Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Using biogenic polyvalent preparation for prophylaxis and treatment of diarrhea in calves

Damir Khussainov[1,2], Zura Yessimsiitova[1,3]*, Ilia Tissen[1], Abylai Sansyzbay[1,2], Zhanikha Lessova[1,4]*, Ayla Kaya[5], Maxat Berdikulov[1,6], Aiqul Yernazarova[1]

1. Scientific Production Technical Center "Zhalyn", Almaty, Kazakhstan
2. Kazakh State Agrarian Research University, Almaty, Kazakhstan
3. Kazakh National University, al -Farabi, Almaty, Kazakhstan
4. Almaty Technological University, Almaty, Kazakhstan
5. Neurox Technology Ltd., Türkiye
6. National Veterinary Reference Center, Astana, Kazakhstan

* *Corresponding author's E-mail: zura1958@bk.ru, zhaniha_lesova@mail.ru*

ABSTRACT

In livestock industry, diarrhea remains the leading cause of illness and death in newborn calves, resulting in mortality, decreased growth rate, and a 10-15% slump in productivity. This complex disease requires comprehensive therapeutic strategies, because it is often caused by a combination of viral, bacterial, and protozoan parasites. Elimination of the pathogens and modification of the immune system are essential in comprehensive therapy. The aim of this study was to assess the cellular and humoral immune response of calves to the treatment and prevention of diarrhea with a new biogenic polyvalent immunomodulatory preparation called "Biodiarim-T." The biopreparation was derived from hyperimmune sera of bulls immunized with viral and bacterial isolates (bovine rotavirus, coronavirus, bovine viral diarrhea virus, herpes virus 3, *Escherichia coli* K99, and *Salmonella* spp). The immunization was completed with tissue antigens from the calves' gastrointestinal tract. 40 calves from birth to 1.5 months of age were split into equal experimental and control groups. 25 days post treatment, the clinical, serological, hematological and immunological parameters were recorded. Calves with diarrhea showed notable immunosuppression. They had a 26% reduction in T-lymphocytes and a 1.79-fold decrease in B-lymphocytes compared to healthy animals. After administering Biodiarim-T, experimental groups displayed quick immune system activation. T-lymphocyte levels rose by 2.9%, and B-lymphocytes increased by 10.6% within 15 days. Hematological parameters improved significantly. Total protein went up by 18.2%, the erythrocyte count by 8.6%, and hemoglobin by 5.52% after 25 days. Clinical results showed impressive efficacy: diarrhea morbidity fell from 67% to 20%, mortality from 22.5% to 6.7%, while daily weight gain increased by 130-140g, and treatment duration shortened from 6.4 to 3.2 days. In conclusion, Biodiarim-T shows strong therapeutic and preventive effectiveness against calf diarrhea by activating both cellular and humoral immunity.

Key words: Diarrhea, Cattle, Serum, Enteral infection.
Article type: Research Article.

INTRODUCTION

Diarrhea is one of the leading causes of morbidity and mortality among newborn calves and is a multifactorial disease caused by the infectious agents, environmental factors and internal predicting factors. Diarrhea can be caused by bacteria, viruses and protozoa, and the disease is often caused by pathogens combination. This pathology causes significant economic losses in livestock industry: it leads to the death of calves, delayed growth and a decrease in future productivity by 10-15% compared to healthy animals (Cho & Yoon 2014; Meganck *et al.*

Caspian Journal of Environmental Sciences, Vol. 23 No. 4 pp. 955-964 Received: March 05, 2025 Revised: June 11, 2025 Accepted: Aug. 26, 2025
DOI: 10.22124/cjes.2025.9212

Publisher: University of Guilan,

2015). In Kazakhstan, as in other countries with developed livestock farming, acute viral diseases of the digestive system of cattle are widespread and often cause outbreaks accompanied by significant economic losses (Khussainov *et al.* 2024; Bulatov *et al.* 2025). Despite the availability of various methods for treating diarrhea in calves, serums are widely used for acute gastrointestinal infections in cattle. In this case, modulation of the immune response plays a role of great importance in prophylaxis and therapy (Mutushev *et al.* 2020). Diarrhea in newborn calves is a polyetiological disease, the development of which is caused by the combined effect of various factors, including nutrition, sanitary conditions, quality of care and the state of the immune system. In many countries, scientists and practitioners have sought to establish the prevalence of infectious agents associated with this disease. The main pathogens causing diarrhea in calves are viruses including bovine rotavirus group A (BRV), bovine coronavirus (BCV), as well as bovine viral diarrhea virus (BVDV); bacteria (*Escherichia coli* K99, and *Salmonella* spp.) and protozoa (*Cryptosporidium parvum*, and *Eimeria* spp.; Uhde *et al.* 2008; Peter *et al.* 2016). Diarrhea in newborn calves leads to serious metabolic disorders, including severe metabolic acidosis, hyperlactatemia, azotemia, hypoglycemia, and hyperkalemia. In addition to fluid and electrolyte loss, the disease is accompanied by decreased absorption of carbohydrates, fats, and amino acids (Berchtold 2009; Trefz *et al.* 2017). To minimize the consequences caused by diarrhea, it is extremely important to promptly identify sick calves and carry out rehydration therapy before severe signs of dehydration appear. The most reliable method for diarrhea diagnostics is considered to be an assessment of faeces consistency, which is carried out by stimulating defecation and then analyzing the sample on a 4-point scale (McGuirk 2008; Curtis *et al.* 2016). Intestinal microbiome plays a key role in regulating the immune system. In this regard, the use of probiotics that help improve the microbial composition of the intestine has a positive effect on the state of the body's immune defense (Gabriel Fernandes *et al.* 2024). Immunomodulators can have both a stimulating and a suppressive effect on the immune system, depending on the dosage, time of use, and functional state of the body (Wang *et al.* 2024; McGill *et al.* 2025). Their use is advisable both for preventive purposes - to activate defense mechanisms, and in the treatment of pathologies accompanied by suppression of local and general immunity, as well as disorders of the gastrointestinal tract. Immunomodulators are widely used to activate suppressed links of the immune system, especially in cases of congenital insufficiency of physiological responses or disorders caused by exposure to microbial and other factors in both the prenatal and postnatal periods. These drugs stimulate humoral immunity and act as an important additional factor coordinating the functions of the digestive and immune systems both in normal and pathological conditions (Raczyńska *et al.* 2025). Biological immunostimulants include bacterial preparations like live bacterial cultures, vaccine materials (e.g. BCG), lipopolysaccharides, as well as components of fungi and yeast, such as zymosan, glucan and acetoxan. Additionally, this group includes herbal preparations (for example, aloe extract, chlorophyllipt, ginseng tinctures, etc.), bee products (propolis, royal jelly, and bee bread), preparations of animal origin (including T- and B-activins, ASD, and Filatov's tissue preparations), as well as various serum products - including hyperimmune and anti-tissue serums (Plaizier *et al.* 2018; Vlasova & Saif 2021; Shichkin & Antica 2022; Boone & Peroni 2023; Melgoza-González *et al.* 2023; Xue *et al.* 2025). Herbal medicines have a pronounced immunomodulatory effect: they have antiviral, antioxidant and antitumor activity, and also help stimulate animal growth. In poultry, it has been established that phytogenic immunomodulators increase the level of antibodies after vaccination against avian influenza with an inactivated oil vaccine (Gu *et al.* 2009). An increase in both the humoral and cell-mediated immune response has also been noted in chickens vaccinated against infectious bronchitis (Zhang *et al.* 2017). In prevention and treatment of diarrhea in newborn calves, special attention is paid to stimulation of both general and local response of the organism, as well as immunomodulation and strengthening of the specific immune response. A significant role is played by drugs that can selectively affect certain tissue systems, activating their functions. In this context, special attention is paid to tissue stimulants and immunomodulators - substances that can restore the disturbed immunological balance and normalize altered immunobiological parameters due to the targeted effect on various body systems. Therefore, the study of the effect of a biopreparation with an immunomodulatory effect in the prevention and treatment of diarrhea is relevant.

MATERIALS AND METHODS

The production of immunomodulators, as well as serological, hematological and immunological blood tests were carried out in the laboratory of biogenic preparations of SPTC "Zhalyn" (Almaty). Scientific and production experiments were conducted in 2025 at the Agora Peasant Farm (Almaty Region) on calves aged from birth to 1.5

months. To achieve the objectives of the study of the immunomodulatory biopreparation, a set of methods was used, including clinical and epizootological, virological, bacteriological, serological, immunological, physicochemical, hematological, biochemical and statistical methods.

Strains of viruses and bacteria

The study used isolates of pathogens of viral diarrhea, as well as rotavirus, coronavirus, herpesvirus infections, escherichiosis and salmonellosis in calves. To reactivate the diarrhea virus isolate, 2-4 vessels with a monolayer culture of Vero cells, aged 1-3 days, showing no signs of spontaneous degeneration, were selected. The inoculum consisted of 3–5 ampoules containing 1 mL of lyophilized virus. The contents of the ampoules were diluted in 60–90 mL of the maintenance medium. The growth medium was first removed from the cell culture vessels, and 30 mL of the prepared viral material was added. The infected cultures were incubated in a roller apparatus at a temperature of 37 ± 1 °C and a rotation speed of 1–2 rpm for 1 hour. Then 300 mL of the maintenance medium was added to each vessel. When a pronounced cytopathic effect appeared, covering 70–90% of the cell monolayer (usually on the 3^{rd}–6^{th} day), the cultures were frozen at a temperature of – 40 °C. To reactivate rotavirus, herpesvirus and coronavirus, the BHK-21 cells of day-old were selected, while the culture must be intact and lack the signs of spontaneous degeneration. The contents of 3-5 ampoules with lyophilized virus (1 mL each) were diluted in 60-90 mL of maintenance medium. The growth medium from the vessels with the cell culture was drained and the diluted virus was added in a volume of 30 mL. The infected cultures were incubated in a roller apparatus at a temperature of 37 ± 1 °C and a rotation speed of 1-2 rpm for 1 hour. After incubation, 300 mL of maintenance medium was added to the vessels. *Escherichia* and *Salmonella* isolates were passed at least once every 10-15 days, growing them at a temperature of 36-37 °C. Daily cultures of *Escherichia* were preserved with a 5% phenol solution. To determine LD_{50}, a daily culture on a liquid dense medium was used. The number of salmonellas in the medium was estimated with counting chamber. With a dose of 100 million microbial units, they were administered subcutaneously to golden hamsters (6 per group) with a 5-10-fold increase in the dose.

To obtain viral antigens, 3-L vessels with a formed continuous monolayer of Vero cells were infected with a virulent isolate of the virus at a dose of 0.01-0.5 $TCID_{50}$. After the monolayer was damaged by at least 80% (usually on the 4^{th}-5^{th} day of cultivation), the cells were removed from the vessel walls mechanically. The resulting suspension was centrifuged at 2500 rpm for 30 minutes, the supernatant was removed, and the sediment was resuspended in a sterile physiological solution in a volume reduced by 100 times relative to the original volume of the virus-containing suspension. Then, after two cycles of centrifugation, the suspension was thermolyzed at -40 °C and centrifuged again at 4000 rpm for 30 minutes. The sediment was removed, and the supernatant with an activity of at least 1:8 in the precipitate diffusion reaction (PDR) is used as a viral antigen. Antigen inactivation was carried out using polyethylenimine (PEI) at a final concentration of 0.03% at a temperature of 22–24 °C for 36 hours. Sorption of the inactivated virus was performed by adding 6% Montanide™ ISA 201 VG at a concentration of 10–20% for 12 hours at a temperature of 4–8 °C. To obtain bacterial antigens, *Escherichia* and *Salmonella* cultures were grown on meat-peptone agar (MPA) at a temperature of 37 ± 1 °C for 18–20 hours, followed by incubation for 24–48 hours. The cultures were regularly checked for purity of growth. Upon completion of incubation, the Tartakovsky flasks were visually inspected to check for contamination with foreign bacterial or fungal microorganisms, and the typicality of the morphological, tinctorial, and cultural characteristics was assessed. Bacterial growth from the flask surface was washed off with sterile saline with a pH of 6.8–7.2 in a volume of 50–100 mL per inoculation dish. Bacterial antigen was inactivated with polyethyleneimine at a final concentration of 0.03% at a temperature of 22–24 °C for 36 hours. For sorption of the inactivated antigen, 6% Montanide™ ISA 201 VG was used at a concentration of 10–20%, keeping the mixture for 12 hours at a temperature of 4–8 °C. To obtain tissue antigens, the abomasum and duodenum of healthy calves aged 1 to 10 days were removed, thoroughly washed from the contents, crushed and homogenized. The resulting suspension was diluted with sterile physiological solution with a pH of 6.8-7.2 in a ratio of 1:10. Tissue antigen inactivation was carried out according to the same method using polyethyleneimine at a concentration of 0.03% at 22-24 °C for 36 hours. Sorption was carried out by adding 6% Montanide™ ISA 201 VG at a final concentration of 10-20% and holding for 12 hours at 4-8 °C. Immunization of bulls with antigens was carried out seven times with an interval of 7 days between injections. The antigen was administered subcutaneously in increasing doses: 5, 5, 10, 10, 20, 20, and 20 mL. Blood sampling was carried out 7-10 days after the last immunization. In this case, the antibody titer in the resulting biopreparation in the ELISA reaction should be registered at a dilution of at least

1:160. Blood was collected from animals using a closed sterile system. The animal was fixed in a stall, the head was fixed, the skin area in the upper third of the neck was treated with 70% ethyl alcohol or iodine tincture. To enhance venous filling, the jugular vein was tied with a rubber tourniquet, after which a sterile needle was inserted into the vein, ensuring that the blood flows through the system into a sterile cylinder. The volume of blood collected was 16 mL per 100 kg of live weight. Blood was collected from clinically healthy oxen with normal body temperature after a preliminary 12-hour fast, with free access to water. Test tubes or cylinders with blood were incubated in a thermostat at 37 °C for 1–2 hours. After the clot has compacted along the edges of the container, it was carefully separated with a sterile glass rod and the blood was placed in a refrigerator at a temperature of 4 ± 2 °C for 10 hours. Then decanting was carried out using centrifugation at 2000 rpm for 20 minutes. The resulting serum, separated from the formed elements of the blood, was collected in a sterile flask. The main object of the study were calves. In each series of experiments, 80 heads were selected, which were divided into experimental and control groups according to the principle of paired analogues. Before the experiments, blood and feces samples were taken from animals of both groups for background laboratory tests. Clinical examination of calves aimed at assessing the effectiveness of the immunomodulatory drug included an analysis of the general condition of the body, appetite, and the identification of pathologies of the digestive system. Body temperature, pulse rate and respiration were determined randomly in individual animals. Serological studies were conducted using blood serum collected from animals with different clinical symptoms in order to detect specific antibodies and identify viral and bacterial pathogens. Identification of T- and B-lymphocytes was performed using the rosette formation method (Svitek *et al.* 2022). Quantitative determination of the content of immunoglobulins of classes G and M (IgG and IgM) in the test sera was performed using the radial immunodiffusion method according to G. Manchini (Manchini 1965). Hematological studies were conducted using standard methods (Kendall *et al.* 2024), the total number of blood leukocytes was counted in a Goryaev chamber. For differential leukocyte counting, smears were prepared and stained according to Romanovsky-Giemsa. The ratio of lymphocytes to other types of blood leukocytes was expressed as a percentage. Determination of the total serum protein was carried out using the generally accepted refractometric method. The bactericidal activity of blood serum was determined by nephelometry (Islam *et al.* 2019). The lysozyme activity of blood serum was determined by the nephelometric method (Culver *et al.* 2017). Biometric processing of experimental data was carried out according to the method of R.B. Strelkov. The statistical significance of differences was determined using Student-Fisher probability tables depending on the sample size. Differences were considered significant at a significance level of $p < 0.05$. As part of the experiment, calves of the 1st experimental group were subcutaneously injected with the biopreparation "Biodiarim-T" at a dose of 0.5 mL kg^{-1} of body weight; animals of the 2nd experimental group - the same drug at a dosage of 1.0 mL kg^{-1}. The control group was injected with normal cattle serum at a rate of 1.0 mL kg^{-1} of body weight. All injections were performed twice with an interval of 3 days.

RESULTS

In the system of treatment and preventive measures for viral respiratory diseases of young cattle, drugs with both antiviral and antibacterial activity are of great importance. The use of specific serums for gastrointestinal pathologies helps to reduce the incidence rate, but their action is directed exclusively at pathogens of viral and bacterial nature, and they do not have an immunomodulatory effect. Antibiotics and sulphonamides are widely used for intestinal infections and demonstrate high efficiency in the fight against bacterial pathogens. However, their use is accompanied by the risk of developing dysbacteriosis and suppression of the immune system. In this regard, the inclusion of biopreparations with an immunostimulating effect in the therapeutic regimen helps to reduce the side effects of antibacterial agents and increase the overall effectiveness of the treatment and prevention of polyetiological diarrhea in calves. The biopreparation "Biodiarim-T" is developed on the basis of the blood of clinically healthy bulls, previously hyperimmunized with viral and bacterial isolates of pathogens causing diarrheal diseases of calves: viral diarrhea, rotavirus and coronavirus infections, herpesvirus infection, escherichiosis and salmonellosis. In addition, the composition of the preparation includes tissue components obtained from the organs of the gastrointestinal tract of calves. The biopreparation contains specific antibodies directed against the listed pathogens, as well as low doses of antibodies that have a stimulating effect on the protective mechanisms of the digestive system. Detection of antibodies to viruses causing intestinal infections in the blood serum of calves in combination with an analysis of the clinical condition of the animals indicates previous viral diseases. During the study, 45 samples of calf blood serum were used for diagnostic purposes.

Antibodies to rotavirus, coronavirus, and the pathogens of escherichiosis and salmonellosis were detected in calves with clinical signs of diarrhea, indicating a viral-bacterial nature of the disease. Viral-bacterial diarrhea in calves is accompanied by severe disturbances in the functioning of the immune system. Both sick calves aged from birth to 20 days with severe symptoms and clinically healthy animals were examined. Data on the state of cellular immunity are presented in Table 1.

Table 1. Cell immune response components in calves with diarrhea.

Parameter	Units	Calves'status	
		Diseased	Intact
Leukocytes	10^9 L^{-1}	7.45 ± 0.23	7.18 ± 0.34
Lymphocytes	10^9 L^{-1}	3.56 ± 0.14	2,94 ± 0.12
T-cells	%	21.41 ± 0.28	27.18 ± 0.27
B-cells	%	12.49 ± 0.24	14.47 ± 0.26

The analysis of the data presented in Table 1 shows significant changes in the indices of cellular immunity in calves depending on their clinical condition. In animals that have recovered from the disease, a marked decrease in the functional activity of immunocompetent cells is observed. The most noticeable change is a decrease in the number of T-lymphocytes and the phagocytic activity of neutrophils; to a lesser extent, a decrease in the level of B-lymphocytes. Thus, in calves with clinical signs of diarrhea, the content of T-lymphocytes was 26% lower compared to clinically healthy animals. The level of B-lymphocytes in sick calves was 1.79 times lower. At the same time, in sick animals, the total number of leukocytes exceeded the same indicator in healthy animals by 3.76%, and the number of lymphocytes by 1.31 times. The obtained results allow us to assume that in viral and bacterial infections of the intestine, the key components of the cellular immune response are suppressed, which is caused by the active replication of viruses and bacteria in the body and their immunosuppressive effect. In addition to changes in the cellular component of the immune system, disturbances in the humoral component of immunity are also of significant importance in gastrointestinal diseases of calves. The components of the specific humoral immune response, in particular, immunoglobulins and antibodies, reflect the body's reaction to the introduction of diarrhea pathogens by producing specific protective proteins. Non-specific immunity indicators, such as lysozyme and bactericidal activity of blood serum, interferon levels, and others, characterize the functional state of innate defense mechanisms and their ability to interact with pathogens. The obtained data on the state of humoral immunity in calves with diarrhea confirm the presence of significant changes in this component of immune protection. The results of the study of humoral immunity after a single administration of the drug are presented in Table 2.

Table 2. Humoral immune response components in calves with diarrhea.

Parameter	Units	Calves' status	
		Diseased	Intact
Gamma globulins	μg mL^{-1}	22.86 ± 2.01	16.98 ± 3.03
BVDV antibodies	μg mL^{-1}	1.75 ± 0.36	0.68 ± 0.02
BRV antibodies	μg mL^{-1}	5.32 ± 0.34	2.47 ± 0.21
BCoV antibodies	μg mL^{-1}	5.12 ± 0.48	4.68 ± 0.34
IRB antibodies	μg mL^{-1}	4.79 ± 0.34	3.26 ± 0.18
E. coli K99 antibodies	μg mL^{-1}	4.62 ± 0.74	4.35 ± 0.61
Salmonella dublin antibodies	μg mL^{-1}	4.68 ± 0.75	3.37 ± 0.34
Blood serum lysozyme activity	%	4.53 ± 0.43	2.95 ± 0.26
Blood serum bactericidal activity	%	27.81 ± 2.36	33.27 ± 3.19

Diarrhea is accompanied by changes in the spectrum of protein fractions of the blood serum. Sick animals show an increase in the level of gamma globulins, which indicates an elevation in the immune response and active production of antibodies against pathogens of intestinal infections. An upraise in the synthesis of gamma globulins in recovered calves is associated with the intensification of immune processes that occur in response to pathological changes in the body. In general, data on humoral immunity indicate an activation of protein synthesis

processes in response to the effects of infectious agents. Diarrhea is also accompanied by the appearance of specific antibodies to pathogens of gastrointestinal infections. Sick calves show a significant increase in the titre of antibodies to viruses that cause viral diarrhea, rotavirus and coronavirus diarrhea, as well as to pathogens of herpesvirus infection, escherichiosis and salmonellosis. This indicates a high level of immune response to past intestinal infections. In addition, in addition to changes in specific humoral immunity, sick calves also have disturbances in the non-specific link of immune protection. Specifically, a decrease in the bactericidal activity of blood serum by 1.19 times and a drop in the lysozyme activity of serum by 1.53 times were noted. Diarrhea in calves is accompanied by pronounced disturbances in both the cellular and humoral links of the immune system.
In this regard, an important element in the prevention and treatment of diarrheal diseases in young cattle is the use of immunomodulatory agents that promote the activation of both components of the immune response. For the prevention and treatment of diarrhea in calves, we have developed the biopreparation "Biodiarim-T", containing specific antibodies to the main pathogens of diarrheal infections, as well as low doses of antibodies that stimulate the protective functions of the gastrointestinal tract. The effect of the preparation on the parameters of cellular and humoral immunity, as well as its effectiveness in combating respiratory infections in calves, was studied. To assess the effect of the preparation, calves of the experimental group were subcutaneously injected with "Biodiarim-T" at a dose of 1.0 mL kg^{-1} of body weight, while animals of the control group were injected with normal cattle serum at the same dosage. Both injections were carried out twice with an interval of three days.
For 10 days, the condition of animals in both groups was clinically observed. A total of 20 calves participated in the study, which were evenly distributed between the experimental and control groups (10 animals in each). The results of the study of cellular immunity response after a single administration of the preparation are presented in Table 3. Analysis of the presented data shows that already on the 5th day after the introduction of the biopreparation, positive dynamics of cellular immunity are observed in the experimental group. The number of leukocytes increased by 0.9%, lymphocytes by 1%, T-lymphocytes by 1.3%, and B-lymphocytes by 9.5%, compared to the initial values. In the control group, the changes were as follows: the number of leukocytes increased by 0.8%, lymphocytes by 0.6%, however, the number of T-lymphocytes decreased by 2%, and B-lymphocytes by 1.1% compared to the initial values. By the 10th day after the introduction of the biopreparation, positive dynamics of cellular immunity was observed in the experimental group: the number of leukocytes increased by 0.4%, lymphocytes by 8.2%, T-lymphocytes by 1.34%, and B-lymphocytes by 13.7% compared to the initial values.

Table 3. Cell immune response components in calves after "Biodiarim-T" administration.

Days after immunization	Group	Leukocytes 10^9 L^{-1}	Lymphocytes 10^9 L^{-1}	T-cells (%)	B-cells (%)
0	Experimental	7.47 ± 0.78	3.16 ± 0.34	24.48 ± 1.48	12.46 ± 0.74
	Control	7.41+0.64	3.27 ± 0.27	25.01 ± 1.36	12.65 ± 0.85
5	Experimental	7.55 ± 0.74	3.31 ± 0.36	24.81 ± 1.34	13.67 ± 0.72
	Control	7.47 ± 0.76	3.29 ± 0.28	24.55 ± 1.57	12.52 ± 0.64
10	Experimental	7.54 ± 0.86	3.42 ± 0.31	25.36 ± 1.41	14.17 ± 0.94
	Control	7.44 ± 0.62	3.24 ± 0.27	24.12 ± 1.67	12.75 ± 0.65
15	Experimental	7.12 ± 0.63	3.31 ± 0.26	25.19 ± 1.46	13.78 ± 4.57
	Control	7.78 ± 0.57	3.10 ± 0.31	24.72 ± 1.07	12.06 ± 3.11

In the control group, the changes were as follows: the number of leukocytes increased by 0.8%, lymphocytes by 2.5%, T-lymphocytes decreased by 1.5%, B-lymphocytes increased by 2.3% compared to the initial data. On the 15th day, the following was noted in the experimental group: the number of leukocytes decreased by 5%, lymphocytes increased by 4.7%, T-lymphocytes remained above the initial level by 2.9%, and B-lymphocytes by 10.6%. In the control group, the dynamics were as follows: the number of leukocytes increased by 4.9%, while lymphocytes decreased by 5.2%, T-lymphocytes by 1.2%, and B-lymphocytes by 4.7% compared to the initial values. Analysis of these data shows that already on the 5th day after the introduction of the biopreparation, an improvement in cellular immunity was observed in the experimental group: the number of T-lymphocytes increased by 1.3%, and B-lymphocytes by 9.5%. By the 10th day, the increase in T-lymphocytes was 1.34%, and B-lymphocytes by 13.7%. On the 15th day, the number of T-lymphocytes remained above the initial level by 2.9%, and B-lymphocytes by 10.6%. These results indicate that the biopreparation "Biodiarim-T" activates key

components of the cellular immune response - T- and B-lymphocytes, which confirms its pronounced immunomodulatory effect. The results of the effect of the biopreparation "Biodiarim-T" on the hematological parameters of calves after two-time administration of the drug are presented in Table 4.

Table 4. Hematological parameters in calves after "Biodiarim-T" administration.

Days after immunization	Group	Protein concentration (g L^{-1})	Erythrocytes (10^{12} L^{-1})	Haemoglobin (g L^{-1})
0	Experimental	86.41 ± 4.6	5.84 ± 4.42	53.47 ± 3.81
	Control	87.19 ± 4.98	5.76 ± 3.71	54.24 ± 5.36
5	Experimental	84.32 ± 4.64	5.86 ± 4.43	56.27 ± 4.48
	Control	80.31 ± 4.18	4.86 ± 3.68	53.36 ± 5.24
10	Experimental	86,34 ± 4.76	5.92 ± 5.24	57.54 ± 3.14
	Control	81.58 ± 4.38	5.14 ± 4.18	53.64 ± 4.21
15	Experimental	88.16 ± 4.61	6.12 ± 5.69	59.35 ± 4.18
	Control	84.15 ± 5.47	5.8 ± 5,12	54.11 ± 5.86
20	Experimental	91.04 ± 6.73	6.27 ± 6.71	61.18 ± 3.17
	Control	85.93 ± 5.38	5.96 ± 5.43	53.92 ± 4.68
25	Experimental	91.18 ± 6.25	6.38 ± 6.14	63.24 ± 5.62
	Control	86.42 ± 6.56	6.05 ± 5.46	56.89 ± 4.81

Laboratory studies of the therapeutic and prophylactic immunomodulatory drug "Biodiarim-T" on calves showed its high efficiency. The use of the drug helps to restore the functional activity of suppressed links of the immune system, ensuring their activation and return to the levels characteristic of healthy animals. On the 5th day after the administration of the biopreparation, the experimental group showed multidirectional dynamics of blood parameters: the content of total protein increased by 4.8%, and erythrocytes by 0.34%, however, the hemoglobin level decreased by 2.5%. In the control group, negative dynamics were observed: the amount of total protein decreased by 1.7%, the number of erythrocytes by 8.7%, and the hemoglobin level by 7.9% compared to the initial values. On the 10th day after the introduction of the biopreparation, an improvement in the indicators was noted in the experimental group: the content of total protein increased by 7.6%, and erythrocytes by 1.36%, however, the hemoglobin level decreased by 1.1%. In the control group, negative dynamics were observed: the amount of total protein decreased by 1%, the number of erythrocytes by 15.7%, and the hemoglobin level by 6.5% compared to the initial values. On the 15th day after using the drug in the experimental group, the content of total protein increased by 10.9%, and the number of erythrocytes by 4.75%, while the hemoglobin level decreased by 1.0%. In the control group, the dynamics were as follows: total protein decreased by 1%, the number of red blood cells increased by 0.4%, and the hemoglobin level dropped by 6.5% compared to the initial values. On the 20th day of observation, a significant increase was observed in the experimental group: total protein increased by 14.4%, the number of red blood cells by 7.3%, and hemoglobin by 4.32%. In the control group, total protein decreased by 0.6%, the number of red blood cells upraised by 2.0%, and the hemoglobin level dropped by 3.5%. On the 25th day after the introduction of the biopreparation, the indicators in the experimental group continued to improve: total protein increased by 18.2%, the number of red blood cells by 8.6%, and the hemoglobin level by 5.52%. In the control group, total protein increased by 4.8%, erythrocytes by 4.6%, but the hemoglobin level dropped by 1.14% compared to the initial values. The data obtained confirm that double treatment of animals contributes to a faster normalization of metabolic processes compared to the control group. Double administration of the biopreparation "Biodiarim-T" to calves activates both cellular and humoral immunity, and also stimulates metabolic processes in the body. In addition, during clinical observation of the experimental animals, better feed consumption was noted, as well as an elevation in daily weight gain by 70-100 g compared to untreated animals (Table 5). The results of laboratory studies of the therapeutic and prophylactic immunomodulatory drug "Biodiarim-T" on calves demonstrated its high efficiency. The use of the drug promotes the activation of

suppressed components of the immune system and the restoration of their functional activity to the level characteristic of clinically healthy animals.

Table 5. The effects of prophylactic "Biodiarim-T" administration in calves.

Items	Experimental	Control
Number of animals	40	40
Diarrhea morbidity	8.20%	30.67%
Mortality	3.67%	9.23%
Dayli weight gain (g)	440	310
Treatment duration (days)	3.2	6.4

CONCLUSION

In recent years, acute gastrointestinal diseases have become increasingly important in the structure of pathologies in young farm animals. The development of these diseases in calves is facilitated by numerous stress factors, violations of zoohygienic standards of maintenance, unbalanced and insufficient feeding, as well as crowded placement of animals. Such conditions contribute to increased virulence of opportunistic microflora, weakening of the body's natural resistance to viruses and bacteria, which in turn leads to the development of respiratory and gastrointestinal diseases, often caused by the same pathogens. The key factor in the etiology of these diseases is a viral infection, manifested in the so-called "viral penetration effect". Its essence lies in the fact that even low-pathogenic viruses with cytopathic action are capable of damaging the epithelium of the respiratory and digestive tract against the background of reduced specific and non-specific immune protection of the body. As part of our research, the etiological structure of pathogens of gastrointestinal infections in calves was studied. Serological activity was determined for antibodies to the main viral agents: bovine rotavirus group A (BRV), bovine coronavirus (BCV), bovine viral diarrhea virus (BVDV), as well as bacterial pathogens - *Escherichia coli* K99 and *Salmonella* spp. The data obtained indicate a high degree of circulation of these pathogens among calves. This is largely due to the lack of targeted specific prevention of these infections, deterioration of animal housing and feeding conditions, as well as the general unfavorable environmental situation. Diarrhea in calves leads to metabolic disorders, decreased immune resistance, suppression of cellular immunity, as well as a drop in the number and activity of T- and B-lymphocytes. The use of the biopreparation "Biodiarim-T" contributes to significant activation of the T- and B-lymphocyte system, strengthening of non-specific humoral mechanisms of immune defense and normalization of metabolic processes. As a result, there is a more than twofold reduction in morbidity, acceleration of recovery processes and an increase in live weight gain.

Funding

This work was carried out as part of the grant-funded project IRN BR24993032 "Development of production technologies for hyperimmune biogenic preparations for the prevention and treatment of animal diseases of bacterial and viral etiology" for 2024-2026, supported by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

REFERENCES

Berchtold, J 2009, Treatment of calf diarrhea: Intravenous fluid therapy. Veterinary Clinics of North America: Food Animal Practice. , 25 (1): 73-99, DOI: https://doi.org/10.1016/j.cvfa.2008.10.001.

Boone, L & Peroni, J 2023, Introduction to equine biologic and regenerative therapies. *Veterinary Clinics of North America*: *Equine Practice*, 39: 419-427, DOI: 10.1016/j.cveq.2023.06.006. Epub 2023 Aug 7. PMID: 37558508.

Bulatov, Y, Kurmasheva, A, Amanova, Z *et al.* 2025, The influence of the associated inactivated vaccine against infectious rhinotracheitis and bovine viral diarrhea on the formation and duration of colostral immunity in Kazakh whiteheaded calves. *Vaccines*, 13: 408, DOI: https://doi.org/10.3390/vaccines13040408.

Cho, YI & Yoon, KJ 2014, An overview of calf diarrhea: infectious etiology, diagnosis, and intervention. *Journal of Veterinary Science*, 15: 1-17, DOI: 10.4142/jvs.2014.15.1.1.

Culver, HR, Sharma, I, Wechsler, ME, Anslyn, EV & Peppas, NA 2017, Charged poly(N-isopropylacrylamide) nanogels for use as differential protein receptors in a turbidimetric sensor array. *Analyst*, 142: 3183-3193, DOI: 10.1039/c7an00787f., PMID: 28745734; PMCID: PMC5570555.

Khussainov, D, Mukhatayeva, K, Ashirova, Zh, Yessimsiitova, Z, Mutushev, A, Idayat, N, Davletova, A & Karbozova, A 2024, Epizootic measures against infectious and viral animal diseases in Kazakhstan. *Caspian*

Journal of Environmental Sciences, 22 (5): 1243-1253, DOI: 10.22124/CJES.2024.8340.

Curtis, GC, Argo, CMcG, Jones, D, Grove-White, DH 2016, Impact of feeding and housing systems on disease incidence in dairy calves. *Veterinary Record*, 179: 512-517, DOI: 10.1136/vr.103895.

Gabriel Fernandes A J *et al.* 2024, Nuxcell Neo® improves vaccine efficacy in antibody response. *Frontiers in Veterinary Science*, *Section Animal Nutrition and Metabolism*. Volume 11. https://doi.org/10.3389/fvets.2024.1248811.

Gu, X, Wang, WJ & Hu, SH 2009, Effect of Yu ping Feng san on the immunization of egg quail against avian influenza vaccine. *Traditional Chinese Veterinary Medicine*, 28: 56-57 [In Chinese].

Islam, LN, Rahman, F & Hossain, A 2019, Serum immunoglobulin levels and complement function of tannery workers in Bangladesh. *Journal of Health and Pollution*, 9: 190308, DOI: 10.5696/2156-9614-9.21.190308. PMID: 30931168; PMCID: PMC6421957.

Kendall, SJ, Green, SE, Edwards, SM, Oetzel, GR & White, HM 2024, Validation of an on-farm portable blood analyzer for quantifying blood analytes in dairy cows. *Research in Veterinary Science*, 171: 105228, DOI: 10.1016/j.rvsc.2024.105228. Epub 2024 Mar 16. PMID: 38531237.

Manchini, G 1965, Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, 2 (3): 235-254.

McGill, JL, Loving, CL & Kehrli, ME 2025, Future of immune modulation in animal agriculture. *Annual Review of Animal Biosciences*, 13: 255-275, DOI: 10.1146/annurev-animal-111523-102209. Epub 2024 Aug 19. PMID: 39159206.

McGuirk, SM 2008, Disease management of dairy calves and heifers. *Veterinary Clinics of North America*: *Food Animal Practice*, 24: 139-153, DOI: 10.1016/j.cvfa.2007.10.003

Meganck, V, Hoflack, G, Piepers, S & Opsomer, G 2015, Evaluation of a protocol to reduce the incidence of neonatalcalf diarrhea on dairy herds. *Preventive Veterinary* Medicine, 118: 64-70, DOI: https://doi.org/10.1016/j.prevetmed.2014.11.007.

Melgoza-González, EA, Bustamante-Córdova, L & Hernández, J 2023, Recent advances in antigen targeting to antigen-presenting cells in veterinary medicine. *Frontiers In Immunology*, 14: 1080238. DOI: 10.3389/fimmu.2023.1080238. PMID: 36969203; PMCID: PMC10038197.

Mutushev, AZh, Yesimsiitova, ZB, Khusainov, DM & Sansyzbay, AR 2020, Hyperimmune sera in the treatment and prevention of diarrhea in neonatal calves. E3S Web of Conferences 175: 03004 INTERAGROMASH 2020 https://doi.org/10.1051/e3sconf/202017503004

Plaizier, JC, Danesh Mesgaran, M, Derakhshani, H, Golder, H, Khafipour, E, Kleen, JL, Lean, I, Loor, J, Penner, G & Zebeli, Q 2018, Review: Enhancing gastrointestinal health in dairy cows. *Animal*, ;12(s2): s399-s418, DOI: 10.1017/S1751731118001921. Epub 2018 Aug 24. PMID: 30139397.

Raczyńska, A, Leszczyńska, T, Skotnicki, P & Koronowicz, A 2025, the impact of immunomodulatory components used in clinical nutrition-A narrative review. *Nutrients*, 17: 752, DOI: 10.3390/nu17050752. PMID: 40077622; PMCID: PMC11902155.

Peter, SG, Gitau, GK, Richards, S, Vanleeuwen, JA, Uehlinger, F, Mulei, CM, *et al.* 2016, Risk factors associated with *Cryptosporidia*, *Eimeria*, and diarrhea in smallholder dairy farms in Mukurwe-ini Sub-County, Nyeri County, Kenya. *Veterinary World*, 9:811-819, DOI: 10.14202/vetworld.2016.811-819.

Shichkin, VP & Antica, M 2022, Key factors for thymic function and development. *Frontiers In Immunology*, 13: 926516, DOI: 10.3389/fimmu.2022.926516. PMID: 35844535; PMCID: PMC9280625.

Svitek, N, Taracha, ELN, Saya, R, Awino, E, Nene, V & Steinaa, L 2022, Analysis of the cellular immune responses to vaccines. *Methods in Molecular Biology*, 2465: 283-301, DOI: 10.1007/978-1-0716-2168-4_15. PMID: 35118627.

Trefz, FM, Lorenz, I, Lorch, A, Constable, PD 2017, Clinical signs, profound acidemia, hypoglycemia, and hypernatremia are predictive of mortality in 1,400 critically ill neonatal calves with diarrhea. *PLoS ONE*, 12: e0182938, DOI: https://doi.org/10.1371/journal.pone.0182938.

Uhde, FL, Kaufmann, T, Sager, H, Albini, S, Zanoni, R, Schelling, E *et al.* 2008, Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. *Veterinary Record*, 163: 362-366, DOI: 10.1136/vr.163.12.362.

Vlasova, AN & Saif, LJ 2021, Bovine immunology: Implications for dairy cattle. *Frontiers In Immunology*, 12:

643206, DOI: 10.3389/fimmu.2021.643206. PMID: 34267745; PMCID: PMC8276037.

Wang, G, Wang, Y & Ma, F 2024, Exploiting bacterial-origin immunostimulants for improved vaccination and immunotherapy: current insights and future directions. *Cell & Bioscience*, 14: 24, DOI: 10.1186/s13578-024-01207-7, PMID: 38368397; PMCID: PMC10874560.

Xue, L, Wang, C & Liu, C 2025, Immunomodulatory effects of gut microbiota on vaccine efficacy against respiratory pathogens. *Frontiers In Immunology*, 16: 1618921, DOI: 10.3389/fimmu.2025.1618921. PMID: 40529354, PMCID: PMC12170667.

Zhang, P, Wang, 1, Wang, W, Liu, X, Liu, H, Li, X *et al.* 2017, Astragalus polysaccharides enhance the immune response to avian infectious bronchitis virus vaccination in chickens. *Microbial Pathogenesis*, 111: 81-85, DOI: 10.1016/j.micpath.2017.0 8.023.

Bibliographic information of this paper for citing:

Khussainov, D, Yessimsiitova, Z, Tissen, I, Sansyzbay, A, Lessova, Z, Kaya, A, Berdikulov, M, Yernazarova, A 2025, Using biogenic polyvalent preparation for prophylaxis and treatment of diarrhea in calves. Caspian Journal of Environmental Sciences, 23: 955-964.