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Boranbayeva T.K., PhD, Senior Lecturer, Department of Technology and food safety, **the main author,** https://orcid.org/0000-0002-1159-1200
«Kazakh National Agrarian Research University», Kazakhstan, 050000, Almaty, Abay Avenue 8, togzhan.boranbayeva@kaznaru.edu.kz
Sansizbay A.R., Doctor of Veterinary sciences, Professor of Department of Biological Safety, Faculty of Veterinary, https://orcid.org/0000-0001-8154-4672
«Kazakh National Agrarian Research University», Kazakhstan, 050000, Almaty, Abay Avenue, 8. sansyzbai.abylai@kaznaru.edu.kz
Myktybayeva R.Zh., Candidate of Veterinary sciences, Associate Professor
«Department Microbiology, virology and immunology», Faculty of Veterinary, https://orcid.org/0000-0001-9459-453X
Kazakh National Agrarian Research University, Kazakhstan, 050000, Almaty, Abay Avenue, 8. raya.myktybayeva@kaznaru.edu.kz
Serkan O., Professor Isparta University of Applied Sciences, Faculty of Agricultural Sciences and Technologies, Department of Animal Science, https://orcid.org/0000-0003-3389-0188**,**
«Isparta University of Applied Sciences», 32200 Bahçelievler Mh. 102. Cd. No:24 ISPARTA, serkanozkaya@isparta.edu.tr
Zhalelov D.B., Master of Technical Sciences, Senior Lecturer, Department of Technology and food safety, https://orcid.org/0000-0002-9688-2639
«Kazakh National Agrarian Research University», Kazakhstan, 050000, Almaty, Abay Avenue, 8. zhalelov.dulat@kaznaru.edu.kz
Khussainov D.M., Professor of Department of Biological Safety, Faculty of Veterinary, https://orcid.org/0000-0002-0244-8381
«Kazakh National Agrarian Research University», Kazakhstan, 050000, Almaty, Abay Avenue, 8., damir.khussainov@kaznaru.edu.kz

EFFICACY OF «EMIX» PREPARATION AND PATHOMORPHOLOGICAL STUDY OF SALMONELLOSIS IN CALVES
"ЭМИКС" ПРЕПАРАТЫНЫҢ ТИІМДІЛІГІ ЖӘНЕ БҰЗАУЛАРДАҒЫ САЛЬМОНЕЛЛЕЗДІ ПАТОМОРФОЛОГИЯЛЫҚ ЗЕРТТЕУ

ANNOTATION

Salmonellosis in calves is a common infectious disease caused by salmonella bacteria, which also pose a danger to humans. It is most often found in young animals under the age of two months, and the largest number of cases occur in spring, especially after calving. It is an infectious disease, common in most regions of the Republic of Kazakhstan.

This scientific article presents the data about pathological and morphological changes in five corpses of calves infected with Salmonellain vivo, as well as the efficacy of the "Emix" preparation used against salmonellosis in calves. Forty calves were used and divided into four groups; Grp1: Healty calf, general feeding program, "Emix" , Grp2: Healty calf, general feeding program (Control group), Grp3: Diseased calf without appetite, general feeding program, "Emix", Grp4: Diseased calf without appetite, general feeding program. At the end of the experiment, Grp1 showed better live weight compared to the other groups. The changes characteristic of salmonellosis that were detected in course of pathomorphological study are as follows: acute catarrhal-hemorrhagic enteritis, acute serous inflammation of mesenteric lymph nodes, acute congestive hyperemia and pulmonary edema, and granular degeneration of the liver and the heart.

Key words: *Salmonellosis, "Emix" probiotical preparation, calf, pathomorphology, histology, edema, intestines, histological studies.*

Introduction. Due to the market economy, animal husbandry has been resumed and started its active development in Kazakhstan. Providing products of livestock breeding to the population is the task

of the agricultural developmentprogram. In the current conditions, livestock breeding has its own peculiarities. Due to the stiff competition in the market, each farm tries reducing its costs and increasing the amount of product. For these purposes, the pedigree livestock is purchased, new equipment is used and the requirements to the veterinary service are becoming increasingly strict. To raise the veterinary service to a new qualitative level, important problems are to be resolved in livestock breeding. Despite numerous efforts for resolving the main veterinary problems, diseases are observed in horses, cattle, and calves, which impede livestock productivity. Very high concentration of cattle at livestock breeding farms contributes to the rapid spread of infectious diseases. Some kinds of diseases in animals also affect humans and reduce the quality of animal breeding products. One of the diseases in this group is salmonellosis in farm animals. Salmonellosis is an infectious disease widely spread in the world, which belongs to the group of zooantroponoses. It has been found that most species of mammals and birds, including almost all species of agricultural animals (sheep, goats, cows, horses, bass, rabbits), are susceptible to salmonellosis in the natural conditions [1, 2]. In Kazakhstan, salmonellosis is mainly the problem for the young cattle at animal farms, the incidence rate and mortality being high [1, 3].

Scientists have been fighting salmonellosis in livestock breeding for many years. This disease causes great economic losses to animal breeding farms through the death of calves, decreased productivity, reduced livestock population, cost of treatment and preventive measures. According to numerous researchers, salmonellosis in calves is observed in all countries in the world and is widely spread among infectious diseases of cattle [4, 5]. Timely diagnosis of some types of infections' characteristics for animal often creates certain difficulties [6].

Besides, a high incidence rate in cattle results in its death. This disease has a very high share among pathologies in humans and animals. Among infectious diseases, salmonellosis belongs to adangerous group of zooantroponoses. Salmonellosis is most frequently observed in humans as a toxic infection, or in the gastrointestinal form [7].

Calves can be infected with various serotypes of Salmonella within a few hours after birth. Subsequent manifestations of the disease are variable, reflecting the balance between host immunity, the dose of the pathogen and virulence. Outbreak of neonatal disease frequently observed in calves aged 4 to 28 days [8].

On a dairy farm, the feces of infected cows are usually the source of infection. It can be difficult to tell which cows shed bacteria because asymptomatic and subclinical affected cows can shed as many organisms in their manure as cows that are sick with salmonellosis. Other sources of infection can be rodents, birds (including waterfowl), flies, wild cats, dogs, raccoons, and, more rarely, humans [9].

Once in the body of newborn calves, Salmonella is attached to the cells of the mucous membrane and is able to destroy enterocytes. Attachment increases if gastrointestinal stasis is present or normal flora has been disturbed or has not yet been established, as is the case in newborns [10].

Salmonella invasion can cause diarrhea through any of a number of mechanisms, including inflammation and necrosis, increased fluid secretion, or decreased absorption or digestive disorders. The inflammatory component received the main emphasis due to the necrotic nature of most Salmonella infections. Salmonella attack the villi along the microfibre border of the intestinal epithelium. These cells break down as the body continues to move until it reaches the propria plate [11]. This initial colonization is very important for the host because bacteria can modulate gene expression in epithelial cells to create a favorable habitat for themselves [12].

The effect of intestinal microbiota is crucial for the nutritional status of the host and is of particular interest to farm animals grown in intensive systems because the balance of the intestinal ecosystem can be altered by agricultural systems [13].

Salmonellosis infection remains the leading cause of morbidity [14] and one of the most common foodborne pathogens in the world, causing approximately 230,000 deaths per year [15]. To reduce the risk of morbidity and mortality, it is necessary to better understand the internal and external stimuli that contribute to the physiological and behavioural responses of calves to stressors. Feeding calves with their first meal is crucial as successful passive transmission reduces the risk of mortality and morbidity [16]. Calf mortality before weaning is 7.8%. The majority of this mortality rate is due to gastroinstestinal infections and diarrhea-related deaths [17]. Research into the pathophysiology of these organisms may

eventually lead to more specific treatment and control recommendations [18]. One of the strategies to improve the microbiota of the gastrointestinal tract of animals is the use of probiotics. Probiotics, which contain live yeast and bacteria, are microbial feed additives that can benefit the animal by imporving the intestinal microbial balance [19] the inclusion of probiotics in the diet of young calves prevents possible imbalances in the normal microbiota of the intestinal tract to improve the growth of young calves placed in stressful conditions, preventing diarrhea [20]. Despite a large amount of information in the literature about this disease, the pathological and morphological studies with the use of various preparations require further research.

Material and Methods. The work was performed at the laboratory of anti-parasitic biotechnology at the Kazakh National Agrarian University and at the Department of LLC Bayserke – Agro. Kazakh National Agrarian University Ethics Committee procedures were applied.

Determining the efficacy of "Emix" preparation. Healthy newborn calves at the age of 1 – 2 days were selected for testing the preparation.

For the prevention purposes, the preparation was given to veal calves at the dosage of 2 cm^3 per one watering twice a day for 10 days. Forty calves were used and divided into four groups; Grp1: Healtyh calf, general feeding program, "Emix", Grp2: Healty calf, general feeding program (Control group), Grp3: Diseased calf without appetite, general feeding program, "Emix", Grp4: Diseased calf without appetite, general feeding program. At the beginning and at the end of the experiment, the calves were weighed.

The disease was diagnosed as a result of comprehensive research; anamnesis, clinical observations, autopsy picture, histological and bacteriological studies. An autopsy was made on 5 calves that died from salmonellosis, accompanied by filling protocols, mostly during the nearest hours after death. Classic autopsy methods were used with the preparation of macro- and micro slides and histological slides from the material selected. A histological examination was performed on the internal organs of the gastrointestinal tract –forestomachs, small and large intestines, liver; respiratory system – lungs, bronchial tubes; the organs of the immune system, mesenteric lymph nodes, spleen; organs of the cardiovascular system – heart and blood vessels. The materials for the histological examination were fixed in a 10 % solution of neutral formalin. After that, serial paraffin slides were prepared with a thickness of 5 and 6 μm. Ultrathin slides were made on semi-automatic microtome HEOTION ERM 3100 and on microtome MS-2.

Celloidin, paraffin and frozen slides were prepared. The sections were stained with conventional and some special histological methods: hematoxylin-eosin on a processor for staining slides Leica No. S4040/No. 000000358. Histological micro slides were studied using binocular microscope MBI-6 at various magnifications.

Statistical analysis: The minitab17 program was used for statistical analysis (ANOVA). Differences were observed between groups according to Tukey test. $p< 0.05$.

Results. Watering newborn calves with the preparation for therapeutic purposes. In the experimental group, the preparation was given for therapeutic purposes to veal calves with symptoms of diarrhea at the dosage of 2 cm^3per one watering, 3 times a day for 10 days. In the reference group, the preparation was not used, antibiotics were used instead.

At the beginning of the experiment, the overall state of the animals was lethargic, the appetite was absent, the coat was rumpled, intestinal disorders with profuse diarrhea were observed, the feces were gray-green.

According to the results of the experiment, in the Grp3 of calves after 5 days of using the preparation, clinical manifestations of the disease disappeared, the overall state as well as the appetite improved.

In the Grp4, clinical manifestations of the disease disappeared only on the 10thday of antibiotic treatment. On the 7th day of the experiment, one veal calf died.The results of the experiment in the Grp3, at the end of the experiment, the average live weight of the calves was 39.51 kg, in the Grp4, it was 39.21 kg (Table 1).

Table 1 – The results of the experiment of watering newborn calves with the preparation for therapeutic purposes

Group	N	Initial LW Mean±S.E.	Final LW Mean±S.E.	Differences Mean±S.E.
1	10	35.97±0.60	42.80±0.26A	6.83±0.53A
2	10	34.90±0.40	38.96±0.23B	4.06±0.52B
3	10	35.08±0.34	39.51±0.29B	4.43±0.40B
4	9	35.19±0.35	39.21±0.48B	4.07±0.75B
P value		0.333	0.000	0.002

According to the results of the experiment on using the preparation for therapeutic purposes, in the Grp1, the average daily weight gain of calves over 10 days was 6.79 kg, in the Grp2,it was 5.0 kg. Therefore, in the Grp1, the average daily gain of live weight was by 1,790 g more than in the Grp2.

By the results of the experiment on using the preparation for therapeutic purposes in the Grp3, the average daily weight gain of calves over 10 days was 4.43 kg, in the Grp4 was 4.02 kg. Thus, in the Grp3, the average daily live weight gain was by 410 g more than in the Grp4.

That is the difference between group averages is statistically significant. Random did not come forward. So there's a difference. ($P<0.05$).

One of the stages of research was studying the pathomorphological changes in the internal organs of calves with salmonellosis. The autopsy of the corpses of dead calves revealed changes in the intestines. In the lumen of the small intestines, there was an accumulation of mucus gases, the mucous membrane was swollen, hyperemic, in some areas small hemorrhages were noted. In the large intestines, the mucosa was covered with bran-like plaque, sometimes petechial hemorrhages and erosions were found (Figure 1). The spleen was enlarged, swollen, of dark red color; increased blood filling of the pulp could be seen on the sections (Figure 2). The liver was enlarged, yellowish-brown, with a greenish tint. Under the capsule and in the thickness of the parenchyma, small foci of necrosis of light grayish color with the yellow tingewere often found, especially when affected by S. gallinarum-pullorum(Figure 3).

Figure 1 – Hemorrhagic enteritis

Figure 2 – Septic spleen

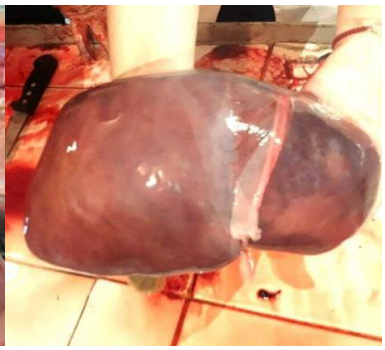

Figure 3 – Parenchymatous degeneration of the liver

The mucosa of the gallbladder was swollen, hyperemic; the gallbladder was filled with dark-olive bile with a mixture of fibrin and mucus.

In the thoracic cavity, serous effusion with flakes of fibrin was found.

The lungs were dark-red, plethoric, had doughy consistency, in the cross-section, the pattern was smoothed (Figure 4).

The heart was slightly increased in the volume due to the enlargement of the right ventricle. The pericardium was grayish-brown, of loose consistency; the right ventricle was distended, filled with uncoagulated blood of the dark-red color. The endocardium had grayish color. Under the epicardium, numerous petechial hemorrhages were found (Figure 5).

In the kidneys, dystrophic processes were found, which were manifested by swelling of the organ and the dark-red color. The capsuleof the kidneys was easily removed (Figure 6).

The vessels in the brain were plethorical, the soft meninges were swollen, pale-yellow, and had flabby consistency.

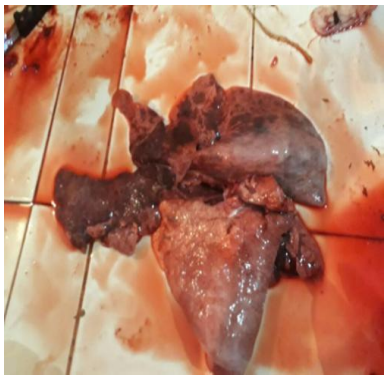

Figure 4 – Acute venous hyperemia and pulmonary edema

Figure 5 – Hemorrhage into the pericardium

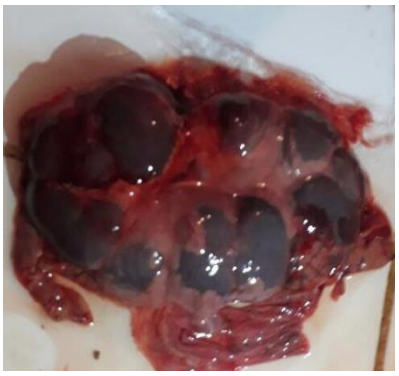

Figure 6 – Parenchymal degeneration and hyperemia of the kidneys

Histological examination in the small intestines revealed serous-mucous catarrhus with epithelium, villi, and glands' desquamation areas (Figure 7).

In the thick intestines, catarrhal-fibrous, rarely diphtheritic inflammation was observed with the development of necrosis in the surface epithelium and partially in the surface layer of the mucous membrane, as well as its delamination resulting in erosion in such areas.

In the spleen, hyperplasia of lymphoid follicles of white pulp was observed. In the kidneys, multiple hemorrhages were observed along with the combination of cortical anemia and the plethora of the medulla blood vessels, as well as granular dystrophy of the epithelium of the convoluted tubules; changes were detected that were characterized by pronounced dystrophic and necrobiotic processes (Figure 8).

In the liver, on the background of dystrophic changes, focal coagulative necrosis of hepatocytes was discovered along with an expansion of bile capillaries and their filling with bile, strong capillary and venous hyperemia with numerous small hemorrhages between the struts, diapedesis of blood elements through the walls of swollen blood vessels (Figure 9). The mucosa of the gallbladder was in the state of catarrhal-fibrinous inflammation. In the myocardium, focal dystrophy of cardiomyocytes was observed with the loss of tinctorial properties and cross striation.

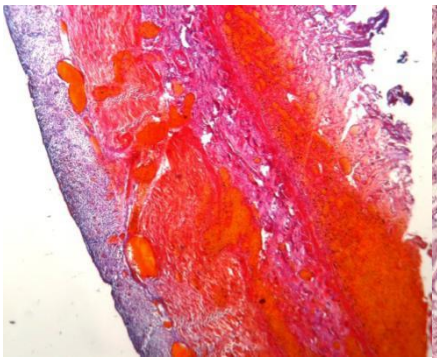

Figure 7 – Small intestine – mucosa. Crypts of the intestine in the stroma (between the intestinal glands, infiltration (accumulation) of histiocytes and lymphocytes was observed). Hematoxylin-eosin x 400

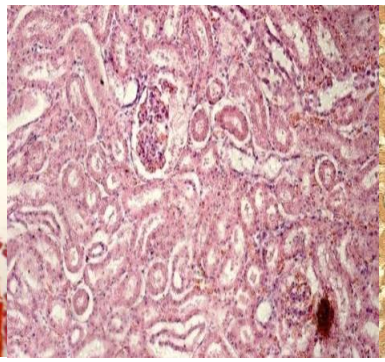

Figure 8 – Granular dystrophy of the kidneys. Hematoxylin-eosin x 400

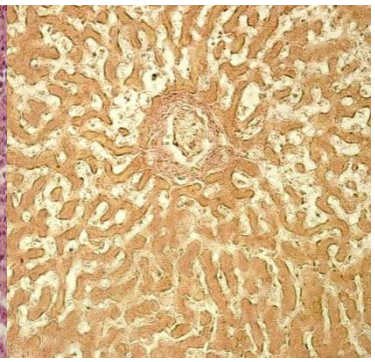

Figure 9 – A large vessel in the liver. Infective embolism of blood vessels, dystrophy, foreign particles in the blood, changes in hepatocytes. Hematoxylin-eosin x 400

Additional bacteriological studies were performed. Samples of parenchymatous organs were taken to the laboratory for bacteriological examination (liver, gall bladder, and lymph nodes, heart,

lungs, spleen, kidney); parts of small and large intestines. The results of the study were positive. Based on anamnesis, clinical research and autopsy, histological and bacteriological examination, the authors came to the conclusion that the calves had died of salmonellosis in the mesenteric lymph nodes.

Discussion and Conclusion. The search for alternative feed additives has begun as a result of the prohibition of antibiotic use in promoting animal growth. Probiotics were started to be used after the banning of antibiotics. The addition of probiotics improves live weight and feed consumption of calves [21, 22]. Intensive herd management in dairy farms causes numerous changes in the gastroinstestinal tract of calves and increases the incidence of diarrhea [23]. Probiotica use improve live weight gain and dry matter consumption of calves [23-25]. In addition, probiotics improve the intestinal flora and immune system, reducing the number of diarrhea days and cases of diarrhea in calves [26-27]. Probiotics improve animal health by balancing the gastrointestinal microbial system. Although probiotics can not delay the arrival of the salmonella pathogen to the target organ, it has been reported to reduce the severity of the infection [25, 28]. Probiotics improve intestine health by promoting healthy microbiota development, preventing colonization of enteric pathogens in the intestine, increasing digestive capacity, lowering pH, and increasing mucosal immunity [25].

Conclusion. In the conclusion of the study, the authors recommend using "Emix" preparation for treating salmonellosis in young farm animals, especially for preventing diseases of the gastrointestinal tract. The efficacy of the preparation is determined by antagonistic properties of the live bacteria contained in it against pathogenic and conditionally pathogenic microorganisms of the gastrointestinal tract and their high degree of adhesiveness.

The pathomorphological study has revealed acute catarrhal-hemorrhagic enteritis, acute serous inflammation of the mesenteric lymph nodes, acute congestive hyperemia and pulmonary edema, and granular degeneration of the liver and the heart.

Authors' Contribution. TB, ZT, AI and DK performed and wrote the experiments. TB and AI analysed data. ZT, AI, DK and SO supervised the research project.

Conflicts of Interest Declaration. The authors declare there are no conflicts of interest

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ТҮЙІН

Бұзаулардағы сальмонеллез-бұл сальмонелла бактерияларынан туындайтын, кең таралған жұқпалы ауру және ол адам өміріне де қауіпт төндіретін ауру түрі болып табылады. Бұл көбінесе екі айға дейінгі жас жануарларда кездеседі, ал бұндай жағдайлардың ең көп саны көктем мезгілі, әсіресе төлдегеннен кейін болады. Бұл жұқпалы ауру, ҚР көптеген өңірлерінде кеңінен таралған.

Бұл ғылыми мақалада in vivo сальмонелла жұқтырған бес бұзау мәйітіндегі патологиялық және морфологиялық өзгерістер, сондай-ақ бұзау сальмонеллезіне қарсы қолданылатын "Эмикс" препаратының тиімділігі туралы мәліметтер келтірілген. Қырық бұзауды қолданып, оларды төрт топқа бөлді; Grp1: сау бұзау, жалпы тамақтандыру бағдарламасы, "Эмикс", Grp2: сау бұзау, жалпы тамақтандыру бағдарламасы (бақылау тобы), Grp3: тәбеті жоқ ауру бұзау, жалпы тамақтандыру бағдарламасы, "Эмикс", Grp4: тәбеті жоқ ауру бұзау, жалпы тамақтандыру бағдарлама. Эксперимент соңында 1-топ басқа топтармен салыстырғанда жақсы тірі массаны

көрсетті. Патоморфологиялық зерттеу барысында анықталған сальмонеллезге тән өзгерістер: жедел катаральды-геморрагиялық энтерит, мезентериялық лимфа түйіндерінің жедел серозды қабынуы, жедел тоқырау гиперемиясы және өкпе ісінуі, бауыр мен жүректің түйіршікті дегенерациясы.

РЕЗЮМЕ

Сальмонеллез у телят - это распространенное инфекционное заболевание, вызываемое бактериями сальмонеллами, которые представляют опасность также и для человека. Оно чаще всего встречается у молодняка в возрасте до двух месяцев, а наибольшее число случаев приходится на весну, особенно после отелов. Это инфекционное заболевание, распространена в большинстве регионов РК.

В данной научной статье представлены данные о патологических и морфологических изменениях в пяти трупах телят, инфицированных сальмонеллой in vivo, а также об эффективности препарата "Эмикс", применяемого против сальмонеллеза телят. Использовали сорок телят и разделили их на четыре группы; Grp1: Здоровый теленок, общая программа кормления, "Эмикс", Grp2: Здоровый теленок, общая программа кормления (контрольная группа), Grp3: Больной теленок без аппетита, общая программа кормления, "Эмикс", Grp4: Больной теленок без аппетита, общее кормление программа. В конце эксперимента группа 1 показала лучшую живую массу по сравнению с другими группами. Характерные для сальмонеллеза изменения, выявленные в ходе патоморфологического исследования, следующие: острый катарально-геморрагический энтерит, острое серозное воспаление брыжеечных лимфатических узлов, острая застойная гиперемия и отек легких, а также гранулярная дегенерация печени и сердца.

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Omarbekova U.Zh., Candidate of Veterinary Sciences, Professor, **the main author**, https://orcid.org/0000-0002-2459-5438
NJSC «Kazakh National Agrarian Research University», Almaty, Abay avenue 8, 050010, Kazakhstan, Urzanoma-58@mail.ru
Umitzhanov M., Doctor of Veterinary Sciences, Professor, https://orcid.org/0000-0003-2734-2943
NJSC «Kazakh National Agrarian Research University», Almaty, Abay avenue 8, 050010, Kazakhstan, m.umitzhanov@mail.ru
Kushmukhanov ZH.S., master of Veterinary Sciences, https://orcid.org/0000-0002-5161-5561
NJSC «West Kazakhstan Agrarian and Technical University named after Zhangir khan», Uralsk, st. Zhangir khan 51, 090009, Kazakhstan, jenis.90@mail.ru
Mussoyev A.M., PhD, associate professor, https://orcid.org/0000-0003-3389-4365
NJSC «Kazakh National Agrarian Research University», Almaty, Abay avenue 8, 050010, Kazakhstan, musoev.a@mail.ru
Turebekov O.T., Candidate of Biological Sciences, Professor, https://orcid.org/0000-0001-7482-7666
NJSC «Kazakh National Agrarian Research University», Almaty, Abay avenue 8, 050010, Kazakhstan, orken_tur@mail.ru
Makhashov E.Sh., Candidate of Veterinary Sciences, Professor, https://orcid.org/0000-0003-1922-7144
NJSC «Kazakh National Agrarian Research University», Almaty, Abay avenue 8, 050010, Kazakhstan, edil57@mail.ru

TOXICOLOGICAL PROPERTIES OF THE DRUG «GLUDEZ» BASED ON GLUTARALDEHYDE

ANNOTATION

Disinfection occupies a special place in the system of basic antiepizootic measures, as an element of a significant reduction in the total number of microorganisms and complete disinfection of pathogenic microflora at environmental facilities. In this regard, the search for new highly effective means for disinfection, prevention and treatment is currently especially relevant against the background of