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# Serological and Hematological Studies of the Blood of Calves in the Experimental Use of the Medication Triazavirin

Tatiana Reshetnikova<sup>1\*</sup>, Tatyana Krylova<sup>2</sup>

**Authors' Affiliation:**  
1. National Research Mordovian State University named after N.P. Ogareva - Russian Federation  
2. Izhevsk State Agricultural Academy - Russian Federation

**\*Corresponding Author:**  
Tatiana Reshetnikova  
Email:  
[reshetnikova\\_ti@mail.ru](mailto:reshetnikova_ti@mail.ru)

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Calves; Antiviral medication; Blood; Antibody titer; Hematological; Serological studies

## Abstract

**Background:** In intensive animal husbandry management, the high disease occurrence in young animals remains an urgent problem, which is a direct consequence of the crowded placement of animals and insufficiently strong immune systems. The paper describes the study of the effect of the antiviral medication Triazavirin on the activity of humoral immunity and the level of specific antibodies in the blood serum of calves.

**Methods:** Hematological and serological studies were carried out. The experiment involved 40 calves of the black-and-white breed, aged one month, with an average weight of 50 kg, without clinical signs of diseases. For this experiment, two groups of calves were formed using the analog method. The animals in the first and second groups received the medication in dosages of 62.5 mg and 1,000 mg by inhalation and orally, respectively.

**Results:** In the first group, hematological studies showed the presence of leukocytosis, lymphocytosis, and agranulocytosis. When studying the level of antibodies in the blood serum, a decrease in the level of antibodies was discovered, which indicates a therapeutic effect and a decrease in the number of viral particles. The titer level of specific antibodies was at the same level, which was associated with taking the medication and activating adaptive mechanisms of the body's defense. In hematological studies of the blood of animals of the second group, leukocytosis was noted. When studying the level of antibodies in the blood serum, it was noted that the level of antibodies had decreased by 40%. The titer level of specific antibodies was at the same level.

**Conclusion:** The results obtained made it possible to recommend the use of the antiviral medication in question for the treatment of respiratory diseases in calves. The authors present conclusions on the effectiveness of the inhalation route of administration of the medication for viral pathogenic agent control.



## Introduction

Modern conditions for the rearing of farm animals are characterized by a significant crowding of livestock, a concentrated type of feeding, and a pharmacological load on the animal bodies, i.e., an intensive type of animal husbandry management [1-6]. Minor deviations and defects in the technological process lead to the appearance and spread of infections among young and newborn animals. In particular, the incidence of respiratory infections in young animals is up to 80%. The causes of occurrence are diverse, such as viruses, microorganisms, fungi, conditions of keeping and feeding, and immunodeficiency of animals (temperature conditions of premises, and colostrum feeding technology) [7-11]. In calves, respiratory diseases are caused by a group of viruses. These are such viruses as parainfluenza-3 (PI-3), infectious rhinotracheitis, respiratory syncytial infection, and viral diarrhea (mucosal disease). To protect against viruses, various methods are used, such as feeding with colostrum, vaccination, and the use of serums. Antiviral medications are practically not in use. The main control of the effectiveness of the means used is the study of the immunobiological reactivity of the organism of young animals [6,12-15].

Our study aimed to evaluate the activity of humoral immunity and the level of specific antibodies in the blood serum to the main respiratory viruses when using the antiviral medication Triazavirin.

## Methods

The study was conducted at the Izhevsk State Agricultural Academy (Izhevsk GSKhA) and the Udmurt Veterinary Diagnostic Center in 2022. For the experiment, two groups of calves were formed using the analog method. The experiment involved 40 calves of the black-and-white breed, aged one month, with an average weight of 50 kg, without clinical signs of diseases. All animals were kept in the same conditions and had the same diet. The design of the experiment is presented in Table 1.

The first group of calves received 1/3 of the median lethal dose (LD50) of the experimental antiviral medication Triazavirin by inhalation, at a dose of 62.5 mg of the medication, dissolved in 2 ml of saline solution. The inhalation treatment was carried out individually using an inhalation mask and a nebulizer device, with a particle size of 3  $\mu$ l. The inhalation treatment was carried out once a day, for five days. The treatment of animals was carried out in special chambers for inoculation. The experimental inhalation administration of various concentrations of Triazavirin exceeding 10 or more times the permissible exposure level equaled 0.5 mg/m<sup>3</sup> [16].

When setting up the inhalation experiment, a plastic individual mask was used, with an OmronCompAirNE-C28 compressor inhaler with an OmronV.V.T. nebulizer chamber (VirtualValveTechnology) connected to it. The particle size MMAD (Mass Median Aerodynamic Diameter) was 3 microns, the required amount of the medication was at least 2 ml and at most 7 ml, the spray level was 0.4 ml/min, the aerosol supply was 0.4 ml, and the aerosol delivery rate was 0.06 ml/min. The second group of calves was injected with Triazavirin in the classical way (orally), at a dose of 1,000 mg of the medication (4 capsules). The medication was given for 5 days. Before and after the experiment, blood was taken from the jugular vein in the experimental calves. During the experiment, hematological, serological, and immunological studies were conducted. Hematological studies were performed on an automatic hematological analyzer BC-2800Vet made by Mindray (China). Serological and immunological studies of blood serum and determination of the presence of antibodies were carried out for PI-3 by the method of hemagglutination inhibition reaction (HAIR), for infectious rhinotracheitis (IRT) and viral diarrhea (VD) by the method of indirect or passive hemagglutination reaction (IHAR), and respiratory syncytial infection (RSI) by the method of diffusion precipitation reaction (DPR).

Statistical processing was performed with the method of variational statistics using Student's t-test and the Microsoft Excel 7.0 statistical analysis software.

## Results

The experiment aimed at studying the hematological and serological parameters of the blood of calves with the inhalation and ingestion of Triazavirin and studying the level of serum antibodies to respiratory viruses demonstrated some changes. The analysis of hematological parameters during the experiment revealed an increase in the level of leukocytes in both groups, in the first group by 673.53%, and in the second one by 65.77%. These indicators exceeded the upper limit of the physiological norm. An increase in the level of leukocytes may be associated with infectious, allergic, viral, purulent, and parasitic diseases. The level of lymphocytes also increases, in the first group by 139%, and in the second one by 22.57%. An increase in the level of lymphocytes occurs due to the presence of a viral or bacterial infection in the body. Monocytes change their percentage ratio but within the average physiological values. In the first group, they decrease by 34.5%, and in the second one, they increase by 7.16%. Granulocytes decrease in the first and second groups, by 66.5 and 18.8%, respectively, and in the first group, the indicator is below the level of the physiological norm.

Group No.	Method of administration and dosage	Number of animals	Administration method
1	62.5 mg of Triazavirin, 1/4 capsule	20 calves, aged 30 days	Inhalation administration, 1 time per day, for 5 days
2	1,000 mg of Triazavirin, 4 capsules	20 calves, aged 30 days	Inside, orally 1 time per day, for 5 days

Table 1: Experimental Design and Dosage Administration in Triazavirin Study on Calves

Indicator	Group 1		Group 2	
	Before the experiment	After the experiment	Before the experiment	After the experiment
Leukocytes, *10 <sup>9</sup> /l	11.37±0.9	87.95±27.7**	13.44±2.77	22.28±1.6**
Lymphocytes, %	30.67±3.6	73.3±14.15**	40.45±3.15	49.58±2.7*
Monocytes, %	10.8±0.99	7.08±0.922**	7.82±1.1	8.38±1.242
Granulocytes, %	58.53±2.76	19.63±18.6*	51.73±4.54	42.03±1.89*
Erythrocytes, *10 <sup>12</sup> /l	8.475±0.59	8.76±0.33	8.345±0.2	8.8±0.06*
Hemoglobin, g/l	92.25±2.1	97.25±1.22*	88.83±2.54	98±3.655*
Hematocrit, %	28.43±0.16	28.83±0.09*	27.23±0.89	29.27±0.43*
Platelets, *10 <sup>9</sup> /l	462.5±22.1	535±16.33**	529±0.7	527.3±0.43*

Note: \*P≥0.950, \*\*P≥0.990, \*\*\*P≥0.999

Table 2: Dynamics of hematological parameters of calves' blood in the experimental groups

Antibody titer	Number of animals with detected antibody titer, %							
	PI-3		IRT		VD		RSI	
	Before	After	Before	After	Before	After	Before	After
<b>Group 1</b>								
0	-	-	-	-	-	-	100	50
1:16	-	-	-	-	-	25	-	25
1:32	-	-	-	-	-	-	-	25
1:64	-	-	50	25	-	-	-	-
1:128	-	25	-	-	25	-	-	-
1:256	25	25	50	75	75	75	-	-
1:512	25	25	-	-	-	-	-	-
1:1,024	50	25	-	-	-	-	-	-
1:2,048	-	-	-	-	-	-	-	-
<b>Group 2</b>								
0	-	-	20	-	-	-	80	60
1:16	-	-	20	-	20	20	-	-
1:32	-	-	-	20	-	-	20	20
1:64	20	-	-	20	20	-	-	-
1:128	-	-	-	20	-	-	-	-
1:256	-	20	60	40	60	80	-	20
1:512	20	60	-	-	-	-	-	-
1:1,024	60	20	-	-	-	-	-	-
1:2,048	-	-	-	-	-	-	-	-

Table 3: Titers of antibodies to viruses causing respiratory diseases in the blood serum of the experimental group calves.

Granulocytes are an important fraction of the leukocyte series (neutrophils, eosinophils, and basophils). The concentration of basophils and eosinophils in the blood in the normal state has a small percentage. Therefore, a change in their number does not have a significant effect. A decrease in the concentration of granulocytes occurs due to a decrease in the level of neutrophils. Granulocytes are responsible in the body for the phagocytosis of viruses, bacteria, and protozoa, and are a link to cellular immunity. Lowering the level of granulocytes, in particular, neutrophils (neutropenia) is possible in case of viral, bacterial, parasitic infections or autoimmune pathologies, as well as when receiving some chemotherapeutic medications (bone marrow toxicity).

The indicators of erythroid lineage (erythrocytes, hemoglobin, hematocrit, platelets) increase in two groups, but remain within the average physiological values (Table 2).

Animals suffer from respiratory diseases on the farm regularly, which allows us to conclude about the presence and circulation of pathogenic agents. It was established that antibody titers for protection against viral infections should be at least 1:16 or more. When studying the level of antibodies in the blood serum of

calves in the first experimental group, with the inhalation administration of Triazavirin, it was observed that the maximum titer of antibodies to the PI-3 virus was 1:1,024 in 50% of serum samples, the minimum was 1:256 in 25% of the samples before taking the medication, and after taking the medication, the maximum titer was 1:1,024 in 25% of the samples, and the minimum one 1:128 in 25% of the samples.

Before the antiviral medication was inhaled, the maximum titer of antibodies to the IRT virus was 1:256 in 50% of serum samples, the minimum one was 1:64 in 50% of the samples, and after taking the medication, the maximum titer was 1:256 in 75% of the samples, and the minimum one was 1:64 in 25% of the samples.

Before the experiment, the maximum titer of antibodies to the VD virus was 1:256 in 75% of serum samples, the minimum one was 1:128 in 25% of the samples, and after the experiment, the maximum titer was 1:256 in 75% of the samples, and the minimum one was 1:16 in 25% of the samples.

During the experiment, before taking the antiviral medication, antibodies to the RSI virus were absent in the blood serum, and after administration of Triazavirin to the calves, the maximum titer was 1:32 in 25% of the samples, and 50% of calves had no

antibodies (Table 3). When studying the level of antibodies in the blood serum of calves in the second experimental group, when the antiviral drug Triazavirin was administered orally, it was observed that the maximum titer of antibodies to the PG-3 virus was 1:1024 in 60% of serum samples, the minimum was 1:64 in 20% before the administration of the medication, and after the medication, the maximum titer was 1:1024 in 20% of the samples, and the minimum one was 1:256 in 20% of the samples. Before the introduction of the antiviral medication, the maximum titer of antibodies to the IRT virus was 1:256 in 60% of serum samples, the minimum was 1:16 in 20% of the samples and in 20% the antibodies were not detected. After taking the medication, the maximum titer was 1:256 in 40% of the samples, and the minimum one was 1:32 in 20% of the samples. Before the experiment, the maximum titer of antibodies to the VD virus was 1:256 in 60% of serum samples, the minimum one was 1:16 in 20% of the samples, and after the experiment, the maximum titer was 1:256 in 80% of the samples, and the minimum one was 1:16 in 20% of the samples. During the experiment, before the administration of the antiviral medication, antibodies to the RSI virus were absent in 80% of blood serum samples, and the maximum titer of antibodies was 1:32 in 20% of the samples. After administration of Triazavirin to the calves, the maximum titer was 1:256 in 20% of the samples, the minimum one was 1:32 in 20% of the samples, and 60% of the calves had no antibodies (Table 3). In the serological diagnosis of viral infections, the titer of antibodies in the blood serum of calves within 1:16 corresponds to the titer of clinically healthy animals. During the IHAR, the titer of healthy animals corresponds to 1:16, while during the HAIR, the titer corresponds to 1:32.

An increase in the titer to 1:256 indicates the formation of passive immunity, and animals are considered protected and healthy. An increase in specific antibodies with serodiagnostics of more than 1:256 and 1:512 indicates the development of the disease.

## Discussion

The present study aimed to evaluate the activity of humoral immunity and the level of specific antibodies in the blood serum to the main respiratory viruses when using the antiviral medication Triazavirin. The findings revealed that both modes of administering Triazavirin caused significant changes in hematological parameters, with an increase in leukocytes and lymphocytes, and a decrease in granulocytes.

These changes may be associated with viral or bacterial infections and could have implications for the health of the animals[17]. The study identified the

maximum titer of antibodies to respiratory viruses and revealed that the drug administration led to the formation of passive immunity in animals.

The findings of this study were consistent with previous research on the effects of antiviral drugs on the immune system of calves[18]. For example, a study by Palomares[19] found that the administration of antiviral drugs led to an increase in leukocytes and lymphocytes in the blood of calves, similar to the findings of our study. However, our study also found a decrease in the concentration of granulocytes in both groups, which was not reported in Palomares[19]. Another study by Razzuoli et al. [20] investigated the impact of antiviral drugs on the antibody titer of respiratory viruses in pigs. The results of their study showed that the administration of antiviral drugs led to an increase in antibody titer against the target viruses, which is consistent with our findings in both groups of calves. However, their study did not measure the hematological parameters of the blood, and thus, the comparison is limited to the serological aspects of the study.

In summary, the findings of our study are consistent with previous research on the effects of antiviral drugs on the immune system of animals [21]. However, our study provides new insights into the impact of Triazavirin on the concentration of granulocytes in the blood of calves. Further research is needed to determine the long-term effects of Triazavirin on the immune system of calves and to compare its efficacy with other antiviral drugs.

In the first experimental group, with the inhalation administration of Triazavirin in the indicated regimen and dose, leukocytosis, lymphocytosis, and neutropenia were noted during hematological blood tests. When studying the level of antibodies in the blood serum of calves in the first experimental group, with the inhalation administration of Triazavirin, it was observed that the level of antibodies on PI-3 had decreased, which indicates a therapeutic effect and a decrease in the number of viral particles. The titer level of specific antibodies on IRT and VD was at the same level of 1:256, which is associated with taking the medication and activating the adaptive mechanisms of the body's defense. The titer level of specific antibodies to the RSI when taking the antiviral medication remained within 1:32 and the animals were healthy. In the second experimental group, where Triazavirin was administered orally in the indicated regimen and dose, leukocytosis was noted during hematological blood tests. When studying the level of antibodies in the blood serum of calves in the second experimental group, with oral administration of Triazavirin, it was found that the level of antibodies to PI-3 had decreased from 1:1024 to 1:512 (by 40%). The titer level of

specific antibodies for IRT, VD, and PSI remained at the same level of 1:256, but the percentage ratio fluctuated. Those animals were considered healthy.

According to the results of the experiment, we recommend using Triazavirin for the treatment of respiratory diseases in calves. The inhalation route of administration of the medication can be used in the treatment of animals. This administration method of the medication gives a positive result in the viral pathogenic agent control.

## Competing Interest

The authors declare that there is no conflict of interest.

## Author Contributions

All authors contributed equally.

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