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#### **Research Article**

# Hyperimmune Sera in the Treatment and Prevention of Diarrhea in Neonatal Calves

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#### **ARTICLE INFO**

#### **ABSTRACT**

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This study assesses the efficacy of hyperimmune sera for the specific prevention of infectious diarrhea in neonatal calves, a major concern in veterinary medicine due to its impact on animal health and farm productivity. The study involved biochemical and hematological blood analyses performed on calves, which were divided into control and experimental groups based on the presence of infectious diseases. The biochemical analysis focused on key enzyme levels, protein fractions, creatinine, and other biomarkers that reflect the functional state of vital organs, such as the liver and kidneys. Hematological analysis was used to identify changes in blood composition indicative of inflammation and immune response. The results showed that the administration of hyperimmune sera significantly reduced the incidence of diarrhea in the experimental group by 60-80%, as compared to the control group. Additionally, mortality rates were also reduced by 30-50% in calves treated with hyperimmune sera. The biochemical findings revealed a decrease in transaminase activity, which suggests reduced liver stress, alongside the normalization of protein fraction levels. Furthermore, erythrocyte-related blood parameters were significantly improved, indicating enhanced overall health and immune function. These findings underscore the potential of hyperimmune sera as a highly effective preventive measure against infectious diarrhea in calves. The treatment not only mitigated clinical symptoms but also improved the general health status of the animals. This approach represents a promising and valuable tool for controlling infectious diseases and enhancing the welfare of neonatal calves in both clinical and farm settings.

**Keywords:** Infectious diseases, calf diarrhea, passive immunity, hyperimmune sera, biochemical parameters, hematological studies.

#### INTRODUCTION

Infectious diseases, including bacterial, viral, and parasitic infections, rank among the leading causes of animal pathologies, significantly impacting veterinary medicine and livestock farming. Their high contagiousness and potential for rapid spread make infectious diseases particularly dangerous, complicating their prevention and control from biological, economic, and social perspectives. Animal health disorders directly affect the productivity of agricultural enterprises, herd welfare, and the economic efficiency of farms [1].

Neonatal calf diarrhea is a multifactorial disease influenced by both infectious and non-infectious factors. Calves exhibit the highest susceptibility during the first weeks of life due to the immaturity of their immune system. Among the infectious agents causing neonatal calf diarrhea, the most significant are enterotoxigenic Escherichia coli (E. coli K99+), Cryptosporidium spp., rotavirus, and coronavirus. These pathogens are the primary etiological agents of intestinal infections [2].

E. coli K99+ is a Gram-negative bacterium capable of colonizing the intestinal epithelium of neonatal calves and producing heat-labile enterotoxin, which induces diarrhea [3]. Viral diarrhea caused by group A rotavirus (RVA) is most frequently observed in calves up to 14 days of age, significantly affecting calf survival rates [4].

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One of the key factors determining neonatal calves' resistance to infections is the level of passive immunity provided by colostral immunoglobulins. Studies indicate that 30–40% of calves fail to receive an adequate amount of antibodies from colostrum during the first hours of life, significantly increasing their risk of infection [5].

To reduce the incidence of neonatal calf diarrhea, comprehensive preventive measures are implemented, including adherence to sanitary and hygienic standards (regular disinfection of housing facilities, reducing stocking density, and controlling temperature conditions), optimizing nutrition, and ensuring timely colostrum intake with high immunoglobulin content. Specific immunoprophylaxis strategies involve vaccinating pregnant cows to transfer antibodies through colostrum and the passive immunization of calves through hyperimmune sera administration. The use of hyperimmune sera enables the rapid formation of passive immunity against the main infectious agents causing diarrhea. Administration of these sera within the first hours of life reduces the incidence of disease by 60–80% and mortality rates by 30–50% [4–7].

This study focuses on analyzing the mechanisms of action, efficacy, and potential applications of hyperimmune sera in veterinary practice. A review of clinical studies highlights the advantages of this approach over other preventive measures. Future research will aim to optimize hyperimmune serum administration protocols to enhance their effectiveness under livestock farming conditions [8,9].

#### MATERIALS AND METHODS

The study involved calves and adult farm animals kept in breeding and commercial farms where respiratory and intestinal diseases of viral etiology are registered. All animals were divided into control and experimental groups depending on the presence of an infectious disease. The control group included clinically healthy animals, and the experimental groups consisted of animals affected by infectious calf rhinotracheitis (ICR), viral diarrhea (VD-BS), parainfluenza-3 (PI-3), salmonellosis, adenovirus, streptococcosis, escherichiosis and respiratory syncytial infection (RSI). For biochemical and hematological studies, blood samples were taken from animals of all groups before infection (control group) and after the development of the disease. Blood analysis included hematological and biochemical studies carried out at all stages of infection. Biochemical markers were determined using standard methods that characterize the functional state of the liver, kidneys, and cardiovascular system. An automatic biochemical analyzer was used for biochemical analysis, evaluating the following parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase, bilirubin, cholesterol, total protein and its fractions. Particular attention was paid to the concentration of creatinine as an indicator of renal pathology, and the de Ritis coefficient, assessed in animals with salmonellosis and leptospirosis. Hematological studies included determining the number of erythrocytes, leukocytes, hemoglobin, platelets, as well as an analysis of the leukocyte formula. The study was conducted using an automatic hematological analyzer. Changes in the content of eosinophils, lymphocytes, neutrophils, monocytes and the color index were analyzed dynamically at various stages of the experiment. Statistical analysis was performed using SPSS Statistics and Microsoft Excel software. The significance of differences was assessed using Student's t-test. Differences were considered statistically significant at a significance level of p < 0.05.

## RESULTS AND DISCUSSION

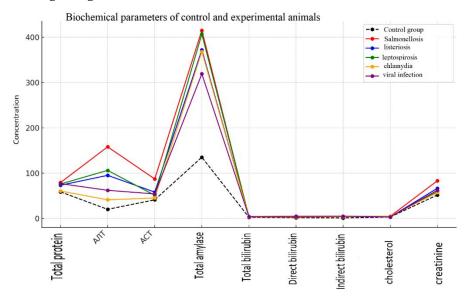
The results of the biochemical study showed that the experimental group of animals exhibited increased levels of transaminases (ALT and AST), total amylase, total protein, and bilirubin, which may indicate the development of an infectious process. Elevated creatinine levels in animals with salmonellosis and leptospirosis suggested potential kidney dysfunction. In groups with viral infections, an increase in globulin levels was observed alongside a decrease in albumins, indicating high immune system activity. The hematological study revealed a significant increase in leukocyte count in the infected animal groups (p < 0.001), with the rise in white blood cells primarily due to an increase in monocytes. Additionally, in infected animals, elevated levels of eosinophils and lymphocytes were observed, suggesting an ongoing inflammatory process. At the same time, a decrease in hemoglobin levels and red blood cell counts was noted, indicating the presence of anemia. Thus, the results of biochemical and hematological analyses provide insights into the severity of pathological processes in infectious diseases of farm animals and can serve as markers for diagnosis and monitoring of their condition. Currently, both breeding and commercial farms

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report cases of respiratory and intestinal diseases of viral etiology in calves and adult animals, including infectious bovine rhinotracheitis (IBR), viral diarrhea (BVD-MD), parainfluenza-3 (PI-3), salmonellosis, adenovirosis, streptococcosis, escherichiosis, and respiratory syncytial infection (RSI). A characteristic feature is that, in the vast majority of cases, young animals are affected by a combination of these pathogens [5]. Significant challenges arise in organizing and implementing preventive and therapeutic measures due to the lack of effective specific drugs for combating several infectious diseases in young farm animals, as well as the difficulties of conducting mass treatments using traditional individual methods. Our research identified variations in multiple parameters, as key indicators of liver diseases, particularly in infectious conditions, are changes in the activity of biochemical blood markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase, bilirubin, and cholesterol. As shown in Table 1, the experimental animal group exhibited an increase in creatinine levels, which may indicate developing kidney pathology. In the group of animals with salmonellosis, the rise in total protein was due to an increase in the globulin fraction, reflecting a heightened immune status in these animals.

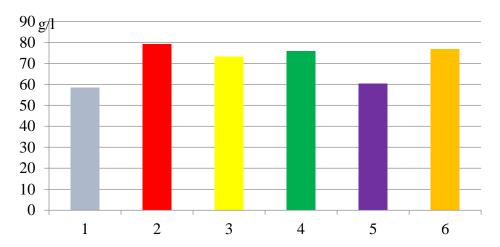


In the groups with salmonellosis and leptospirosis, the De Ritis ratio was 4 and 3, respectively, which may indicate the presence of cardiac pathology in the animals. As shown in Table 2, compared to the control group, the serum parameters of the experimental animals differed significantly, displaying an increase in most of the studied indicators. For instance, elevated levels of transaminases (ALT and AST), total amylase, total protein, and bilirubin were observed. These parameters serve as markers for diagnosing infectious diseases in animals, such as salmonellosis, leptospirosis, listeriosis, chlamydiosis, and viral infections. Experiments analyzing the blood protein spectrum showed that in infectious diseases, the total protein content in animals increased by 1.4 and 1.03 times, respectively.

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Designation: ordinate axis – unit of measurement, g/l; abscissa axis – stages of the experiment, 1 – control group, 2 – salmonellosis, 3 – listeriosis, 4 – leptospirosis, 5 – chlamydia, 6 – viral infections.

Figure 1 – Biochemical indices of total protein in animals of the control group and after diseases

The highest total protein levels in the blood were recorded on the 30th day after the onset of the disease in the respective groups of animals. It was found that the total protein content in the blood of the control group animals remained within the range of 58.5 ± 2.8 g/L throughout the study period. The most significant increase in total protein was observed in animals with salmonellosis and viral infections. In all groups, a decrease in albumin levels was noted, along with a simultaneous increase in the gamma-globulin fraction. The albumin levels remained within the physiological norm, while in the experimental group animals, albumin content increased 1.04-2.06 times on the 20th day compared to the control values. From the beginning of the experiment, changes in the biochemical blood parameters of the animals relative to the control group became more pronounced. In the groups of diseased animals, an increase in the globulin fraction of the protein was observed, which may indicate the progression of the infectious process. An increase in bilirubin levels, as well as the elevated activity of the liver enzyme alanine aminotransferase (ALT) in the diseased animal group, could serve as markers of early-stage liver diseases or hemolytic anemia. The activity of aspartate aminotransferase (AST) in the experimental groups also continued to rise, indicating the development of cardiac pathology. A high level of alkaline phosphatase, along with increased bilirubin and maximal ALT values in the group of animals with salmonellosis and leptospirosis, as well as in the group with viral infections, may serve as a marker of hepatobiliary system diseases. The increase in creatinine levels in the serum of animals may indicate the onset of renal failure, as creatinine concentration rises in renal failure earlier than urea concentration (Figure 2).

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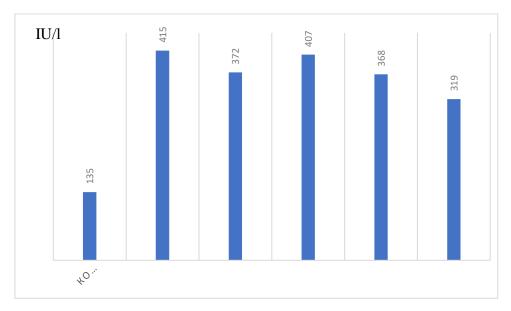


Figure 3 - Total amylase levels in animals of the control group and after diseases

Besides biochemical parameters, we conducted a study on the hematological indicators of the blood of experimental animals. The analysis of these indicators showed that, with the onset of infection, changes occur in the concentrations of various blood cells. These hematological parameters, including eosinophils, lymphocytes, erythrocytes, and hemoglobin concentration, demonstrate a direct correlation with the development of infectious diseases such as salmonellosis, listeriosis, leptospirosis, and other viral infections.

During the infectious process, there was a significant increase in leukocyte and platelet concentrations, as well as a decrease in hemoglobin levels and the number of erythrocytes. It was noted that before infection, these indicators were 130.9  $\pm$  4.38 g/L for hemoglobin and 5.63  $\pm$  0.25  $\times$  10<sup>12</sup> for erythrocytes, while after infection, these values decreased to  $112.5 \pm 3.96$  g/L and  $4.44 \pm 0.31 \times 10^{12}$ , respectively. Infectious diseases were also accompanied by an increase in the color index, mean hemoglobin content, and mean hemoglobin concentration in erythrocytes. These changes occurred against the background of a decrease in the mean erythrocyte volume and hemoglobin distribution by volume. As various infectious diseases progressed, a significant increase in the number of leukocytes was observed, from  $7.56 \pm 0.81 \times 10^9/L$  to  $10.96 \pm 0.51 \times 10^9/L$  (P < 0.001). The increase in white blood cells was primarily due to monocytes. After infection, their number reached  $10.96 \pm 0.51 \times 10^9$ /L, varying in some experiments between  $9.12 \pm 0.67 \times 10^9/L$  and  $9.59 \pm 0.33 \times 10^9/L$ . Significant changes were also observed in the levels of eosinophils and lymphocytes. Specifically, both the absolute and relative numbers of eosinophils increased. Before infection, the total number of eosinophils was  $0.08 \pm 0.03 \times 10^9$ /L, with a percentage of  $0.77 \pm 0.35$ %. By the 30th day of the experiment, these values had increased by 9.6 and 7.7 times, respectively. A significant increase in both the relative and absolute number of lymphocytes was also recorded on the 30th day post-infection:  $30.0 \pm 3.14\%$  and 3.37 ± 0.35 × 109/L, respectively. Changes in lymphocytes primarily affected their percentage ratio, while the absolute number of cells remained relatively stable throughout the experiment. The proportion of lymphocytes in the blood of animals before infection was 16.34 ± 1.98%. Similar changes were observed in the number of segmented neutrophils. By the 12th day after invasion, a significant increase in their absolute number was recorded  $(4.76 \pm 1.36$  $\times$  10<sup>9</sup>/L), while by the 30th day, their relative number had increased (46.94 ± 6.76%).

## **CONCLUSION**

The conducted study demonstrated the significant impact of infectious and parasitic diseases on the health and productivity of farm animals, highlighting the need for early diagnosis and comprehensive preventive measures. The detected biochemical and hematological changes in the blood of experimental animals closely correlated with the severity of the infectious process and can be used as markers for monitoring animal health in veterinary practice. The analysis of the effectiveness of hyperimmune sera confirmed their significant contribution to reducing the incidence

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of neonatal calf diarrhea caused by major etiological agents such as enterotoxigenic *Escherichia coli* K99+, rotavirus, coronavirus, and *Cryptosporidium* spp. The administration of hyperimmune preparations not only reduced mortality rates among young animals but also improved their overall condition, confirming the high effectiveness of this method as a key component of infectious diarrhea prevention in veterinary practice. The study results also showed that optimizing immunoprophylaxis strategies, including colostrum quality control, specific vaccination of pregnant cows, and the use of hyperimmune sera, represents an effective strategy for protecting calves from infectious diarrhea. In addition, strict sanitary and hygienic measures in animal housing facilities remain essential for preventing the spread of infectious diseases. Thus, the obtained data expand the understanding of the pathogenesis of infectious diarrhea in calves and confirm the importance of a comprehensive approach to disease prevention and control. Further research should focus on improving specific immunoprophylaxis methods, developing new highly effective hyperimmune preparations, and implementing more accurate and accessible diagnostic techniques for early detection and effective control of infectious diseases in farm animals. In the long term, this will significantly improve the health and productivity of livestock, positively impacting the economy and sustainability of livestock enterprises.

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