, tryptic soy agar (dry) was mixed with 39.5 g of nutrient medium per 1 liter of distilled water and boiled for 2 minutes until the agar was completely dissolved. The medium was then poured into test tubes of 7-8 cm3 and 500 ml flasks, which were sterilized at 121°C for 15 minutes in an autoclave. After cooling the medium (45-50)ºC, sheep erythrocytes were added and thoroughly mixed.

Sheep erythrocytes were prepared by taking sheep blood into a sterile mounted flask with beads, defibrinating for 10-15 minutes, and washing with sterile saline until a clear supernatant was obtained at 2500-3000 rpm for 15 minutes.

The nutrient medium was poured into Petri dishes and tested for sterility in a thermostat at 37°C for 48 hours. A sterile saline solution was added to the biological material before inoculation until the sample was completely immersed. Inoculations were made on a nutrient medium by applying the seed material with a loop on the surface of the nutrient agar and placing it in a thermostat at 37°C. A visual inspection for culture growth was performed daily, and when characteristic colonies appeared, they were marked with a marker,

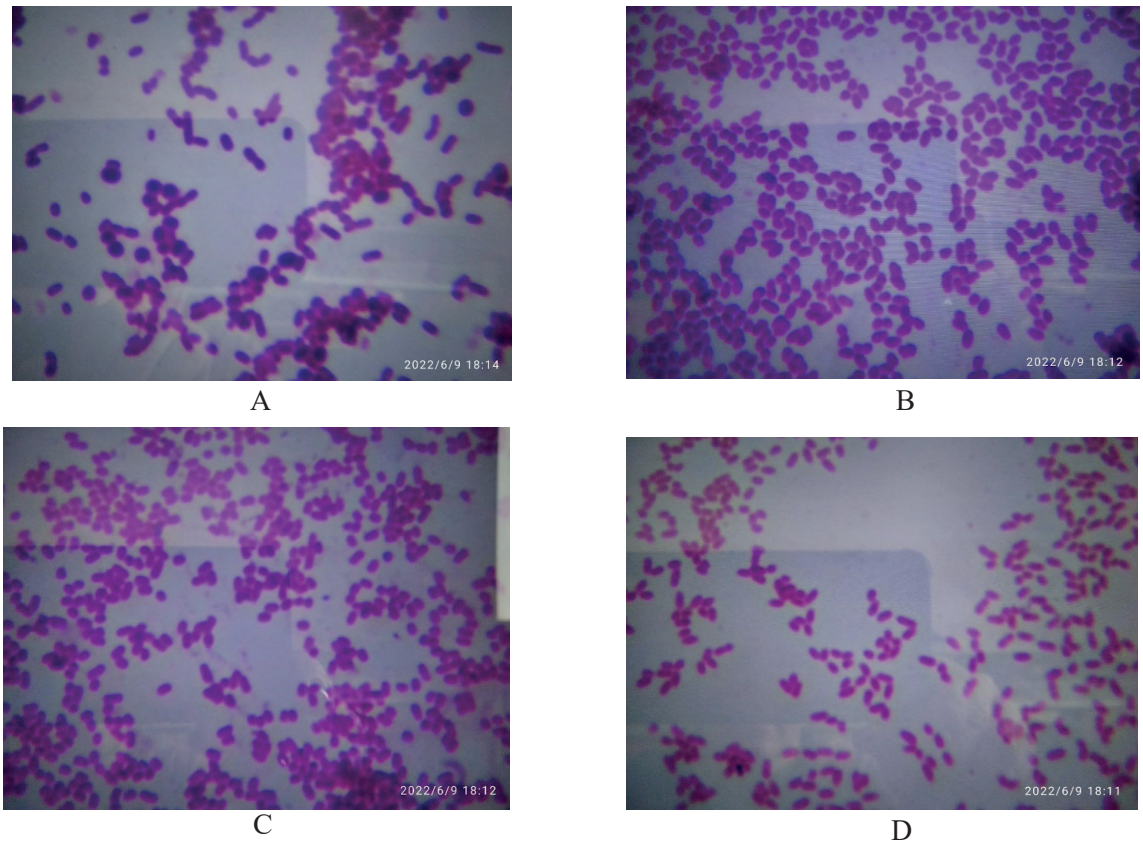
subjected to microscopy, and subcultured into test tubes with slant agar.

Biochemical research was conducted using the following materials: a Gram stain kit, Hiss medium, Himedia, India, 0.7% meat-peptone agar, 1.2% meat-peptone agar (MPA), meat-peptone broth (MPB), 3% hydrogen peroxide solution, and 1% tetramethyl paraphenylenediamine dihydrochloride solution. The culture was sown in test tubes with MPA and Hiss medium, cultivated at 37ºC for one day, and the results were taken into account.

Prepared MPA in 4 test tubes. Three of them were cultured and one tube served as a control. Then, the daily culture was inoculated into 24 tubes with Hiss medium (with glucose, sucrose, lactose, maltose, mannitol, and sorbitol) with semiliquid agar (Himedia, India). The crops were cultivated at 37 ºC for one day, then the results were taken into account.

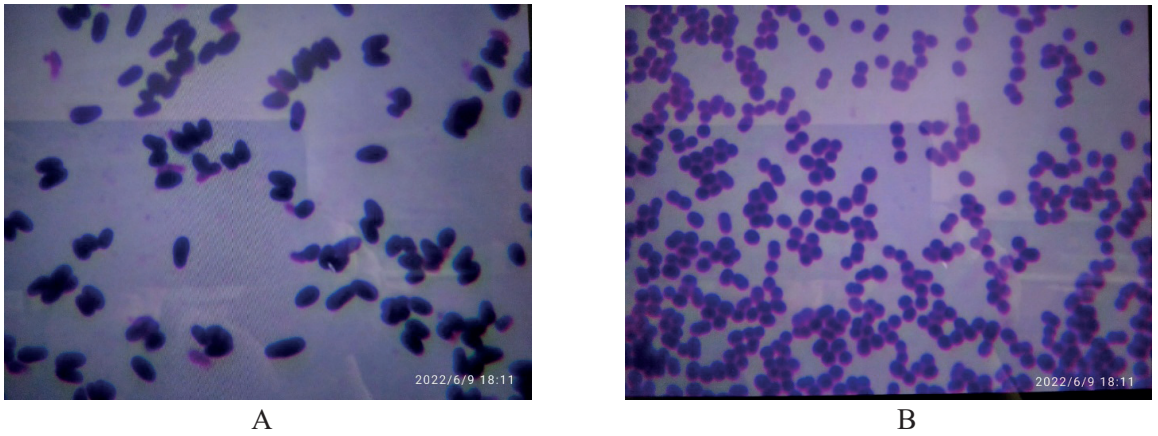
Results

The results of bacteriological studies of pools from the exercise pen and soil showed that smears were stained according to Gram. Gram-negative bacteria were stained in purple or blue, and Gram-positive bacteria were stained in red. *E. coli, Staphylococcus, Streptococcus, Diplococcus, Tetracoccus,* rod-shaped microorganisms, and fungi were found. In the study of manure in 27 pools, micrococcus, short, non-motile, Gram-positive rods were found in 12 (Figures 1, 2), and they were subject to genetic analysis. The ranking showed that 5 of them are from the Almaty region, 4 from Zhambyl, and 3 from Turkestan.



A B C D

Figure 1 - Swabs prepared from the feces of horses belonging to the farms of the Almaty region (A, B, C, D - smears)



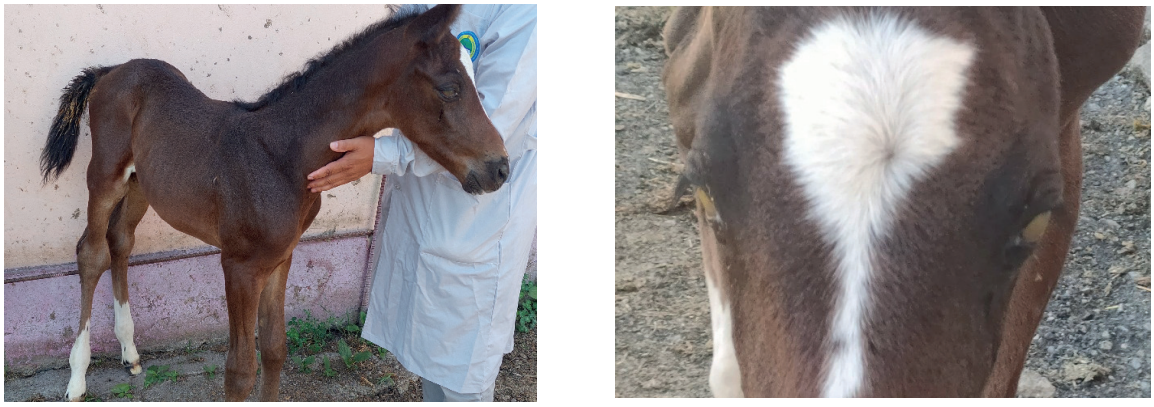
A B C D

Figure 2 - Swabs prepared from the feces of horses belonging to the farms of the Zhambyl region (A, B, C, smears; D - growth of cultures in blood agar)

Of note, blood agar showed growth of small, smooth, shiny white colonies after 24 hours of incubation.

Bacteriological studies of 40 fecal samples from farms belonging to the East Kazakhstan region also showed a negative result for *R. equi.*

We conducted clinical studies of horses and foals in a horse breeding farm located in the village of Arkabay, Talgar district, Almaty region, where there was a sporadic case among foals. During the examination, we found one foal exhibiting the following clinical signs: diarrhea, emaciation, pollution of the rear part of the body, swelling of the joints of the fore and hind limbs. Additionally, opacities were also observed in both eyes (Fig. 3).



A B

Figure 3 - Foal with swelling of the joints (A), blurred eyes (B)

Feces were taken from this animal and from the mother mare for bacteriological examination. Blood agar cultures of both showed growth of small, smooth, shiny and non-hemolytic colonies after 24 hours of incubation, but became larger, slimier, and salmon pink in color with age (Fig. 4). The smears were Gramstained positively.

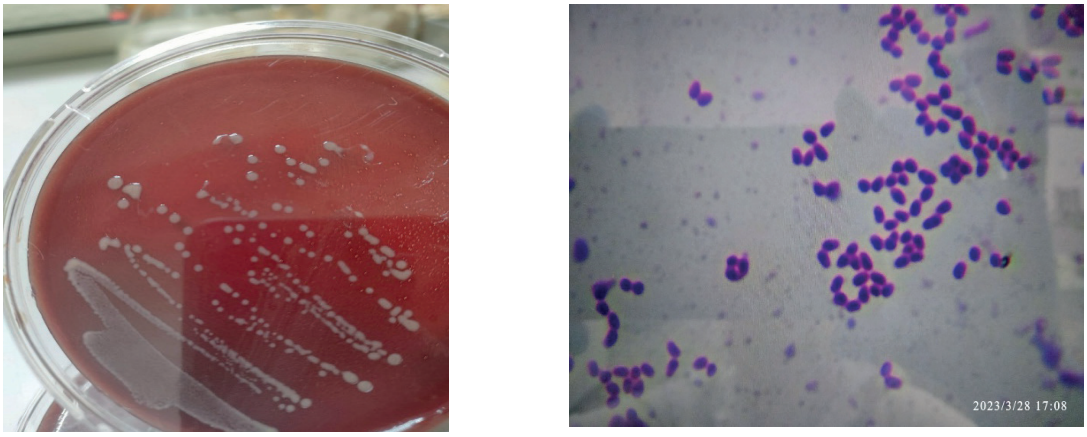


Figure 4 – Colony and smear from foal feces

To differentiate the isolated bacterial culture, its biochemical properties were studied on Hiss nutrient media. The *Rhodococcus equi* culture was found to be catalase-positive and oxidase-negative. It tested negative for glucose, lactose, sucrose, maltose, and mannitol.

Discussion

The isolated bacterial culture from the sick foal was identified as *Rhodococcus equi.* This bacterium is well-known for causing pyogranulomatous pneumonia in foals and as an opportunistic pathogen in other animals and humans. However, there is limited information available on the biochemical properties of animal isolates of this species.

The culture was found to be catalase-positive, oxidase-negative, and negative for glucose, lactose, sucrose, maltose, and mannitol in biochemical studies. The colonies of the culture grew irregularly, were smooth and slimy, and turned salmon-pink to yellow after a week of growth. The smears were Gramstained positively. The morphology of *Rhodococcus equi* varies from bacillary to coccoid, depending on the growth conditions. Some strains have pili or appendages, but they do not have flagella.

The sick foal showed various clinical signs, including diarrhea, emaciation, hindquarters soiling, joint swelling, and eye clouding.

According to other researchers, the morphology of *Rhodococcus equi* varies from bacillary to coccoid, depending on the growth conditions. If the bacteria are rod-shaped after 4 hours of growth in a culture broth, then after a day of growth in a liquid medium or on blood agar, they become coccoid [11]. *Rhodococcus equi* do not have flagella [12], but some strains have pili or appendages [13]. Rhodococcus equi grow in irregular, smooth and slimy colonies that turn salmon pink to yellow after a week of growth [12]. The positive result for acid resistance found in some studies probably depends on the growth conditions and the technique used. Rhodococcus equi is a Gram-positive obligate aerobic bacterium. They are catalase-positive, mostly urease-positive and oxidase-negative, and their optimum growth temperature ranges from 30 to 37ºC. *Rhodococcus equi* produce soluble "equi factor(s)" associated with phospholipase and cholesterol oxidase activity [14]. This factor interacts with phospholipase D from Corynebacterium pseudotuberculosis, b -toxin from Staphylococcus aureus or hemolysin from Listeria monocytogenes, causing complete hemolysis of erythrocytes in sheep and cattle [12]. Nutrient requirements are simple, and carbon can be used from many different sources, including simple organic acids such as propionate or acetate [15], which are found in abundance in herbivore manure [16, 17]. Predilection *R. equi* to lipids as a carbon source is also supported by analysis of the *R. equi* chromosome, which encodes - like in mycobacteria - many genes involved in lipid metabolism, and no gene for sugar transport [18].

The *R. equi* isolate is immobile, and growth in semi-liquid agar is localized strictly in the upper

part of the surface of the medium, indicating that aerobic conditions are optimal for this culture.

The most common clinical signs in foals with *R. equi* reflect lower respiratory tract infections and include cough, fever, increased respiratory rate and effort (including flaring of the nostrils), increased heart rate, and abnormal breath sounds in the trachea (often referred to as tracheal rales) and in the lungs (coughs, rales, or both may be heard) [1, 19]. Foals may also show extrapulmonary signs (EPS) of R. equi infection [20]. The most common EPS are diarrhea, ulcerative enterocolitis, suspected immune-mediated synovitis, intra-abdominal lymphadenitis or abscess, and uveitis.

Polysynovitis can occur in 40% or more of affected foals. The most commonly affected joints are the tarsocral, carpal, and pastern joints, but other synovial structures may also be involved. These swellings usually cause little more than mild pain and reduced range of motion, whereas foals with septic arthritis usually show severe lameness.

Conclusion

During bacteriological studies of feces in a horse breeding farm, where a foal with clinical signs characteristic of *R.equi* (diarrhea, emaciation, swelling of the joints of the fore and hind limbs, clouding of both eyes) was found, an isolate was isolated. It showed the growth of small, smooth, shiny and non-hemolytic colonies on blood agar and was Gram-positive.

In terms of cultural, morphological, tinctorial and biochemical properties, in general, the isolate was a typical representative of the *Rhodococcus equi* species.

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PREVALENCE AND ANTIBIOTIC RESISTANCE OF MICROORGANISMS OF THE ENTEROBACTERIACEAE FAMILY ISOLATED FROM DOGS WITH PARVOVIRUS ENTERITIS AND CLINICALLY HEALTHY CONTROLS IN THE NORTHERN REGION OF THE REPUBLIC OF KAZAKHSTAN

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Abstract

One of the main problems in the treatment of infectious diseases in pets is the spread of strains of microorganisms resistant to antimicrobial drugs. The aim of the study was to isolate conditionally pathogenic microorganisms of the *Enterobacteriaceae* family from dogs with parvavirus enteritis and clinically healthy animals, with the determination of phenotypic and genotypic resistance to antimicrobial drugs. In the period from March 2021 to March 2023, biological material from dogs with parvavirus enteritis (n = 152) and healthy dogs (n = 196), taken in veterinary clinics of Kostanay, was analyzed. Eighty-two isolates of conditionally pathogenic microorganisms of the *Enterobacteriaceae* family were isolated and studied from 348 biomaterial samples taken from dogs with enteritis. Conditionally pathogenic *Enterobacteriaceae* were isolated in 26.3% of cases (40 strains), among them: 65% of *E.coli* strains, 12% - *Klebsiella*, 10% - *Proteus*, 8% - *Citrobacter,* 5%- *Enterobacter.* Forty two microorganisms were isolated from healthy dogs: 83% are *E.coli* microorganisms, 5% are Klebsiella and Proteus isolates, and 2% are *Citrobacter* and *Enterobacter* microorganisms. In all isolated isolates, the resistance/sensitivity to the action of antibacterial drugs was determined by the disco-diffuse method. It was revealed that all isolated strains of microorganisms showed sensitivity to the action of streptomycin, belonging to the group of aminoglycosides, showed resistance to tetracycline, doxycycline, ofloxacin, ampicillin, amoxicillin. Resistance genes have been identified to beta-lactams, aminoglycosides, tetracyclines, and sulfonamides. The resistance gene to fluoroquinolones was isolated from 1 *E. coli i*solate, from a healthy animal. Resistance genes were not detected in the DNA of *Citrobacter, Enterobacter* and *Proteus* isolates isolated from clinically healthy dogs. The most common genes were genes encoding resistance to aminoglycosides - 28% of strains, to beta-lactams - 21.9%, to tetracyclines - 13.4% of animals. It was concluded that the uncontrolled and frequent use of antibacterial drugs of the beta-lactam group and tetracyclines in dogs leads to the spread of genotypic resistance among microorganisms of the *Enterobacteriaceae* family.

Key words: antibiotic resistance; *E. coli; Enterobacteriaceae; Klebsiella;* parvovirus enteritis; *Proteus;* the resistance gene.

Basic position and Introduction

Parvovirus infection characterized by severe enteritis and vomiting, as well as dehydration, fever, leukopenia and diarrhea. Treatment of this infection is mainly symptomatic, antimicrobial and antiemetic drugs are also used. One of the main problems in the treatment of infectious diseases in pets is the spread of strains of microorganisms resistant to antimicrobial drugs [1, 2]. Infectious diseases caused by such strains of microorganisms are characterized by a long course and worsen the further prognosis of the disease. If the drug used in the treatment of the disease is not effective, then it is necessary to use other stronger antimicrobial agents that are unsafe [3]. All this increases the risk of the spread of resistant strains of microorganisms in the environment.

Every year the list of drugs increases, in particular, antibiotics used in the treatment of diseases of small domestic animals (cats and dogs), the exception is not drugs used in human medicine (cephalosporins and fluoroquinolones) [4]. The transmission of microorganisms that have developed resistance to these drugs at the genetic level occurs between pets, owners and veterinary staff, where pets can act as reservoirs of bacteria, which creates the possibility for interspecific transmission of resistant forms of microorganisms. All of this may affect the use of antimicrobials in human medicine [5]. The increase and spread of antimicrobial resistance in domestic animals leads to an increased risk of therapeutic failures, i.e. inefficiency of treatment, increased costs of animal treatment and health complications [6].

In the infectious pathology of dogs, a significant part falls on viral infections. According to many researchers, parvovirus enteritis of dogs, despite the widespread use of effective vaccines, remains the most dangerous and most common viral infection, with 100% morbidity at all ages, 10% and 91% mortality in adult dogs and puppies, respectively [7, 8]. It leads to significant economic losses, which consist of direct losses due to high mortality of especially valuable animals, a decrease in service and breeding qualities in sick and ill individuals, as well as the costs of diagnostic, therapeutic and preventive measures.

Studies of recent decades have significantly expanded the understanding of epizootology and specific prevention of parvovirus enteritis as a monoinfection [9]. However, such important issues as the peculiarities of the course of the pathological process and the treatment of dogs with parvovirus enteritis complicated by associations of conditionally pathogenic bacteria remain poorly understood.

The aim of the study was to isolate conditionally pathogenic microorganisms of the *Enterobacteriaceae* family from dogs with parvavirus enteritis and clinically healthy animals, with the determination of phenotypic and genotypic resistance to antimicrobial drugs.

Materials and Methods

The research work was carried out in the period from March 2021 to March 2023. The selection of biomaterial from dogs was carried out in veterinary clinics of the city of Kostanay. Laboratory studies were carried out in the Department of Microbiological Analysis of the Research Institute of Applied Biotechnology of the A. Baitursynov KRU.

The object of research is biological material from dogs clinically healthy and diagnosed with parvavirus enteritis. A total of 348 samples were examined - flushes from the oral cavity and anal opening.

All animals underwent a clinical examination according to generally accepted methods, with the necessary diagnostic tests, a blood test, rapid tests to confirm infectious pathology (tests for parvovirus enteritis) and sampling from animal biological material to isolate conditionally pathogenic microorganisms with determination of resistance to antimicrobial drugs. After the final diagnosis was made, the animals were treated with data on sensitivity to antibacterial drugs.

Isolation and accumulation of pure cultures of microorganisms was carried out using universal chromogenic, differential diagnostic media. The identification o*f E.coli, Klebsiella, Citrobacter, Enterobacter* and *Proteus* cultures was performed according to the approved guidelines for the microbiological diagnosis of diseases caused by microorganisms of the *Enterobacteriaceae* family [10], as well as in accordance with the Bergi bacterial determinant.

To isolate microorganisms from the studied material, crops were sown on BCH, incubated for 18-20 hours at a temperature of 36-37 ° C, after which they were transplanted onto cups with chromogenic CHROMagarOrientation,

which were re-cultivated. When distinct colonies characteristic of the growth of E.*coli, Klebsiella, Citrobacter, Enterobacter* and *Proteus* appeared on this medium, smears were prepared and colored by Gram. When gram-negative straight rods with rounded ends were found in smears typical in morphology, their biochemical properties were studied (Table 1).

Table 1 – Differentiation of Enterobacteriaceae by enzymatic properties

	The microorganism / Test or substrate	*Escherichia coli*	*Klebsiella*	*Citrobacter*	*Enterobacter*	*Proteus*
According to the results of seeding on a combined medium (Kligler)	Lactose (bevel)	+/-	+	+/x	+	-
	Glucose (column)	+/-	+	+	+	x
	Hydrogen sulfide	-	-	+/-	-	+/-
Additional tests to determine ancestral affiliation	Simmons Citrate	-	+/-	+	+	+/-
	Lactose	+	+/-	+/-	+	-
	Mannitol	+	+	+	+	-
	Mobility	+/-	-	+	+	+/-
	Indole	+/-	-/+	-/+	-	+/-
	Urea by Christensen	-	+	-/+	+/-	+/-
	Test with methyl red	+	-/+	+	-	+
	Voges-Proskauer Test	-	-/+	-	+	-

+ 90% or more positive reactions
- 90% or more negative reactions
+/- more often positive, less often negative
-/+ more often negative, less often positive
X Various biochemical reactions

The ability of bacteria to ferment lactose, glucose, as well as to form gas and hydrogen sulfide was determined by the change in the color of the medium, the appearance of gas bubbles in the Kliegler medium. The change in the medium was taken into account after 24-hour incubation at t 37 ° C.

The enzymatic properties of bacteria were studied on Gis media with lactose, mannitol. Utilization of sodium citrate during culture growth was studied by changing the color of the Simmons medium, and the formation of indole was studied by the appearance of a red ring on the surface of the medium after the addition of Kovacs reagent (4–dimethylaminobenzaldehyde, amyl alcohol and hydrochloric acid). The mobility of the studied isolates was studied by their growth when seeded with an injection into semi-liquid agar. The change in media during growth was taken into account after 2 days of incubation.

The Voges-Proscauer test was performed based on the detection of acetoin by adding α-naphthol and potassium hydroxide to a 2-day culture of microorganisms on Clark's medium. In the presence of oxygen, acetoin is oxidized into a diacetyl-forming compound of red color.

The methyl red test was used for a certain concentration of ions (pH) in the medium of glucose fermenting microorganisms by adding 5 drops of the methyl red indicator to the culture of the microorganism and observing the color change.

To detect indole, a reaction was carried out using Kovacs reagent, by adding it to the culture of the microorganism on BCH, with a positive reaction, the formation of a red ring was observed.

The antibiotic sensitivity of isolated isolates of *E.coli, Klebsiella, Citrobacter, Enterobacter* and *Proteus* was studied by applying standard antibiotic discs to a freshly sown lawn of the culture using Muller–Hinton agar. The results were taken into account after 18-24-hour incubation at a temperature of 37 ° C by the presence of microbe growth retardation zones around the discs, which, according to the instructions, indicates either the sensitivity of the pathogen to the drug or its resistance to this antibiotic (Table 2). Interpretation

of the results was carried out:

- according to methodical instruction 4.2.1890-04 "Determination of the sensitivity of microorganisms to antibacterial drugs" [11];

- in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 11.0 [12];

- in accordance with the recommendations of the Institute of Clinical and Laboratory Standards (CLSI) [13].

Table 2 - Interpretation of the results of determining the sensitivity of Enterobacteriaceae

№	Name of disks with drugs	≤R	I	S≥	Interpretation of the base
1	2	3	4	5	6
1	Ampicillin (AMP) 10 mcg	14		14	Eucast 11.0
2	Amoxicillin (ACC) 25 mcg	15	15-20	21	methodical instruction
3	Cefoperazone (CPR) 75 mcg	15	16-20	21	CLSI, МУК
4	Cefoxitin (CFN) 30 mcg	9		19	Eucast 11.0
5	Cefpodoxime (CFM) 10 mcg	17		17	CLSI
6	Meropenem (MPN) 10 mcg	16		22	Eucast 11.0
7	Streptomycin (STR) 10 mcg	11	12-14	15	CLSI
8	Kanamycin (CAN) 30 mcg	13	14-17	18	CLSI, methodical instruction
9	Gentamicin (GEN) 10 mcg	17		17	Eucast 11.0
10	Levomycetin (LEV), 30 mcg	17		17	Eucast 11.0
11	Tetracycline (TET) 30 mcg	19		19	Eucast 11.0
12	Doxycycline (DOC C) 30 mcg	0	11-13	14	CLSI
13	Enrofloxacin (ENR) 5 mcg	17	18-21	22	methodical instruction
14	Ciprofloxacin (CIP) 5 mcg	22		25	Eucast 11.0
15	Norfloxacin (NOR) 10 mcg	22		22	Eucast 11.0
16	Ofloxacin (OF) 5 mcg	22		24	Eucast 11.0
17	Hemifloxacin (HEME) 5 mcg	15	16-19	20	CLSI
18	Nalidixic acid (NC) 30 mcg	13	14-18	19	CLSI
19	Trimethoprim/sulfamethoxazole (KTZ) 1.25/23.75	11		14	Eucast 11.0
20	Furazolidone (FRN) 300 mcg	14	15-16	17	CLSI
21	Furadonin (FD) 300 mcg	14	15-16	17	CLSI

Determination of resistance genes

DNA material for molecular research was obtained by bacterial lysis according to the recommendations of the Reference Laboratory for Resistance to Antibacterial Drugs of the European Union (Community Reference Laboratory for Antimicrobial Resistance) with minor changes. Identification of genes encoding antimicrobial resistance was carried out by PCR.

Results

In a clinical study, parvovirus enteritis was detected in 152 animals. The group of clinically healthy animals (n=196) included dogs whose owners applied to a veterinary clinic for preventive examination, as well as vaccination and deworming. Biological material was taken from all animals (flushes from the mouth and anus). Eighty two isolates of opportunistic *Enterobacteriaceae* were isolated and studied from 348 samples of biomaterial. Among them: 61 (57.9%) strains of *E.coli,* 7 (10.5%) strains of *Klebsiella,* 4 (8.8%) strains of *Citrobacter,* 4 (12.3%) strains of *Enterobacter* and 6 (10.5%) strains of *Proteus* (Figure 1).

Morphological, tinctorial and cultural properties of the isolated isolates were characteristic of their family and genus.

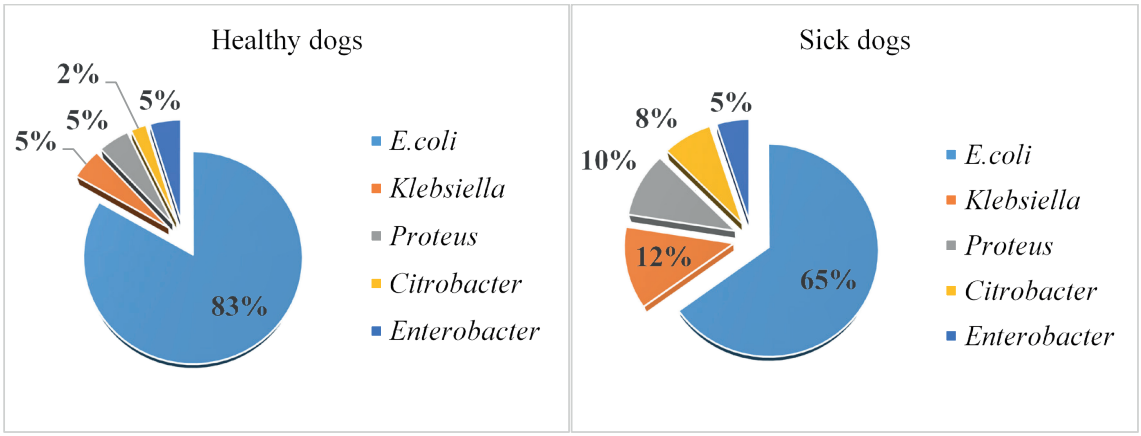

Figure 1- Percentage of isolates isolated from dogs

From 152 samples of biomaterial from dogs with enteritis, conditionally pathogenic enterobacteria were isolated in 26.3% of cases (40 strains). Among them: 26 (65%) *E.coli* strains, 5 (12%) *Klebsiella* strains, 4 (10%) *Proteus* strains, 3 (8%) *Citrobacter* strains, 2 (5%) and *Enterobacter* strains.

Forty two microorganisms were isolated from 196 samples of biomaterial from healthy dogs, which is 21.4% of the total number of samples. Among 83% (35 strains) are *E.coli* microorganisms, 5% of *Klebsiella* and *Proteus* isolates (2 strains each), and 2% of *Citrobacter* and *Enterobacter* microorganisms.

Antibiotic resistance. The next stage of the research was to determine the sensitivity to antibacterial drugs of the isolated strains and the determination of resistance genes. The results of antibiotic resistance of microorganisms of the *Enterobacteriaceae* family isolated from dogs with parvovirus enteritis and clinically healthy controls are shown in Figures 2 and 3.

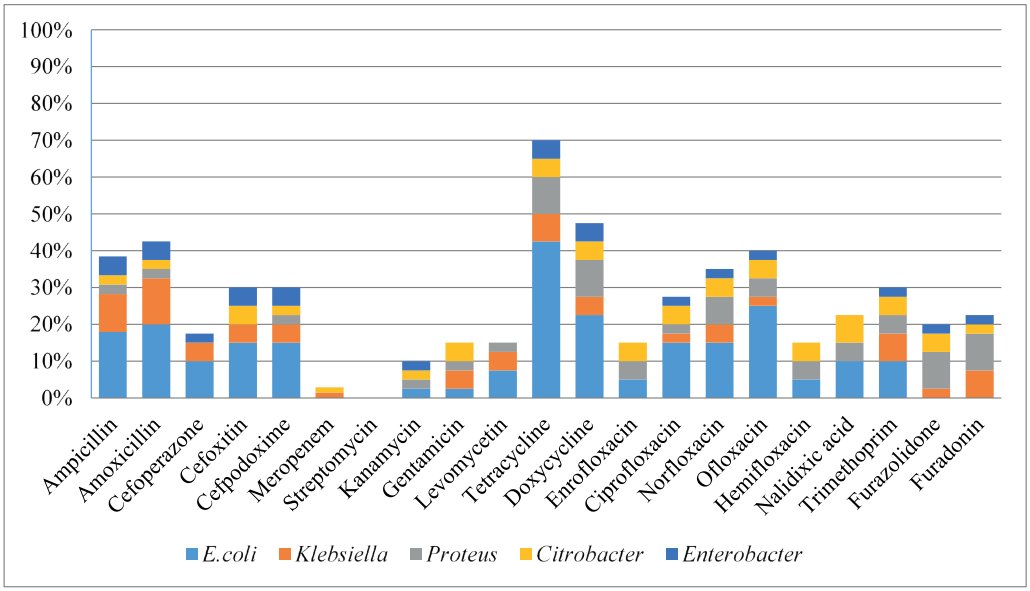

Figure 2 – Results of antibiotic resistance of strains isolated from dogs with paravavirus enteritis

Of the 40 studied strains of opportunistic microorganisms, 70% were resistant to tetracycline - 28 strains (17 - *E.coli,* 4 - *Proteus,* 3 - *Klebsiella*, 2 *Citrobacter* and *Enterobacter*), 47.5% were resistant to doxycycline (9 strains - *E.coli*, 4 - *Klebsiella*, 2 *Proteus*, *Citrobacter* and *Enterobacter*), to amoxicillin – 42.5% (7 strains - *E.coli*, 4 - *Klebsiella*, 2 *Enterobacter* and 1 *Proteus, Citrobacter*), ofloxacin - 40% (10 strains - *E.coli*, 2 - *Citrobacter* and *Proteus*, and 1 strain of *Klebsiella* and *Enterobacter*), 37.5% were resistant to ampicillin (7 *E.coli*, 4 - *Klebsiella*, 2 - *Enterobacter,* 1 strain - *Proteus, Citrobacter*), 35% to norfloxacin (6 strains of *E.coli,* 3 - *Proteus,* 2 - *Klebsiella* and *Citrobacter,* and 1 - *Enterobacter*), 30% of microorganisms

were resistant to cefpodoxime (6- E.coli, 2 strains of *Enterobacter* and *Klebsiella*, 1 *Citrobacter* and *Proteus* strain,), cefoxitin (6 - *E.coli,* 2 strains of *Klebsiella, Citrobacter* and *Enterobacter*) and trimethoprim/sulfamethoxazole (4 - *E.coli*, 3 - *Klebsiella*, 2 – *Citrobactera* nd *Proteus* and 1- *Enterobacter*), 27.5% to ciprofloxacin (6 strains of *E.coli,* 2 – *Citrobacter* and 1 *Klebsiella, Enterobacter, Proteus*) and nalidixic acid (4- *E.coli*, 3 - *Citrobacter* and 2 - *Proteus*), 22.5% were resistant to furadonin (4 - *Proteus*, 3 - *Klebsiella* and 1 *Citrobacter* and *Enterobacter*), 20% were resistant to furazolidone (4- *Proteus*, 2- *Citrobacter* and 1 *Klebsiella* and *Enterobacter*), 17.5% to cefoperazone (4 *E.coli*, 2 - *Klebsiella* and 1 *Enterobacter*), 15% of microorganisms were resistant to gentamicin (2 strains of *Citrobacter* and *Klebsiella*, 1 each – *Proteus* and *E.coli*), levomycetin (3 *E.coli*, 2 *Klebsiella* and 1 *Proteus*), enrofloxacin and hemifloxacin (2 strains of *E.coli, Citrobacte*r and *Proteus*), to kanmycin – 10% (1 strain of *Proteus, Citrobacter, E.coli* and *Enterobacter*), 5% - 1 strain of *Citrobacter* and 1 strain of *Klebsiella* was resistant to meropenem. No streptomycin-resistant strains were detected.

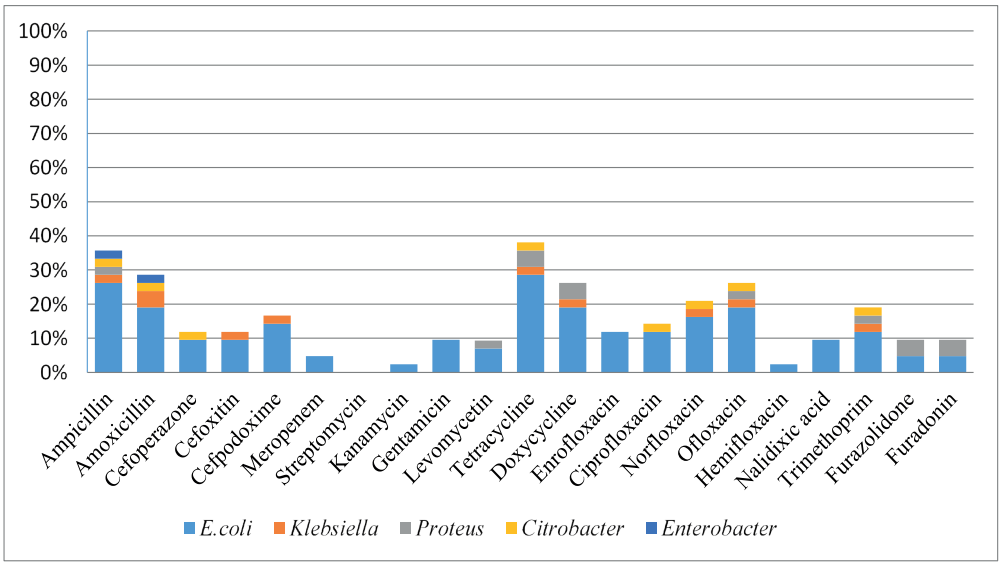

Figure 3 – Results of antibiotic resistance of strains isolated from healthy dogs

Of the 42 studied strains of *Enterobacteriaceae* isolated from healthy dogs, 38% were resistant to tetracycline - 16 strains (12 - *E.coli*, 2- *Proteus* and 1 strain of *Klebsiella* and *Citrobacter*), 35.7% - to ampicillin (11 *E.coli* and 1 strain of *Proteus, Klebsiella, Citrobacter, Enterobacter*), 28.6% - to amoxicillin (8- *E.coli*, 2- *Klebsiella* and 1 – *Citrobacter and Enterobacter*), 26.2% to doxycillin (8- *E.coli*, 2- *Proteus,* 1- *Klebsiella*) and ofloxacin (8- *E.coli,* 1 – *Proteus, Klebsiella* and *Citrobacter*), 21.4% to norfloxacin (7 *E.coli*, 1 strain of *Klebsiella* and *Citrobacter*), to trimethoprim/sulfamethoxazole – 19% (5 strains of *E.coli* and 1 *Proteus, Klebsiella, Citrobacter*), to cefpodoxime – 16.7% (6 *E.coli* and 1 *Klebsiella*), 14.3% to ciprofloxacin (5 *E.coli* and 1 strain of *Citrobacter*), 12% of microorganisms were resistant to cefoperazone (4 strains of *E.coli* and 1 *Citrobacter*), cefoxitin (4 strains of *E.coli* and 1 *Klebsiella*) and enrofloxacin (5 strains of *E.coli*), 9.5% of microorganisms are resistant to gentamicin (*E.coli*), levomycetin (3 *E.coli* and 1 *Proteus*), nalidixic acid (*E.coli*), furazolidone and furadonin (2 strains of *E.coli* and *Proteus* for each drug), 4.8% - to meropenem (*E.coli*), 1 isolate *E.coli* (2.4%) was resistant to the action of kanamycin and hemifloxacin. No streptomycin-resistant strains were detected.

To determine the genetic profiles of resistance of microorganisms, primers were used, which were selected by us taking into account the use of classes of antibiotics and antimicrobials in veterinary practice.

As a result of the conducted studies, 82 samples that showed phenotypic resistance to antibacterial drugs were tested by PCR for the presence of genes encoding resistance. The results are presented in table 3.

Table 3 - Microbial resistance genes

Group of antibiotics	Gene	*E.coli*	*Klebsie-lla*	*Citro-bacter*	*Entero-bacter*	*Proteus*	*E.coli*	*Klebsie-lla*	*Citro-bacter*	*Entero-bacter*	*Proteus*	Total
		animals with enteritis					healthy animals					
Beta-lactams	BlaTEM	5	2	1		1	3	1				13
	OXA	1					3	1				5
Aminoglycosides	StrA	4			1		2	1				8
	StrB	4			1		1	1				7
	aadB					1	1					2
	aphA1	2	1		1		2					6
Tetracyclines	tetA	2	1	1			4					8
	tetB	3										3
Sulfonamides	SUL1		1			1						2
	SUL3	1				1	3					5
Fluoroquinolones	qepA						1					1
	qnrA											0

Based on the data presented in Table 3, it can be seen that resistance genes were identified for all the studied groups of antibiotics in the DNA of microorganisms of the *Enterobacteriaceae* family isolated from dogs. Most often, in 28% of strains, genes encoding resistance to aminoglycosides were isolated (in 37.5% of animals with enteritis and in 19% of healthy animals). Beta-lactam resistance genes were found in 21.9% of isolates (in 25% of sick dogs and in 19% of healthy ones). Genes encoding resistance to tetracyclines were detected in 13.4% of cases (in 17.5% of dogs with enteritis and in 9.5% of healthy animals). Genes encoding resistance to sulfonamides were also identified in 10% of cases from sick animals and in 7.1% in healthy ones. The smallest number of genes were identified that cause resistance to fluoroquinolones (qepA, n=1). It should be noted that in the DNA of *Citrobacter, Enterobacter* and *Proteus* isolates isolated from clinically healthy dogs, no genes encoding resistance to antibacterial drugs have been identified.

Discussion

The acquisition and spread of resistance to antibacterial drugs by microorganisms has been noted for many years and has become one of the most important therapeutic problems in human medicine and veterinary medicine. Our study analyzed the spread of antimicrobial resistance in strains of microorganisms of the *Enterobacteriaceae* family isolated from healthy domestic dogs and dogs with diagnosed parvavirus enteritis.

As a result of the conducted studies, 348 samples of biological material (flushes from the oral cavity and anus) were selected from dogs in veterinary clinics of Kostanay, among them clinically healthy dogs (n=196) and dogs with parvavirus enteritis (n=152). 23.6% of animals (n=82) were found to carry conditionally pathogenic microorganisms of the *Enterobacteriaceae* family, including 48.7% (40 isolates) isolated from sick animals and 51.3% (42 isolates) from healthy ones. Among them: 61 (57.9%) strains belonged to *E.coli,* 7 (10.5%) strains of *Klebsiella,* 4 (8.8%) strains of *Citrobacter*, 4 (12.3%) strains of *Enterobacter* and 6 (10.5%) strains of *Proteus*. The results obtained by us correlate with the data obtained in a number of studies in Western Europe, where the prevalence of colonization by Enterobacteriaceae in dogs (including healthy and sick animals) ranged from 3.1 to 55% [14, 15]. From 152 samples of biomaterial from dogs with enteritis, conditionally pathogenic *Enterobacteriaceae* were isolated in 26.3% of cases (40 strains). Among the microorganisms isolated from the biomaterial of sick animals, 65% of the strains belonged to *E.coli,* 12% of the strains to *Klebsiella,* 10% of the strains to *Proteus*, 8% - *Citrobacter*, 5% - *Enterobacter.* Forty two microorganisms were isolated from 196 samples of biomaterial from healthy dogs, which is 21.4% of the total number of samples. Among them, 83% are *E.coli* microorganisms, 5% are *Klebsiella* and *Proteus* isolates, and 2% are *Citrobacter* and *Enterobacter* microorganisms.

In all isolated isolates, antimicrobial resistance/ sensitivity was determined by the disco-diffuse method. Analyzing the data obtained during this study, it should be noted that the maximum number of microorganisms (53.6%) isolated from both sick animals and healthy ones showed

resistance to the action of tetracycline. These results are consistent with a number of studies conducted over the past decade in the UK and Portugal, where *Enterobacteriaceae* also had the maximum percentage of resistant strains to this antibacterial drug [16, 17]. *E.coli* isolates showed increased resistance to ampicillin, amoxicillin, cefpodoxime, as well as low resistance to carbapenems (meropenem), these results correlate with the results of studies obtained in Australia [18]. The maximum resistance to the action of amoxicillin (100%) and ampicillin (71%) and the minimum to the action of meropenem were also found in microorganisms of the genus *Klebsiella.* Studies conducted in 2017-2019 in Thailand and Portugal confirm that this microorganism has a high resistance to this drug [19; 20]. There are not many studies on the prevalence of resistance to antibacterial drugs in *Citrobacter* isolates from dogs all over the world. The results of our studies show that the maximum number of microorganisms were resistant to nalidixic acid, tetracycline, ciprofloxacin, norfloxacin, ofloxacin, trimetaprim/ sulfamethoxazole. In studies conducted in Nigeria, the data were partially similar, *Citrobacter* showed resistance to amoxicillin, ciprofloxacin [21]. All microorganisms of the genus Proteus have resistance to tetracycline, doxycycline and nitrofurans, as well as a decrease in resistance to norfloxacin and hemifloxacin. Similar results were obtained in Japan, where similar resistance to the action of antibacterial drugs was obtained in *Proteus* and *Enterobacter* [22, 23]. It was revealed that *Enterobacter* strains exhibit the greatest resistance against ampicillin, amoxicillin, tetracycline and doxycycline.

It should be noted that in general, the largest number of all isolated microorganisms showed resistance to the group of tetracyclines and beta-lactams, which, according to the State register of Veterinary drugs and feed additives of the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan, are the drugs of choice in the treatment of infectious diseases of animals. In a study conducted in Spain, beta-lactams were the antimicrobial drugs most commonly prescribed to dogs [24].

Among the isolated microorganisms, the majority of isolated strains of microorganisms (56%) had polyresistance, with the exception of 21 strains isolated from healthy animals (20 isolates of *E.coli* and 1 *Enterobacter*) and 14 isolates of *E.coli* obtained from sick dogs.

In general, as a result of studies of isolates, conditionally pathogenic *Enterobacteriaceae*, it was noticed that strains isolated from dogs with diagnosed parvavirus enteritis have a greater degree of resistance to antibacterial drugs, unlike strains isolated from healthy animals. Isolates from sick animals are on average 8.7% more resistant to the beta-lactam group than strains from healthy dogs, 4.4% more resistant to the aminoglycosides group, 5.5% more resistant to amphenicols, 25.6% more resistant to tetracyclines, 11.24% more resistant to fluoroquinolones, 18% more resistant to quinolones, 11% more resistant to sulfonamides, to nitrofurans by 11.7%.

To determine the bacterial resistance profiles, primers were used, which were selected taking into account the use of classes of antibiotics and antimicrobials in veterinary practice. As a result of DNA studies, 82 strains of opportunistic microorganisms of the *Enterobacteriaceae* family were tested by PCR for the presence of genes encoding resistance. Resistance genes were identified for all the studied groups of antibiotics in the DNA of microorganisms of the *Enterobacteriaceae* family isolated from dogs. Most often, in 28% of strains, genes encoding resistance to aminoglycosides were isolated (in 37.5% of animals with enteritis and in 19% of healthy animals). Beta-lactam resistance genes were found in 21.9% of isolates (in 25% of sick dogs and in 19% of healthy ones). Genes encoding resistance to tetracyclines were detected in 13.4% of cases (in 17.5% of dogs with enteritis and in 9.5% of healthy animals). Genes encoding resistance to sulfonamides were also identified in 10% of cases from sick animals and in 7.1% in healthy ones. The smallest number of genes were identified that cause resistance to fluoroquinolones. It should be noted that in the DNA of *Citrobacter, Enterobacter* and *Proteus* isolates isolated from clinically healthy dogs, no genes encoding resistance to antibacterial drugs have been identified.

In the course of the study, it was found that in 18 DNA samples with genotypic resistance to antibiotics, their connection with phenotypic profiles is traced. However, in 6 *E.coli* samples (3 from sick animals and 3 from healthy ones), resistance genes to antibacterial drugs of the aminoglycoside group (StrA, strB, aadB, aphA1) were detected, while phenotypic resistance in these microorganisms to this drug was not detected. Probably, these may be the so-called "silent"

genes, which are found in a number of studies, but have not been studied enough yet [25, 26].

Thirty seven strains of microorganisms had phenotypic resistance to the group of fluoroquinolones (enrofloxacin, ciprofloxacin, norfloxacin, ofloxacin, hemifloxacin), while genotypic resistance, i.e. the presence of qepA, qnrA genes was absent. This is probably due to differences in the mechanisms of resistance [27].

Conclusion

In the Northern region of the Republic of Kazakhstan, dogs have a high prevalence of opportunistic microorganisms of the *Enterobacteriaceae* family, as in dogs with parvavirus enteritis (*E. coli* - 26 (65%), *Klebsiella* - 5 (12%), *Enterobacter* – 2 (5%), *Proteus* – 3 (8%), and 3 isolates *Citrobacter*), and in healthy animals (*E. coli* - 83%), *Klebsiella* 5%, *Enterobacter* – 2%, *Proteus* – 5% and *Citrobacter* - 2%). The study of antibiotic resistance of the isolated strains showed high resistance to beta-lactams and tetracyclines. Resistance to these groups of antibacterial drugs is due to the presence of resistance genes blaTEM, OXA, tetA and tetB in microorganisms.

In general, as a result of studies of isolates, conditionally pathogenic *Enterobacteriaceae,* it was noticed that strains isolated from dogs with diagnosed parvavirus enteritis have a greater degree of resistance to antibacterial drugs, unlike strains isolated from healthy animals. Isolates from sick animals are on average 8.7% more resistant to the beta-lactam group than strains from healthy dogs, 4.4% more resistant to the aminoglycosides group, 5.5% more resistant to amphenicols, 25.6% more resistant to tetracyclines, 11.24% more resistant to fluoroquinolones, 18% more resistant to quinolones, 11% more resistant to sulfonamides, to nitrofurans by 11.7%.

Uncontrolled and frequent use of antibacterial drugs of the beta-lactam group and tetracyclines in dogs leads to the spread of genotypic resistance among microorganisms of the *Enterobacteriaceae* family.

Thus, the results obtained made it possible to assess the existing level of prevalence of antibiotic-resistant forms of conditionally pathogenic microorganisms detected from dogs in the Northern region of Kazakhstan and to determine their phenotypic and genotypic profile.

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GENOTYPIC RESISTANCE DRUGS OF ESCHERICHIA COLI STRAINS ISOLATED FROM KAZAKHSTANI PRODUCERS' CHEESES TO ANTIMICROBIAL

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Abstract

Global spread of antimicrobial resistance among pathogens is alarming for the modern society. The research aimed to study distribution of *Escherichia coli* in cheeses produced in Kazakhstan and to assess its resistance to antibacterial drugs. There were collected 101 samples of cheeses at retail outlets in different regions of Kazakhstan in 2021-2023, from which 55(54.4%) *E. coli* strains were isolated using conventional microbiological methods. The strains were phenotypically evaluated for antibiotic resistance, and the resistant isolates were tested for antibiotic resistance genes for the β-lactams group-penicillins (blaTEM, blaSHV, and OXA genes), aminoglycosides (aphA1, aadB), tetracyclines (tetA, tetB), quinolones (qnrA, qepA) and sulphonamides (sul1, sul2, and sul3 genes). Among studied E. coli strains 61% were resistant to at least one of the 21 antibiotics tested and were multi-resistant to the 15 antibacterial drugs. The greatest resistance was to sulfamethoxazole (43.6%), tetracycline (32%), followed by cefoxitin (29%). Isolates showed resistance to gentamicin (18.1%), ofloxacin (12.7%), furadonin (11%), amoxicillin (9.1%), doxycycline (7.2%). A gene encoding resistance to sulfonamides (sul3) was identified in eight *E. coli* strains. Thus, genotypic antibiotic resistance has been established in *E. coli* populations contaminating cheeses produced in the central, northern and eastern regions of Kazakhstan.

Key words: antimicrobials; cheeses; *Escherichia coli*; genotypic resistance; Kazakhstan; multi-resistance.

Basic position and Introduction

E. coli species belongs to the Enterobacteriaceae family and considers the normal commensal bacteria of animals and humans [1]. However, *E. coli* turns into a comprehensive pathogen infecting humans and animals through mobile or horizontal transfer of pathogenic genetic material in bacterial colonies [2]. Currently, antimicrobial resistance has become one of the biggest global threats to public health [3]. Agricultural products can be carriers of antimicrobial-resistant bacteria with resistance genes to humans [4, 5, 6]. It is predicted that by 2050 antimicrobial resistance will lead to millions of deaths, a financial burden and a significant reduction in livestock production. Extensive studies related to the genetic characterization and

antimicrobial control of resistant bacteria, which are often recorded in the environment, in humans and animals, show that extended-spectrum beta-lactamase-producing (ESBL) *E.coli* have negative consequences for humans and animals [7,8].

To control this pathogenic bacterium, a "One Health" approach was introduced, aimed at preventing the distribution of antimicrobial resistance among various local ecosystems. Intensive using of antimicrobials as a growth stimulants to increase cattle production in addition to therapeutic purposes in animal husbandry systems has allowed resistant bacteria to spread from animals to humans [8,9]. The rapid and global distribution of resistance mechanisms in gram-negative bacteria is the main reason for the increase in antimicrobial resistance. Data registered in the USA and the European Union on the spread of resistant bacteria confirmed that pathogenic bacteria isolated from food products have high antimicrobial resistance [10,11].

Recently epidemiological studies the genetic characteristics of ESBL E. coli in humans have shown that, in general, they were identified from animal products [12, 13]. Among the food goods offered to consumers the most dangerous are food products of animal origin. This is a case when the cheeses occupy a special place among a wide range of dairy products. The sanitary conditions of cheese industry enterprises, equipment, food products contaminated with bacteria through workers pose a potential danger to human health and life.

The purpose of these studies was to determine the distribution and genetic characteristics of antibiotic-resistant *E.coli* strains isolated from domestic cheeses in various geographical regions of Kazakhstan.

Materials and Methods

Microbiological and molecular genetic studies were carried out in the period from May 2021 to September 2023 at the Kazakh-Chinese Biosafety Laboratory of the S. Seifullin Kazakh Agrotechnical Research University, the Laboratory of Molecular Diagnostics of Food Pathogens of the İstanbul University-Cerrahpaşa, and the Research Institute of Applied Biotechnology of the A. Baitursynov Kostanay Regional University. In total, 101 samples of various cheeses from enterprises of the central, northern and eastern regions of Kazakhstan were examined, which were randomly sampled at retail outlets in six cities: Astana, Karaganda, Kostanay, Petropavlovsk, Semey and Uskaman (Figure 1).

Figure 1 - Geography of sampling by regions

Microbiological studies were carried out according to the methods of microbiological analysis of the GOST 32901-2014 "Milk and dairy products". Isolation and identification of *E.coli* strains were done by seeding to conventional nutrient selective media and re-culturing into appropriate differential diagnostic media. Isolated strains were Gram-stained, their lactose fermentation, catalase test, and indole formation were determined.

Antibiotic sensitivity testing was performed by Kirby-Bauer disco diffusion in accordance with EUCAST recommendations [14]. Discs containing the following 21 antibiotics were

used for testing: beta-lactams (ampicillin-10 mcg, amoxicillin-25 mcg, cefoperazone-75 mcg, cefoxitin 30 mcg, cefpodoxime-10 mcg), aminoglycosides (streptomycin - 10 mcg, kanamycin-30 mcg, gentamicin-120 mcg), amphenicols (levomycetin-10 mcg 30 mcg), tetracyclines (tetracycline-30 mcg, doxycycline-30), fluoroquinolones (enrofloxacin-5 mcg, ciprofloxacin-5 mcg, norfloxacin-10 mcg, ofloxacin5 mcg), quinolones (nalidix acid-30 mcg), sulfonamides (sulfamethoxazole with trimethoprim-1.25/ 23.75), nitrofurans (furadonin-300 mcg, furazolidone-300 mcg).

To detect the genes encoding resistance to antibacterial drugs, the PCR method, represented by 1.5% agarose gel, was used. DNA isolation was carried out by thermal lysis. The reaction mixture contained water without DNAase, a Drean TaqGreen master mixture, direct and reverse primers, and DNA being tested. The primers listed in Table 1 were used to determine the presence of genes by PCR.

Table 1 – Resistance genes' detection primers

Primer	Length	T^0	Sequence (5'–3')	A source
Beta-lactams group: penicillins				
blaTEM	857	56	blaTEM-F-GAGTATTCAACATTTTCGT blaTEM-R-ACCAATGCTTAATCAGTGAG	15
Beta-lactams group: carbapenems				
OXA	276	48	OXAI-F-TCAACAAATCGCCAGAGAAG OXAI-R-TCCCACACCAGAAAAACCAG	15
Tetracyclines				
tetA	210	60	tetA F-GCTACATCCTGCTTGCCT tetA R-CATAGATCGCCGTGAAGA	16
tetB	930	64	tetB F-CATTAATAGGCGCATCGCTG tetB R -TGAAGGTCATCGATAGCAGG	17
Aminoglycosides				
aadB	634	68	aadB-F-ATGGACACAACGCAGGTCGC aadB-R-TTAGGCCGCATATCGCGACC	15
aphA1	500	55	aphA1-F-AAACGTCTTGCTCGAGGC aphA1-R-CAAACCGTTATTCATTCGTGA	
strA	546	55	strA-F-CCTGGTGATAACGGCAATTC strA-R-CCAATCGCAGATAGAAGGC	
strB	509	56	strB-F-ATCGTCAAGGGATTGAAACC strB-R-GGATCGTAGAACATATTGGC	
Sulfanilamides				
SUL 1	547	65	sul1-F-TTCGGCATTCTGAATCTCAC sul1-R-ATGATCTAACCCTCGGTCTC	15
SUL 3	880	53	sul3-F-GAGCAAGATTTTTGGAATCG sul3-R-CATCTGCAGCTAACCTAGGGCTTTGA	
Fluoroquinolones				
qepA	218	60	qepA F-GCAGGTCCAGCAGCGGGTAG qepA R -CTTCCTGCCCGAGTATCGTG	18
qnrA	516	53	qnrA F- ATTTCTCACGCCAGGATTTG qnrA R-GATCGGCAAAGGTTAGGTCA	15

The amplification mode consisted the denaturation at 94º C for 30 seconds, annealing temperature according to Table 1, elongation at 72º C for 1 minute 30 seconds. The amplification time was 1 hour and 45 minutes. The determination of amplification products was carried out by electrophoresis in 105v on 1.5% agarose gel for 1 hour and 25 minutes. To carry out the reaction, specific markers, the TBE buffer solution of, the SYBR Safe DNA gel stain dye were used.

Results

There were isolated 55 *E. coli* strains from 101 cheese samples, which amounted to 54.4% (Table 2).

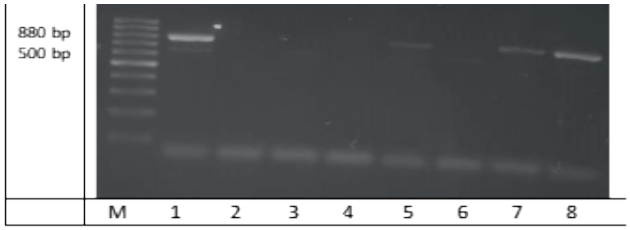
Table 2 - Contamination of Kazakhstani producers' cheeses by *E.coli* strains

№	Country regions	Number of samples	Identified *E. coli* strains (%)
1	Northern	33	18(32,6)
2	Eastern	48	25(47,6)
3	Central	20	20 (19,8)
	Total	101	55

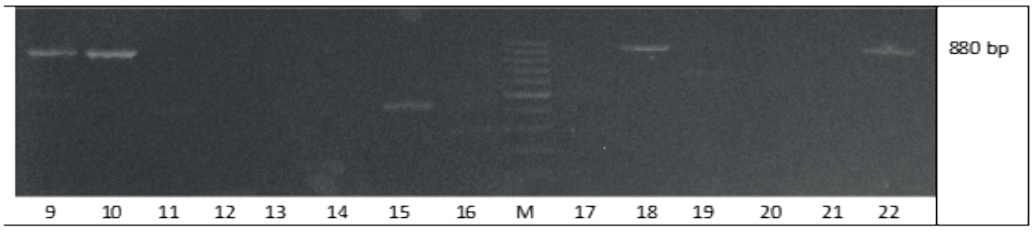
According to the phenotypic antimicrobial resistance's study of *E.coli* strains, a high level resistance was found in all sampling regions to most tested antibiotics. Among them, the greatest resistance was to sulfamethoxazole containing trimethoprim (43.6%), to cefoxitin (29%), followed by tetracycline (32%). Isolates showed also resistance to gentamicin (18.1%), ofloxacin (12.7%), furadonin (11%), amoxicillin (9.1%), doxycycline (7.2%).

In addition, resistance to three or more classes of different antibiotics was found in 15 of *E. coli* strains (27%). Similar results in Venezuela, as well as in Brazil and Egypt confirm that E. coli strains isolated from dairy products have multi resistance to many antimicrobial drugs [19, 20].

In the antibiotic resistance genotyping period, the sul3 resistance gene was detected in three samples of *E. coli* strains from the East Kazakhstan region, in two samples from the North Kazakhstan region and in three samples from Central Kazakhstan region. Thus, the molecular genetic study of strains having phenotypic resistance to six groups of antibacterial drugs shown that the sul3 resistance gene to sulfonilnamides (sulfamethoxazole with trimethoprim) is identified in eight samples (Figure 2).



M – marker, 1-8 samples (1, 5, 7, 8 – positive)



M – marker, 9-22 samples (9, 10, 18, 22 – positive)

Figure 2 - Determination of genotypic resistance

Discussion

One of the most alarming problems of this century is the continuous distribution of infections caused by resistant microorganisms, in particular bacterial pathogens with multidrug resistance [22].

The problem of antibiotic resistance, along with food safety, requires study in the veterinary, agricultural and environmental sectors. There is ample scientific evidence that drug-resistant bacteria can be transmitted through direct contact between animals and humans or through contaminated food. In 2015 the World Health Organization has adopted the Global action plan on antimicrobial resistance and called on each country to develop the National Action Plan to prevent global distribution of antimicrobial resistance.

Escherichia coli is recognized as a significant foodborne pathogen, so its identification is important for food hygiene management and rapid epidemiological studies. This studies are pilot research in terms of investigating cheeses of domestic producers for *E.coli* contamination. Strains of the bacterium were isolated in 54.4% of samples from the central, northern and eastern regions of Kazakhstan. Phenotypic analysis of resistance shown a high level of resistance of isolated *E.coli* strains to several antimicrobial drugs. Among *E. coli* isolates 61% were resistant to at least one of the 21 antibiotics tested and 27.3% were multi-resistant. At the same time, 43.6% of strains had intermediate sensitivity to cefoperazone and amoxicillin, 32% of strains shown resistance to sulfamethoxazole with trimethoprim. Similar studies conducted in Ethiopia confirm that E. coli strains isolated from dairy products have a similar level of resistance to the drugs sulfamethoxazole, tetracycline, doxycycline and cefoxitin [20].

Thus, the highest prevalence of *E. coli* strains' resistance was observed for β-lactams antibiotics cefotaxime (29%) and then for tetracyclines. It is known that beta-lactam antibiotics are widely used for therapeutic purposes in human and veterinary medicine. However, gram-negative ESBL E. coli is able to hydrolyze most β-lactams, which makes them resistant to β-lactam antibiotics. Resistance to these antibiotics has become a particular problem in recent years. Bacterial strains producing extended-spectrum beta-lactamases are highly resistant to many antibiotics, and infections caused by these strains are difficult to treat [8, 21].

As a result of these studies, it has been proved that in *E. coli* populations contaminated cheeses of Kazakhstani producers, there are strains that preserve the sul3 gene encoding resistance to sulfonilamides. Genes encoding resistance to trimethoprim are commonly found in many integral profiles. Integrons can exchange between species and play an important role in spreading antimicrobial resistance among bacteria [22].

At the next stage of the research, it is planned the computer modeling of the contamination risk of domestically produced cheeses by resistant to antibiotics *E. coli* strains, in order to develop measures ensured the sanitary safety of cheese products in the Kazakhstani dairy market.

Conclusion

The phenotypic and genotypic resistance of *E.coli* strains isolated from retail cheeses in the central, northern and eastern regions of Kazakhstan to 21 antimicrobial drugs was 61%. The highest prevalence of antibiotic resistance was observed for cefotaxime and tetracyclines, followed by β-lactams. There was established the genotypic resistance to sulfonilamides in the bacterial populations of this species.

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THE EFFECT OF IMPROVAC ON TESTOSTERONE LEVEL AND LIVE WEIGHT OF BULLS

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Abstract

There are many castration methods of bulls and it is believed that the best ones are those that lead to gonadopause or inhibit the function of the gonads, contribute to a significant increase in the quantity and quality of the products obtained. It is currently considered that immunocastration, compared with chemical and surgical castration, is painless and effectively inhibits testosterone production and spermatogenesis. In order to suppress sexual activity in bulls, it is recommended to accomplish immunocastration with Improvac at a dose of 2 ml subcutaneously, with an interval of at least 4 weeks. After 4 weeks of the first injection of Improvac testosterone level decreased to 7.86 ± 1.9 n.gml. With the second injection of the medication the testosterone level decreased to 1.8 ± 0.6 ng/ml in 80% of the animals.

The immunocastration method in bulls with a live weight of 248.6±11.0 to 268±12.4 kg showed the increase in live weight of 34.9±12.9 kg in 60 days. Based on the data obtained the Improvac can be recommended for suppression of activity in bulls, while this method is less invasive compared to surgical methods of castration. There is a fairly high percentage of decrease of testosterone level. This accordingly affects meat productivity, which tends to increase.

Key words: bulls; castration; chemical castration; growth; immunocastration; testosterone.

Basic position and Introduction

Castration is one of the most common operational effects on the animal body in order to improve the quality of the products obtained. As a result of castration, the function of the endocrine glands changes in animals and affects the metabolic processes. Ultimately there are changes in the biochemical status of animals, and a certain shift in hormonal metabolism. As a result, animals gain live weight and are fattened, while their meat becomes tender, fine-fibered, contains more fatty layers.

There are many castration methods and the best are those that will lead to gonadopause or inhibit the function of the gonads, contribute to a significant increase in the quantity and quality of the products obtained.

According to I.F Gorlov., A.A. Kaidulina [1], the determining factor defining the level of animal improvement and its meat productivity is the live weight. The results of studies by some authors show that the intensity of growth of castrated and non-castrated bull-calves of the Kalmyk breed under identical conditions of keeping and feeding is not the same in different age periods. If at the initial stage of the experiments the live weight was almost identical, then in the future, non-castrated animals were superior in productivity to castrated peers, but at the same time they were inferior in quality of meat, the yield of internal fat, and marbling. D.M. Henricks and others [2] relate such changes after castration with the corresponding changes in the restructuring of the hormonal status.

According to K. Stafford & Mellor, D. [3], W. Y. Pang and others [4], G. Ripoll, and others [5], after castration the production of reproductive hormones suspends or decreases, which leads to a change in the physiological status of the organism and its behavioral reactions. Males

have an increased appetite, do not show sexual activity, less mobile, which ultimately stimulates an increase in live weight.

Choosing a method of castration plays an important role. So according to I.T Dzhakupov., K.M. Kamsaev, D.I Domanov [6], live weight increase was higher when using percutaneous method of castration than the bloody method by an average of 35-40%.

But at the same time, in particular, Moreira and others [7], indicate the beneficial effect of castration on the productivity of animals, including the immunogical method, but the authors believe that the quality indicators of the carcass do not depend on the chosen castration method.

Given that the existing methods of castration performed in most cases are highly painful, it should be considered when choosing a castration method. J.F. According to Coetzee, [8], D. Gellatly and others [9], open bloody methods of castration, in particular the complete surgical removal of the gonads, causes severe pain, which is especially evident in the first two hours. Elimination or reduction of pain during castration performance plays an important role in the subsequent development and growth of bulls. According to the literature data, the pain experienced by animals causes severe changes in the body: vascular tone increases, breathing quickens, secretion of the glands of the gastrointestinal tract is inhibited, all types of metabolism are intensified towards catabolism, acidosis, which leads to a decrease in their productivity.

E. L. Ribeiro, and others [10], note that castration, regardless of the chosen method, reduces the overall growth rate of the body, but at the same time, according to the authors, castrated and immunized animals had a greater marbling and carcass fat percentage than non-castrated bulls.

In recent years, in order to reduce sexual behavior of bulls, various methods have been used to inhibit the spermatogenic function of the gonads. According to G. Ripoll and others [5], the elimination of the spermatogenic function of the gonads and the preservation of their hormonal activity allows obtaining high quality meat without reducing the growth rate of animals.

According to P.H. Yamada and others and others [11], immunocastration, compared with chemical and surgical castration, is practically painless, effectively suppresses testosterone production and spermatogenesis. In addition, P.H. Yamada and others [12], the authors studying the effect of castration methods, indicate that during immunocastration a decrease in pain reaction favorably affects the general condition of the animal.

P.R. Huenchullan and others [13], studying the effectiveness of immunocastration, came to the conclusion that this method of castration is safer in relation to post-castration complications and has a beneficial effect on the animal's organism.

In addition to the above, biological castration influences on the quality of the resulting product, which does not lose its appearance and does not contain any impurities that affect the purity of the product. Among medications applied for biological castration of bulls "Bopriva" developed by "Zoetis Inc", USA, is mainly used against Gonadotropin releasing hormone (GnRH), which was proposed for sterilization of males. On its basis the Improvak was developed for pigs which can also be used for bulls according to A. Noya, and others [14].

The proposed medications allow keeping the aggressive sexual behavior of animals under control for a certain time by producing antibodies against gonadotropin-releasing hormone (GnRH), thereby reducing the level of testosterone in the blood of males.

In accordance with the abovementioned, the purpose of our research was to study application of Improvak for biological castration of bulls and to study the dynamics of testosterone in animal's body. Considering the effectiveness of the drug on boars and the absence of contraindications for its use on bulls, it was decided to conduct a study of "Improvak" on bulls.

Materials and Methods

Animals. As a study object 30 heads of bulls at the age of 12-14 months in feeding platform were chosen. The animals were injected with Improvak for immunocastration twice at a dose of 2 ml subcutaneously with an interval of 4 weeks. The effect of the medication on the level of testosterone and live weight of bulls was identified.

Blood was taken from the jugular vein before castration and 30, 60 days after it. All analyzes were carried out in the scientific laboratory of veterinary medicine and in the Republican Diagnostic Center.

Detection of testosterone. In order to study the functional activity of the endocrine glands and metabolism in the peripheral blood of animals, the

presence of the testosterone hormone in the blood serum was measured. Studies on testosterone were performed with a Roche C8000 analyzer (Germany) by photometric method.

Statistical analysis. The materials of experimental and clinical studies were analyzed biometrically using Student's test, as well as the constant method. The digital indicators obtained in the process of research were processed by the method of variation statistics according to V.K. Kuznetsov [15], as well as using the statistical functions wizard of Microsoft Excel.

Results

To analyze the effectiveness of castration the Improvak of "Zoetis Inc" for immunocastration was applied at a dose of 2 ml subcutaneously with an interval of at least 4 weeks [16].

The average age of the tested bulls was 15.2±0.26 months, and the average live weight was 263.6±18 kg.

Clinical changes in animals were not observed as a result of using Improvak. The second dose of Improvak was given 4 weeks after the first injection (Table 1).

Table 1 - Interval between injections and duration of Improvak for bulls

Interval between injections, weeks (after 2 injections)	Minimum duration period, weeks
3 - 4	12
6	14
8	16

Table 1 shows that, subject to the intervals between injections, the period of action of the Improvak allows for a certain time to control the aggressive sexual behavior of animals, producing antibodies against gonadotropin-releasing hormone (GnRH), thereby reducing the level of testosterone in the blood at bulls.

As noted above, the level of testosterone is an important criterion for the effectiveness of the method for reducing sexual activity. It was significantly reduced when using the above method. The research results are shown in table 2.

Table 2 - Dynamics of testosterone levels in bulls after immunocastration

	Before injection	30 days after injection (ng/ml) (M±m)	60 days after injection (ng/ml) (M±m)
Castrated	14,5±1,2	7,86±1,9	1,8±0,6
Non- castrated	16,5±1,8	-	-

When analyzing table 2, in particular, it was found that after using the Improvak 30 days after the injection, the testosterone level decreased by almost two times and amounted to 7.86 ± 1.9 ng/ml. And after repeated injection of the medication after 4 weeks and checking the level of testosterone in the blood 60 days after the first injection, it was 1.8 ± 0.6 ng/ml i.e. decreased by 4.36 times.

It should be especially noted that the use of Improvak did not cause reactive changes at the injection site. The animals took the injection calmly, which indicates a low invasiveness. As it is known, surgical methods of castration cause a pain reaction, which accordingly affects the physiological state of the animal, especially in the first days after the procedure.

Table 3 - Dynamics of the growth rate in bulls after immunocastration

Group	Castration age, month (M±m)	Live weight before castration (M±m)	Live weight after 60 days (M±m)	Live weight gain	
				For 2 months, (kg) (M±m)	Daily (g) (M±m)
After immunocastration with Improvac	15,7±0,29	263,6±4,6	298,5±17,5	34.9±12,9	581±4.6
Non- castrated bulls	15,5±0,21	252,6±21,3	284±11,8	31,4±9,5	523±2.3

When studying the effect of the drug "Improvak" on live weight, according to Table 3, it was noted that before immunological castration, the live weight of bulls averaged 263.6 ± 4.6 kg, and 60 days after the manipulation 320.5 ± 9.2 kg. This amounted to an increase in live weight by an average of 34.9±12.9 kg. It should be noted that the average increase in non-castrated bulls on the farm for the same period was 31.4±9.5 kg (n=30).

Discussion

One of the ways to increase meat productivity is to reduce the sexual behavior of animals. There are various ways influencing the activity of animals. One of the optimal cost-effective methods is castration, orchidectomy of male live-stock animals. The meat of fattening castrates becomes tender, loses its unpleasant odor and taste. In castrated animals loses their violent temper, which facilitates their operation and group housing. Castration methods have both positive and negative aspects affecting both the effectiveness of castration and the increase in the live weight of animals.

Recently, study of various immunological medications that are used to suppress the sexual activity of bulls has been much considered.

There is a lot of literature information about the positive effect of castration on the meat productivity of animals, in particular Moreira, Aline D., Siqueira, Gustavo R., Lage, Josiane F. and others [7], which indicate the beneficial effect of castration on the productivity of animals, including and immunological method. But the authors believe that the quality indicators of the carcass do not depend on the chosen method of castration.

Medications of the immunological method of castration allow for a certain time to keep the aggressive sexual behavior of animals under control by producing antibodies against gonadotropin-releasing hormone (GnRH), thereby reducing the level of testosterone in the blood of bulls. A decrease in testosterone levels, contributing to a decrease in aggressiveness, reduces injuries among fattening bulls, increases their productivity, and facilitates group housing. In addition, L.S. Leal-Karolewski, and others [17], indicate that the use of immunocastration, in addition to suppressing testosterone production, also affects the size of the testes.

Our studies showed a decrease in testosterone levels to 1.8 ± 0.6 (ngml) at day 60 after injection, which indicates the inhibition of secretion of sexual hormones. Similar results were obtained by J.L Bolado-Sarabia and others [18], who noted a decrease in testosterone levels in Holstein bulls after the application of the Bo-priva vaccine. The decrease in the amount of testosterone to a residual level is reflected in the results of studies by A. Noya and others [14].

J.A. Withoeft and others [19], note that after immunocastration of bulls there is reduction in size of testes and a decrease in spermatogenesis, which is associated with inhibition of testosterone production.

At the same time, P.H. Yamada and others [12] believe that immunocastration is a good alternative to surgical castration, since this method allows suppressing testosterone production and spermatogenesis, but is less invasive.

But at the same time, there are different opinions on the effect of immunocastration on the growth of animals according to A.D. Moreira and others [20], when comparing surgical and immunocastration, the qualitative characteristics of carcasses improve, regardless of chosen castration method.

V. M. De Freitas and others [21], note that immunocastrated animals were similar in characteristics to non-castrated animals, and had lower productive indicators.

The average daily weight gain for 2 months was 581 ± 4.6 g for immunocastrated and 523 ± 2.3 g for non-castrated was identified. The results obtained indicate a favorable effect of the vaccine on the growth and development of animals.

To suppress the sexual activity of bulls a scheme of using Improvac needs to be drawn up. We recommend 1 injection of Improvac at the beginning of May, and the second injection after 4 weeks in June, the sexual activity of bulls will be suppressed from July to September during the grazing of animals on pasture.

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Conclusion

The use of imunnocastration in bulls with a live weight of 248.6±11 to 268±12.4 kg showed that in 60 days the increase in live weight was 34.9±12.9 kg. The testosterone level 4 weeks after the first injection of the drug "Improvac" was 7.86 ± 1.9 n. gml, and after the second injection of the drug, the testosterone level decreased to 1.8 ± 0.6 ngml in 80% of animals. The data obtained allow us to recommend Improvac at a dose of 2 ml subcutaneously, with an interval of at least 4 weeks, to suppress activity in bulls.

At the same time this method is less invasive compared to surgical methods of castration. There is a rather high percentage of testosterone level reduction that accordingly affects meat productivity, which tends to increase.

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DETERMINATION OF IMMUNIZING DOSES OF THE COWPOX VACCINE CANDIDATE: PRELIMINARY RESULTS

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Abstract

Determining the effective immunizing dose of a vaccine is one of the important pillars of a vaccination strategy. In this regard, this article presents the results of studies to determine the effective immunizing dose of a candidate cowpox vaccine prepared from the BIEMG-51 strain of the vaccinia virus. At determining a immunizing dose of the vaccine, it was found that in all animals vaccinated at doses of 103 TCID50, 104 TCID50, 105 TCID50, antibodies were not detected in the serum neutralization test (SNT). However, these vaccinated calves at the indicated doses were resistant to the challenge, while the unvaccinated group responded to the challenge and fell ill with the characteristic clinical signs of cowpox. The obtained primary results give grounds for improving the technology of vaccine preparation and in-depth study of the factors of cellular immunity of vaccinated animals in case of poxvirus infections.

Key words: cowpox; minimum immunizing dose; safety; vaccine; virus.

Basic position and Introduction

Cowpox is a contagious disease characterized by intoxication of the body, fever and nodular-pustular rash on the skin and mucous membranes. The causative agent of this disease is a viral agent that belongs to the genus *Orthopoxvirus* of the *Poxviridae* family [1]. Many *Orthopoxviruses* have an animal reservoir and can be transmitted by contact from an infected animal to a person. The main reservoirs and carriers of the cowpox virus (CPXV) are wild and predatory rodents [2].

Until the 70s of the XX century, it was believed that CPXV causes outbreaks of the disease only in the population of cattle, the clinical picture of which is more often manifested in the form of local (lesions on the skin of the udder and on the nipples), less often generalized form infection (more typical for calves). Later, it was found out that a much wider range of animals are susceptible to the virus, in addition, CPXV is pathogenic to humans and can cause generalized infection in people with weakened immunity [3-5]. In people with weak immunity, exposure to Orthopoxvirus can lead to severe forms of the disease or even death [6].

Recently, human infection with cowpox associated with domestic rats has been reported in Europe, with usually mild and self-healing lesions [7, 8]. In many countries, the reappearance or emergence of other orthopoxviruses in human and animal populations is an urgent global health and veterinary problem. There are literature data on human infections and diseases caused by zoonotic *Orthopoxviruses*, such as Monkeypox virus (MPXV) [9], CPXV [10], vaccinia virus (VACV) [11] and Ahmet virus [12]. Also, Research Institute for Biological Safety Problems (RIBSP)

employees found seropositive animals to cowpox virus during monitoring studies on the territory of Zhambyl region (data not published). These facts raise concerns about the habitats and distribution of orthopoxviruses, as well as their potential to cause outbreaks among animals and humans, thereby having a further impact on the health of animals and the population. This indicates the need for special efforts to develop modern means of rapid diagnosis of the etiological agent of this disease, the search for antiviral drugs and vaccination of susceptible animals. So, vaccination is one of the most important achievements of science, and is an effective, safe, economical means of controlling and eliminating life-threatening infectious diseases. Taking into account the above situations, research on the creation of a live vaccine against cowpox based on the VACV was started at the RIBSP.

Therefore, the aim of the study was to determine the immunizing dose of an experimental cowpox vaccine for target animals.

Materials and Methods

2.1 Virus strains

The BIEMG-51 strain from the VACV obtained from the Moscow Research Institute of Viral Preparations in 1996 was used as an object of research. The virus was adapted by three consecutive passages on the chorion-allantois membrane (CAM) of embryonated chicken eggs (ECE). It is stored in vials under vacuum at a temperature of minus 40 ° C. There is no information about its genetic characteristics. The lyophilized CowPOX strain obtained from All-Russian Research Institute of Veterinary Virology and Microbiology, which passed 12 passages on the CAM of ECE, was also used in the work.

2.2 laboratory animals

To study the immunogenicity of the vaccine against CPXV, local breeds of one-year-old twelve calves were used in the experiment, and fifteen white mice weighing 18-21 g, ten guinea pigs weighing 700-800 g were used to determine the safety of the vaccine and ten rabbits weighing 1.5-2 kg. Maintenance and feeding of laboratory and target animals was carried out in accordance with the instructions [13].

2.3 Virus propagation in cell culture and determination of infectious activity

The CPXV growth in lamb kidney cell culture was carried out according to the method [14]. Further, microscopy, collection, preparation of viral suspensions and determination of their infectivity titers in cell culture were carried out according to the above method. The titer of the virus was considered to be its greatest dilution, causing CPE in 50% of infected vials with cell culture. The virus titer was calculated using the method of Reed L.J. and Muench H.A. [15].

2.4 Preparation of the protective environment and formulation of the vaccine

As a protective environment for the CPXV strain, we used 5% peptone and 3% sucrose in final concentrations with double sterilization by liquid steam at (100±1) °C for 30 minutes (the interval between sterilizations is 18-20 h.). A sterile protective environment was combined with a viral suspension before lyophilization in a ratio of 1:1. The resulting mixture was poured into vials of 1 mL. Then mixture frozen at minus 60°C for (12±4) hours and dried in a lyophilic apparatus “Usifrua” under the following mode: freezing temperature – minus (55-60)°C; pressure in the chamber - from 3 to 7 Pa; the heating temperature of the shelves is from 10 to 40°C; the final temperature of the viral suspension is (22±2)°C. After drying the material, the vials were sealed under vacuum on a carousel-collector apparatus at a residual pressure of 25 to 30 Pa.

2.5 Determination of the safety of the vaccine in laboratory animals

To determine the safety of the developed CPXV vaccine, laboratory white mice weighing 16-18 grams, guinea pigs 1,5 months old, and rabbits 3 months old weighing 4,5-5 kg were used. The lyophilized vaccine was diluted with saline solution to the initial volume and injected into each animal, subcutaneously in the area of the hairless area of the axillary region at the appropriate dose: 10 white mice, 5 guinea pigs at doses of 0,1 cm3/head and 0,3 cm3/head and 8 rabbits at a dose of 0,5 cm3/head. As a control, 5 mice, 2 guinea pigs and 2 rabbits were left unvaccinated. The observation period for vaccinated animals was 10-14 days. According to the results of the research, the animals should be alive and clinically healthy.

2.6 Determination of the immunizing dose of the vaccine

The immunizing dose of the CPXV vaccine was determined on one-year-old calves, on a cut, shaved and treated with ethyl spirit area of the skin in the neck by intradermal immunization.

To determine the immunizing dose, the following vaccine doses were tested: group I (n=3) – 1000 TCID50, group II (n=3) – 10000 TCID50, group III (n=3) – 100000 TCID50, which were applied to test calves by intradermal immunization at different points in a volume of 1,0 cm3. The immunized calves were monitored daily in case of the appearance of characteristic clinical signs of smallpox disease and body temperature was measured. Blood serums were taken from vaccinated animals on days 7, 14, 21 after immunization to determine virus neutralizing antibodies to the cowpox virus in the SNT.

2.7 Determination of the titer of virus neutralizing antibodies

The activity of virus neutralizing antibodies in blood sera was determined in the SNT with a constant dose of virus and different serum dilutions according to the method [16]. The titer of antibodies of the serum under study was taken to be the largest dilution of serum that inhibits the development of the CPE virus in at least 50% of the infected cell culture.

Results

3.1. Determination of the safety of the developed vaccine on laboratory animals

In order to determine the safety of the developed vaccine against cowpox from the strain Biemg-51, experiments were carried out on white mice weighing 16-18 grams, guinea pigs 1,5 months of age and rabbits weighing 2,5-3 kg, which were injected subcutaneously with an experimental vaccine against cowpox in the amount of 0,1, 0,3 and 0,5 cm3/head, respectively. The experimental animals were clinically observed with daily thermometry for 14 days, paying special attention to the general condition of the animals and the local reaction at the injection site. At the same time, the general reaction (according to the presence and severity of hyperthermia) and the local reaction in terms of the size and nature of seals at the site of vaccine administration were taken into account. Before and during the experiment, the animals were kept under standard vivarium conditions under natural light, on a balanced diet with free access to water. The results of the conducted studies are presented in table 1.

Table 1 - Evaluation of the safety of an experimental sample of the vaccine against cowpox in mice, guinea pigs and rabbits

Type of animals	Number of animals (head)	Animal reaction	
		local reaction	general reaction
White mice	15	absent	absent
Guinea pigs	6	absent	absent
Rabbits	6	slight swelling	absent
Control (saline solution)	2 heads from each type of animal	absent	absent

The results of the experiments and the data in Table 2 showed that no signs of deviation from the physiological norm were observed in all laboratory animals when the vaccine was administered subcutaneously. At the end of time, the animals remained alive and healthy.

3.2. Determination of the immunizing dose of the vaccine

To determine the immunizing dose of the experimental vaccine, we tested three doses of the strain BIEMG-51 of the vaccinia virus: 103 TCID50, 104 TCID50, 105 TCID50. At the same time, the animals were immunized intradermally in a volume of 1 cm3.

According to the results of the experiment, it was found that all vaccinated animals didn't have a temperature reaction and any post-vaccination complications to the introduction of the BIEMG-51 strain of the vaccinia virus.

Further, when studying the humoral immune response to the CPXV in SNT, it was found that in all animals vaccinated with vaccinia in different doses on days 7, 14, and 21, virus neutralizing antibodies was practically absent in the blood sera.

Table 2 – Results of determining the immunizing dose of the vaccine on calves

Group and doses of the vaccine	Animal number	SNT results, log2			Results of the control infection		
		7	14	21	temperature	skin lesion	other clinical signs
I (10^3 $TCID_{50}$)	1	0	0	0	38,5	-	-
	2	0	0	0	38,7	-	-
	3	0	0	0	38,5	-	-
II (10^4 $TCID_{50}$)	4	0	0	0	38,6	-	-
	5	0	0	0	38,5	-	-
	6	0	0	0,75	38,7	-	-
III (10^5 $TCID_{50}$)	7	0	0	0	38,8	-	-
	8	0	0	0,5	38,6	-	-
	9	0	0	0,75	38,5	-	-
IV (control)	10	0	0	0	39,7	+	+
	11	0	0	0	39,5	+	+
	12	0	0	0	39,8	+	+

Notes:
(–) – absence of clinical signs
(+) – presence of clinical signs.

However, despite the absence of virus neutralizing antibodies in calves, all vaccinated animals did not respond to the control infection with the virulent strain «Cowpox-CAM». At the same time, in the vaccinated groups during the observation period, no deviations from the physiological norm were noted, regardless of the immunizing dose of the vaccine. Whereas in the control group of animals on the 3rd day there was a lack of appetite, a slight increase in body temperature, as well as lesions (variolas) on the skin of animals developed at the site of the introduction of the virus.

Discussion

At present, the human population has practically no immunity to orthopoxvirus infections that cause smallpox viruses, monkey pox, cowpox, buffalo pox. Every year, more and more massive outbreaks of orthopoxvirus infections among humans and animals are registered on different continents [17]. Therefore, vaccination is the only way to protect people, animals and birds from this infectious disease. In this study, we used the BIEMG-51 strain of the vaccinia virus, which was successfully used for preventive immunization of camels against camel pox in 1996 in the Mangystau region [18]. In addition, this strain was successfully used until 1980 in the production of smallpox vaccine for healthcare during the Soviet Union. In this regard, in this study, the specified strain was chosen as a comprehensively tested and most safe strain for creating a vaccine, and studies were carried out to determine its immunizing dose for cattle, and its immunogenicity was determined. It is known that the immunogenicity of vaccines, as a rule, is directly dependent on the concentration of the antigen in the vaccination dose, that is, the higher the activity of the drug, the more significant immunogenicity it has, while causing intense immunity in animals in the short term and for a long period after vaccination. [19]. According to some authors [18], the duration of post-vaccination immunity against chicken pox in birds depends on the vaccination dose of the vaccine preparation. At the same time, the duration of immunity in birds that received small doses of the vaccine was relatively short. Such similar studies on the selection of the minimum immunizing field dose were carried out during the development of monovaccines against sheep pox, *peste des petits ruminants* (PPR), lumpy skin disease and associated vaccine against sheep pox and PPR [19-21]. When determining the immunizing dose of the associated vaccine, the authors prepared 4 samples of a vaccine preparation with different immunizing doses of 10, 100, 1000, 10000 TCID50. According to the results of the conducted studies, it was found that animals vaccinated at a dose of 100 TCID50/cm^3

of the PPR and sheep pox virus acquired protect to challenge with sheep pox, and at the same time the effectiveness of immunization was 67%. At a dose of 1000 – 10000 TCID50/cm3, the virus neutralizing antibodies to the sheep pox virus was in titer 1:8 – 1:32, to the PPR virus in titer 1:2 - 1:8 and the animals didn't respond to the control infection, the effectiveness of immunization was 100%. While animals vaccinated at a dose of 10 TCID50/cm^3 and intact animals became ill after control infection with epizootic sheep pox virus. A similar study conducted on calves that received different doses of the vaccine showed protect to challenge with the lumpy skin disease virulent virus of cattle without showing any clinical signs of the disease [19]. In our study, no antibodies were produced in immunized animals regardless of the administered dose concentration of the vaccine preparation. But, despite this, during experimental infection, vaccinated animals did not show clinical signs characteristic of cowpox, remained healthy and alive during the entire observation period. A possible explanation for this conclusion may be a decrease in immune properties during attenuation of poxviruses due to a long passage in the biological system or a simultaneous increase in paraspecific effects in poxvirus infections [22]. It is important to note that in poxvirus infections, cellular factors play a more prominent role as protective factors of immunity. Humoral factors may be absent or present at a low level of antibodies, which cannot be detected using available tests (SNT, ELISA). Animal protection or the presence of immunity in such cases is confirmed by resistance to infection with a virulent virus. These data are based on such results of studies in which animals whose blood serum lacked specific antibodies, and they remained immune to the virulent virus during experimental infection [23]. The same data were obtained by other researchers who experimentally studied the noted phenomenon regardless of previous authors. Based on the results of their research, they attribute this phenomenon to cellular immunity observed in smallpox diseases [24]. However, this question remains open and requires additional research.

Conclusion

Thus, the results of the experiments showed that the experimental sample of the vaccine against cowpox was evaluated as safety against laboratory animals. Also, when determining the immunizing dose of the vaccine, it was found that animals vaccinated at doses of 10^3 $TCID_{50}$, 10^4 $TCID_{50}$, 10^5 $TCID_{50}$ were resistant to control infection, while the unvaccinated group reacted to challenge and fell ill with characteristic clinical signs of cowpox.

Based on the foregoing, the optimal immunizing dose of the vaccine for calves was 1000 $TCID_{50}$. However, taking into account unforeseen circumstances during storage and transportation, we propose a fivefold immunizing dose - 5000 $TCID_{50}$.

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EVALUATION THE DAIRY COWS' WELFARE IN NORTHERN KAZAKHSTAN FARMS

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Abstract

In modern society animal welfare is an important attribute of the food quality general concept. Research purpose was to identify the dairy cows' welfare problems in Kazakhstan. In 2022 and 2023, studies were organized at two industrial dairy farms in the central (Kamyshenka LLP) and northern (Ayna Dairy Farm LLP) zones of the Akmola region. Here, the welfare of 110 cows was measured in accordance with 33 parameters, 12 criteria and 4 principles (according to the Welfare Quality® protocol). On both farms, the main animal welfare indicators were approximately within the same limits. The criterion "Absence of prolonged thirst" was 60.0 scores. In both farms, such criteria indicators as "Absence of prolonged hunger", "Absence of diseases" had relatively low values in the range of 3.5-19.98 points. The criteria "Absence of injuries", "Lameness", "Integument alterations", "Positive emotional state" were also below 53.9 points. Scores of criteria "Comfort during rest", "Absence of pain caused by management procedures", "Manifestation of other forms of behavior", "Good relations between man and animal" exceeded 55 points and reached a maximum value of 80.01 points at the Ayna Farm. Overall, farms scored from 20.1 to 50.4 points on the basic principles of welfare, with the exception of the principle of "Good Health", according to which Ayna Dairy Farm scored 64.4 points, but Kamyshenka Farm had a lower level – 38.2. Thus, both farms were classified by the Welfare Protocol as "Acceptable". The study has been shown that the housing of dairy cows needs to be improved for increasing the comfort of rest and reduce cow injuries.

Key words: Akmola region; cattle; criteria of welfare; dairy farm; measurements of welfare; principles of welfare; welfare of dairy cows.

Basic position and Introduction

The dairy herd in Kazakhstan has more than 1.2 mln cows, which are bred in more than 3.415 productive and 821 breeding farms. Over the past 10 years, the number of productive farms has increased by more than 7.5 times, and breeding farms by 3.3 times [1]. Repeated multiple fertilization, short intervals between calving, overproduction of milk, restrictive maintenance systems, poor nutrition and physical disorders worsen the welfare of animals in industrial dairy enterprises. Due to the milk yield increase cows have additional health problems for high metabolic productivity. In the 20th century, selective breeding of dairy cattle was mainly focused on increasing dairy productivity with insufficient attention to improving qualities important for health and welfair [2, 3]. According to the WOAH definition "Animal welfare means the physical and mental state of an animal in relation to the conditions in which it lives and dies" [4].

The lactating and dry cows' farming systems are determined by the climate and the vast majority of dairy enterprises in the world keep cows mainly indoors, and only in 9.9% of farms the lactating cows are raised mainly on pasture [5], although the preventing access to pasture had been considered

among the main hazards to welfare of dairy cows [6].

Thus, different ptoduction technologies determine different levels of welfair of the dairy herd, the definition of which is currently considered one of the main criteria for assessing the overall management of livestock enterprises. In countries with industrialized animal husbandry, public concern and expectations in this matter are constantly growing. Consequently, the dairy sector should actively work to ensure high standards of animal welfare in order to maintain consumer confidence.

In addition, over the past decade, new scientific data, tools and animal welfare standards have been published (WOAH, 2018; ISO, 2016), which are regulated by the legislation of many countries (for example, Directive 98/58/EC in Europe) [4, 7, 8]. The European Food Safety Authority (EFSA) has identified lameness, mastitis, metabolic disorders, low fertility and short life expectancy as the main problems affecting the welfare of the dairy herd [9].

Based on the research of various experts from different animal sciences' area a Welfare Quality® protocol for assessing the welfare of dairy cows was developed [10]. The overall rating scale allows comparing the results according to different criteria, so that it is easier to set priorities. Thus, the results should serve as a guide to identify the main risks to welfare on the farm and, in turn, at the population level. One of the important questions is to what extent the characteristics of the farm inform us about the risks to animal welfare.

These studies aimed to identify the most important problems of welfare on Kazakh dairy farms based on their assessment according to the Welfare Quality® protocol.

Materials and methods

In 2022 and 2023, field studies were organized on two industrialized dairy farms in central (Kamyshenka LLP) and northern (Ayna Dairy Farm LLP) Kazakhstan, when cows were indoors. Here the welfare of 110 cows was measured according to 33 parameters, 12 criteria and 4 principles. Figure 1 shows the moments of animal-oriented measurements in conditions of the Kamyshenka Dairy Farm.

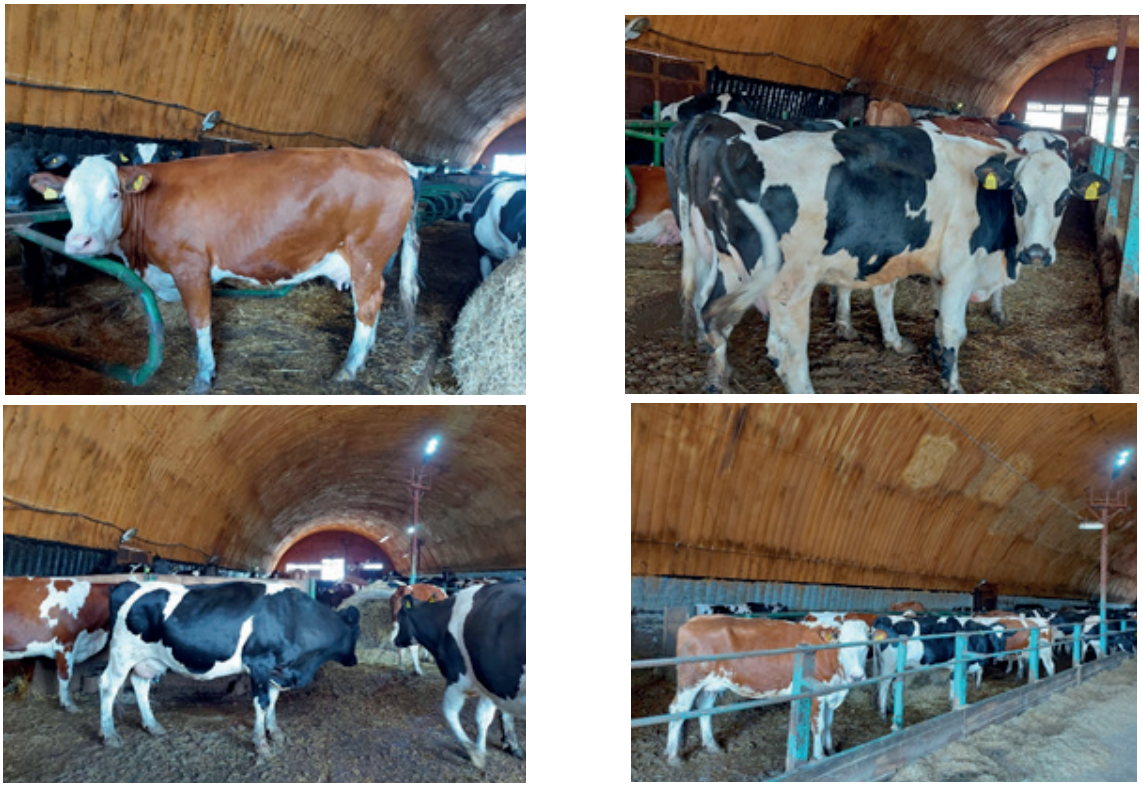

Figure 1 – Housing conditions in Kamyshenka Dairy Farm

Five characteristics of dairy farms were selected as stratification parameters. They included: location (central and northern parts of the Akmola region steppe zone), breed of cows (Holstein), milking system (automated milking system – AMS), housing system (loose) and the number of cows on each farm (Table 1).

Table 1- Characteristics of the farms studied

Housing system	Breed	Milking system	Geografic zone	Number of lactating cows	Farm
Loose housed	Holstein	AMS	Steppe	395	Ayna Dairy Farm
Loose housed	Holstein	AMS	Steppe	439	Kamyshenka

There was used a Protocol for Assessing the Quality of Welfare for Dairy Cattle (Welfare Quality ® 2009), which is based on four principles ("Good feeding", "Good housing", "Good health" and "Appropriate behavior") [10]. They are divided into 12 criteria (for example, the "Good feeding" principle includes the criteria "Absence of prolonged hunger" and "Absence of prolonged thirst"). Each criterion was evaluated using from one to seven measurements, resulting in a total of 33 measurements giving baseline data, such as the % of animals with a given problem or the frequency of social interactions. Most of the measurements were performed on animals (clinical and behavioral observations). Some measurements are based on resources (for example, the number of water points) or on management (for example, the method of dehorning). Measurements on animals made at the individual level were carried out on a sample of animals randomly selected in the herd, depending on the size of the herd, according to the protocol. The data obtained using measurements that relate to this criterion were collected into scores that reflect how well the farm meets this criterion. The assessment summarizes information on the prevalence and severity of problems according the protocol. The score was expressed on a scale of 0-100, where 0 means very low welfare and 100 means excellent welfare.

There were performed three main types of calculations:

Measurements the criteria "Absence of prolonged hunger", "Absence of injuries", "Expression of social behaviours", "Expression of other behaviours", "Good human-animal relationship", "Positive emotional state" provided continuous data on similar scales. The severity of the problem was taken into account (for example, the % of non-lame, moderately lame and severely lame cows). Then a weighted sum was calculated (for example, the % of lame animals weighted taking into account the severity of lameness). Then cubic functions were used to convert the weighted sum into an estimate of the criterion. According to the "Absence of injuries" criterion, two partial scores were calculated – one for integument alterations (i.e. skin changes: hairless areas and lesions/swellings with a diameter of 2 cm or more) and one for lameness, which were then combined into a criterion score.

Measurements of the criteria "Comfort around resting" and "Absence of diseases" gave continuous data expressed in different scales. For each type of data (proportion of affected animals, average lying time, etc.), three levels were determined: the data collected on the farm corresponded to the absence of problems, moderate problem or serious problem. The number of problems noted on the farm was then converted into an estimate using cubic functions (as indicated above).

Measurements of the criteria "Absence of prolonged thirst", "Ease of movement" and "Absence of pain induced by management procedures" gave data expressed in a limited number of categories, and a decision tree was used to calculate discrete scores. For example, to assess the "Absence of pain induced by management procedures", the procedure used for dehorning (without dehorning, disbudding by thermocoagulation, chemical disbudding, dehorning in adult cows) and the use of drugs (no, anesthetics, analgesics, both) were taken into account.

Data collection began immediately after morning milking and ended in the afternoon. The assessment was made on one visit per day.

Criteria scores of the farm in relation to such measurements as "Absence of hunger", "Comfort around resting", "Absence of injuries", "Absence of lameness", "Absence of diseases", "Expression of social behavior", "Good human-animal relationship", "Positive emotional state" were calculated based on the primary data using the I-spline function according to the general formula:

Score = $a + b \times I + c \times I^2 + d \times I^3$ with the specified values for each coefficient.

Partial assessment of skin changes was calculated by the index for integument alterations

$I_s = \left(100 - \frac{(\%mild)+5\,(\%severe)}{5}\right)$.

Partial score for lameness was determined by the index

$L_l = \left(100 - \frac{2(\%moderate)+7(\%severe)}{7}\right)$.

Index of social behavior was determined by the formula

I = 100 x [(43,8) – (x (head butts) +y(displacements))]/43.8,

Index for good human-animal relationship – by

$I = \left(100 - \frac{3(\%cat2)+11(\%cft3)+26(\%cat4)}{26}\right)$.

Behaviour scores are converted to an index using a weighted sum:

$$\text{Index} = -3.40496 + \sum_{k=1}^{20} W_k N_k, \text{ where}$$

N_k is the value obtained by the farm for a given period k, W_k – the weight attributed to this term *k*.

Then these indicators were recalculated by the I-spline function. Two partial estimates for the absence of injuries were combined using the discrete Choquet integral of a function $g: N \rightarrow R$ defined by:

$$C_\mu(g_1, \ldots, g_n) = \sum_{i-1}^{n} \left(g_{(i)} - g_{(i-1)}\right) \mu(A_{(i)}), \text{ where}$$

μ is a fuzzy measure, $\mu: 2^N \rightarrow [0,1]$. (i) means that the indices are permutated as $\{0 \leq g_{(1)} \leq g_{(2)} \leq g_{(3)} \ldots \leq g_{(n)}\}$; $A_{(i)} = \{(i), \ldots (n)\}$, and $g_{(0)} = 0$.

Criterion scores for measurements “Absence of prolonged thirst”, “Ease of movement”, “Absence of pain induced by technological procedures" were determined according to the decision tree. Then the obtained criterion scores were combined into principle scores by the Choquet integral. For each principle, a score (on a scale of 0-100) was calculated, summarizing the estimates received by the firm according to various criteria related to a specific principle, which served as the basis for assessing the welfare of cows on the farm.

Results

General assessment the welfare of dairy cows in studied farms

These studies have shown that in both firms, the principal scores for assessing the state of animal welfare are approximately within similar limits, with some excess in Ayna Dairy Farm (Figure 2).

Overall, farms scored from 20.1 to 50.4 points on the main principles of welfare with exception the "Good Health" principle, according to which the Ayna Dairy Farm’s scores were 64.4, but Kamyshenka Fairy Farm had a lower level - 38.2. Thus, both farms were classified by animal welfare as “Acceptable”.

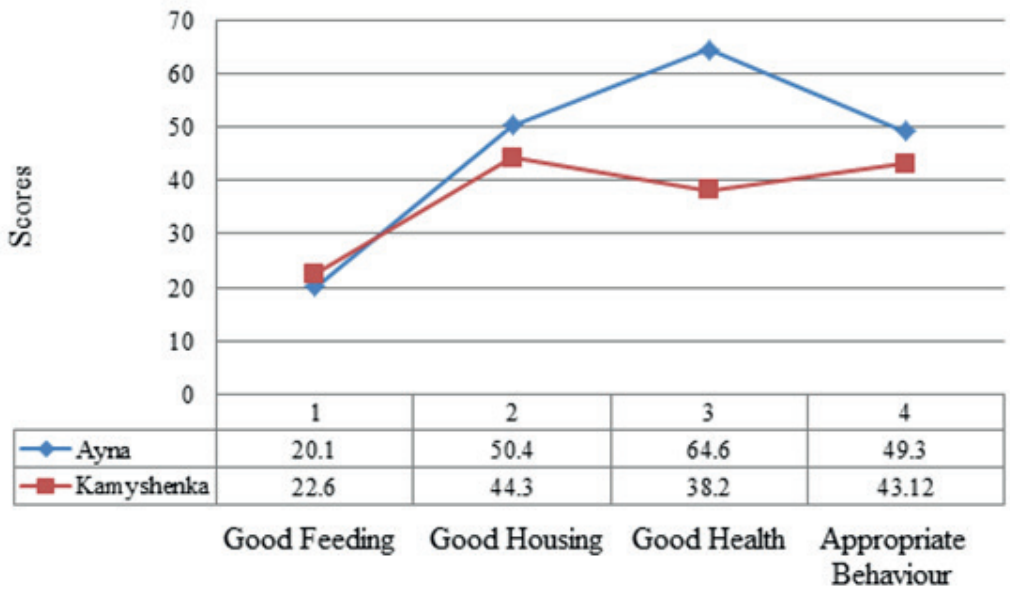

Figure 2- Welfare indicators of dairy herds by principal scores
(on a scale of 0-100, where 0 is a low level and 100 is an excellent level)

Determination the criterion-scores

In both farms, criterion scores showed similar results, while in Ayna Dairy Farm, relatively high values of indicators of the welfare of dairy cows were observed. As for the criterion "Absence of prolonged hunger", more than 18% of very lean cows were observed in both farms, which is explained by the breeding of Holstein cows on both farms (Table 2).

Although on farms, in general, the water supply is sufficient, the criterion "Absence of prolonged thirst" reached a value of 60.0, since the length of the water points and the water pressure was below the standard specified in the protocol.

Table 2 - Main indicators of welfare of dairy herds by criterion score (on a scale of 0-100, where 0 means low and 100 – excellent welfare) for two farms

Welfare Criteria	Scores	
	Ayna Dairy Farm	Kamyshenka Dairy Farm
Absence of prolonged hunger	3.50	5.7
Absence of prolonged thirst	60.00	60.0
Comfort around resting	66.3	55.7
Ease of movement	100	100
Absence of injuries	53.90	49.3
Lameness	22.9	28.8
Integument alternations	42.50	43.7
Absence of disease	19.98	4.18
Absence of pain induced by management procedures	60.0	65.0
Expression of social behaviours	80,01	61.93
Expression of other behaviours	0	0
Good human-animal relationship	56.09	49.09
Positive emotional state	24.45	22.06

In both farms such criteria indicators as “Absence of prolonged hunger”, “Absence of disease” had relatively low values in the range of 3.5-19.98 scores. Criteria “Absence of injuries”, “Lameness”, “Integument alternations”, “Positive emotional state were also below 53.9 scores. Criterion points for “Comfort around resting”, “Absence of pain induced by management procedures” “Expression of other behaviours”, “Good human-animal relationship” exceeded 55 points and reached a maximum value of 80.01 scores at the Ayna Dairy Farm. The value of the criterion indicator “Accessibility of movement” was absolute in both farms, since here loose keeping of livestock is practiced (Table 2).

Results of primary animal-, housing- and resource-oriented measurements.

The primary data collected on farms show the presence of serious problems on certain aspects of welfare in the dairy herds of both farms (Table 3).

Table 3 - Main indicators of dairy herds’ welfare at the farms

Measures	Dairy Farms	
	Ayna	Kamyshenka
Absence of prolonged hunger % of very lean cows	18.33	29.6
Comfort around resting		
Mean time to lie down (in seconds)	6.87	6.8
% of animals colliding with housing equipment during lying down	30	13.3
% of animals lying artly/completely outside lying area out of all lying animals	0	0

% of animals with dirty udder	31.66	16.6
% of animals with dirty hindquarters	73.3	16.0
% of animals with dirty lower hind legs	83.3	57.4
Absence of injuries		
% of moderately lame animals	30	18.5
% of severely lame animals	18.33	14.8
% of animals with mild integument alterations	21.7	22.2
% of animals with severe integument alterations	25	24.1
Absence of disease		
Mean number of coughs per animal and per an hour		0.6
% of animals with nasal discharge	8.33	27.7
% of animals with ocular discharge	0	12.6
% of animals with hampered respiration	0	7.4
% of animals with diarrhoea	11.7	14.8
% of animals with vulvar discharge	8.33	1.85
% of cows with somatic cell count of 400,000 or above	8.75	19.0
% of dystocia	0	11.3
% of downer cows	0	8.8
% of mortality on the farm during the last 12 months	5.4	15.9
Expression of social behaviours		
Mean number of head butts per animal an hour	1	0.8
Mean number of displacements (agonistic behaviours except head butts) per animal an hour	0	2.0
Expression of other behaviours		
Number of days with access to pasture per year	0	0
Number of hours per day on pasture	0	0
Good human–animal relationship		
% of animals that can be touched	73.3	48.0
% of animals that can be approached closer than 50 cm but not be touched	10	28.0
% of animals that can be approached as closely as 100 to 50 cm	3.3	12
% of animals that cannot be approached as closely as 100 cm	13.3	12

In both enterprises, there are problems with the herd health that significantly affect and determine the dairy animals' welfare.

Discussion

According to our research on Ayna and Kamyshenka dairy farms, respectively, more than 48.8 and 32.6% of animals suffer from moderate and severe lameness and the proportion of cows with severe lameness is quite high. Similarly high rates of traumatic skin injuries were observed in studied cows (46.7 and 46.3%, respectively).

It should be noted that lameness is one of the most serious problems of welfare in dairy farms and at the most of industrialized dairy enterprises described a similar situation [17]. For example, in the USA, the level of lameness in a herd of dairy cows reached 24.6% [11]. In the review of the main causes of cow mortality, lameness and injuries occupy the highest place – 20%, followed by mastitis – 16.5% and calving problems – 15.2%. It is also known that after mastitis and calving problems, lameness is the third most common reason why dairy cows are culled [12]. Lameness causes pain and discomfort. Cows suffering from lameness develop hypoalgesia; they change their behaviour in an attempt to relieve pain

by changing the position of the body, reducing activity when walking and more frequent weight transfer from one leg to the other [13]. The main cause of lameness is damage to the hooves, and they are associated with a concrete floor. There is an assumption that the frequency of lameness increases with increasing milk yield. Lameness is also associated with insufficient physical activity. Increased physical activity and access to pasture can improve the gait of cows and have a positive effect on hoof health [14].

Studies have shown that in both dairy farms, percentage of cows with a high number of somatic milk cells reach a significant value: 8.75% in Ayna Dairy Farm and 19.0% in Kamyshenka LLP (Table 3). This indicator serves as proof of the presence of mastitis in the herd of dairy cows.

Clinical mastitis is the most frequently reported herd health problem in the dairy industry and causes the death of 16.5% of animals with this disease. Injury of nipple tissues by milking machines and genetic selection to obtain extremely high milk yields are considered the main predisposing factors of painful swelling of the udder. Most cases of mastitis are caused by infections with pathogenic microflora penetrating through the nipple opening [15]. Thus, poor cleanliness of the premises and the cows themselves increases the frequency of mastitis [16]. The results of these studies indicate that in Ayna Dairy Farm, the proportion of cows with dirty udders, sides and upper limbs, as well as lower limbs, is, on average, 31.66, 73.3 and 83.3%, respectively. In Kamyshenka Dairy Farm, these indicators were, respectively, 16.6, 16.0 and 57.4%, which indicates the omissions of farms on animal welfare (Table 3). Nevertheless, it is known that frequent change of bedding and good sanitary conditions in the milking parlor can reduce the risk of udder inflammation. Reducing the density of cows in loose housing systems can also reduce the risk of mastitis by improving hygiene and reducing the frequency of nipple injuries.

Apparently, insufficiently satisfactory sanitary conditions and poorly balanced feeding contributed to the fact that proportion of cows with nasal discharge, eye discharge, difficulty breathing, diarrhea and vulva discharge at Kamyshenka Dairy Farm averaged 27.7, 12.6, 7.4, 14.8 and 1.85%, respectively. These indicators at Ayna Dairy Farm were an lower, with exception of the vulva discharge rate, which was for 4.5 times higher here (Table 3). Such unfavorable factors of hausing and managing are reflected in fairly high mortality rates of lactating cows, which is 5.4% in Ayna Dairy Farm and 15.9% in Kamyshenka Dairy Farm.

It should be noted that in traditional cattle breeding dairy cows graze on pasture throughout the day, but in modern dairy farms cows are fed only once or twice a day [18]. Even if the diet contains all necessary nutrients, the cow may still have a behavioural need to perform oral feed manipulation, as would be normal in nature conditions [19]. As a result of genetic selection for high milk yields, used in modern dairy production cows are unable to receive all necessary energy only from feed to maintain their abnormally high milk productivity. Thus, the feed for industrially raised dairy cows has become very concentrated with the use of high-calorie nutrients, and the diet of lactating cows consists of feed concentrates by 30-60% [20].

An abnormally concentrated diet leads to the formation of organic acids, which can lead to rumen acidosis in cows [14]. Another problem closely related to concentrate feeding is laminitis, which can lead to lameness [21]. Excessive mobilization of fat reserves causes ketosis, which in serious cases can lead to signs of neurological dysfunction, such as walking in circles, excessive self-care, wandering and excessive salivation [22].

As a result of described above factors, an increase in the number of cows with dystocia and downer cows is observed in industrialized farms. At the studied farms the proportion of cows with dystocia and downer cows is fixed only at Kamyshenka Dairy Farm with percentage 11.3 and 8.8%, respectively (Table 3). Dairy producers have to cull cattle before they become physically unsuitable for transportation to the slaughterhouse [23].

This investigation had shown nessesity for future research of animal welfare in different regions of the country for understanding the core problems of industrialised dairy farms and developing domestic standards for quality assessing the management of row milk producing entities.

Conclusion

Welfare issues vary depending on the conditions in which animals are housed. As a result of a cross-sectional study, two geographically remote farms in the center and north of the

Akmola region were recognized as "Acceptable" from welfare points according to the classification of Welfare Quality® protocol. Scores for feeding, hausing, health, and behavior ranged from 20.1 to 64.6.

Dairy cows in the studied farms have various welfare problems, the most important (in terms of severity and prevalence) of which are health disorders, including diseases and injuries. The results can be used by stakeholders to prioritize corrective actions in welfare plans, paying particular attention to characteristics that are at high risk of specific health problems.

Dairy technology management should be prioritized with respect to these core issues. More specifically, our study shows that the housing of dairy cows needs to be improved for increasing the comfort of rest and reduce cow injuries, as well as the fact that Holstein cows are at high risk of thinness and diseases. This shows that the cows' welfare depends not only on the characteristics of the farm, but also on its management.

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Dear author!

The scientific journal "Bulletin of Science of S.Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences" aims to be included in international databases such as Scopus, Web of Science and AGRIS (International information system for the Agricultural sciences and technology), etc. In this regard, the editorial board of the journal decided to consider and accept for publication from 2023 articles prepared in English.

Basis

In accordance with the order of the Minister of Education and Science of the Republic of Kazakhstan No. 170 dated April 30, 2020, the editorial office of the journal "Bulletin of Science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences" has developed a website with an online system for submission and review of articles.

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The editorial board of the journal asks the authors to familiarize themselves with the rules and adhere to them when preparing papers sent to the journal.

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Nevin, A. (1990). The changing teacher education special education. Teacher Education and Special Education: The Journal of the Teacher Education Division of the Council for Exceptional Children, 13(3-4), 147-148.

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SAMPLE DESIGN OF THE ARTICLE

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IDENTIFICATION OF WHEAT GENES CONDITIONING RESISTANCE TO PATHOGENIC FUNGI

Aitbay K. Bulashev[1] (ID), Kairat N. Nabiyev[2] (ID)...

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Corresponding author: Aitbay K. Bulashev, e-mail: tech@mail.ru
Co-authors: Kairat N. Nabiyeva, e-mail:naruk@mail.ru

Abstract: The author of the article, on the basis of his own research, proves that the presence of wheat resistance genes to pathogenic fungi is a key factor for use in breeding work. The article presents the results of identification of wheat genes Sr32, Bt9 and Bt10 responsible for drought resistance to pathogenic fungi that cause diseases of stem rust, as well as common smut ... [not less than 100 words and not more than 300 words].

Key words: resistance genes; stem rust; hard smut; pathogenic microscopic fungi; electrophoresis; PCR; wheat. (7 words or phrases).

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