Study of the immune status in the treatment of calves with respiratory syndrome with the antiviral drug "Triazavirin"

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Abstract. The article discusses the use of various treatment regimens for calves with respiratory syndrome using antibacterial and antiviral drugs, including Triazavirin. During the experiment, a study of immunological blood parameters was carried out - phagocytic activity of leukocytes (PAL), phagocytic number (PN), phagocytic index (PI). In relation to the control, the phagocytic activity of leukocytes changed significantly in the first and second experimental groups; there was an increase in this indicator by 26.14% and 44.61%. In turn, the absorption capacity also actively increases, the phagocytic number increases by 58.33% and 72.92%, the phagocytic index is higher by 28.81% and 33.9% in groups, respectively. In a comparative analysis of experimental groups with different treatment regimens, it was noted that in the second group, when taking the antiviral drug "Triazavirin" and the antibacterial drug "Lexoflon", the indicators of phagocytic activity of leukocytes, phagocytic number, phagocytic index were higher by 1.05%, by 7.69 %, by 4.69% respectively. When using various medications and treatment regimens, including triazavirin, we observe activation of protective components and increased immune activity. The maximum quantitative value in terms of phagocytic activity and absorption capacity of leukocytes was observed in the second experimental group, with the use of the antiviral drug "Triazavirin" and the antibacterial drug "Lexoflon".

1 Introduction

Animal immunity plays an important role in the occurrence, development of the disease, in the fight against the pathogenic agent and recovery. The use of various drugs affects the quality of immunity. The use of direct-acting antiviral drugs for the treatment of animals is being studied [1-2, 8, 13]. Of particular interest is the state of the immune system. The use of various drugs can lead to both suppression and stimulation of immunity. Viral diseases

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cause significant damage to agriculture and animals. They lead to a decrease in productivity, a decrease in the quality of products, and the culling of animals [3-5, 17-18]. Timely treatment of viral infections of the respiratory system, the use of antiviral drugs, monitoring the state of the body and especially the immune system are very important for veterinary medicine [7, 10, 14]. Natural resistance depends on many indicators of the body, but one of the most important properties is phagocytosis. It consists of four main stages: 1) chemotaxis, capture of a pathogenic object and fusion with it to form a phagosome; 2) activation of lysosomes and their fusion to form phagolysosomes; 3) further destruction, dissolution, and breakdown of the pathological object. Defective phagocytosis or its violation causes various deviations and failures in immunity, and the development of pathological conditions of the body. Therefore, monitoring the phagocytic activity of blood leukocytes and other related indicators (phagocytic number, phagocytic index), reflecting cellular immunity, is an essential diagnostic criterion and can also be used to assess the immunocorrective effect of various drugs, in particular antiviral agents in veterinary medicine [6, 9, 11-12, 15-16].

2 Materials and methods

Scientific research was conducted at JSC "Uchkhoz Iyulskoye Izhevsk State Agricultural Academy" of the Votkinsk district of the Udmurt Republic, the village of Iyulskoye. To participate in the experiment, experimental groups of calves were formed, according to the principle of analogues. Identical zoohygienic conditions were provided, with the same diet in accordance with the age and technology of maintenance. Calves with clinical manifestations of respiratory tract damage were selected. The age of the animals was taken into account.

For the experiment, calves with symptoms of damage to the respiratory system were selected. Calves sick with non-specific pneumonia are characterized by the presence of clinical signs: depression, decreased appetite, an increase in body temperature by 1 - 2 °C, increased heart rate and respiratory rate, disheveled fur, cyanotic mucous membranes, shortness of breath, cough, nasal discharge of various nature. Auscultation revealed wheezing and harsh bronchial breathing, and percussion revealed foci of dullness in the lungs.

The experiment involved 30 calves aged 60 to 90 days. Each experimental group consisted of 10 animals.

In the first experimental group, the antiviral drug "Ingavirin" was used for treatment - orally, in a dose of 1 capsule per day per head, the antibacterial drug "Azitronit" - in a dose of 3 ml, intramuscularly, and subcutaneous injections of "Glucose, 5% solution" were also carried out in a volume of 40 ml, once a day. The drug for expectoration "Ambroxol" was used - one tablet, orally, three times a day. "Diphenhydramine" - 1 ml, intramuscularly, in the neck area, once a day. The therapy lasted 7 days. From the third day of treatment, the calves were additionally administered "Eufeline" - 2 tablets per head, "Furosemide" - 2 ml, intramuscularly, once a day, and were given warm herbal "tea" with soda - 5 g of soda per head, for five days. On the 5th day of treatment, the calves were given ASD-2, 6 ml per head, orally, once a day for 8 days.

In the second experimental group, the antiviral drug "Triazavirin" was used - orally, at a dose of 1 capsule per day per 1 head, the antibacterial drug "Lexoflon" - 2 ml, once a day, intramuscularly in the middle third of the neck. To remove sputum, "Mukaltin" was used, one tablet orally, three times a day. Subcutaneous injections of "Glucose, 5% solution" were performed - in a volume of 40 ml once a day, "Diphenhydramine" - 1 ml intramuscularly in the neck once a day. The therapy was carried out for 7 days. From the third day of treatment, the calves were additionally given "Eufelin" - 2 tablets per head,

"Furosemide" - 2 ml intramuscularly once a day, and were given warm herbal "tea" with soda - 5 g of soda per head, for five days.

The third experimental group of calves was a control group and was not subjected to treatment (Table 1).

Table 1. Experimental scheme.

Experiment No.	Drug	Method of administration	Administration regimen
1 experimental group	"Ingavirin"	internally	1 capsule per day, per head, 7 days
	"Azitronit"	intramuscularly	3 ml, 1 time per female, 7 days
	"Ambroxol"	internally	1 tablet, 3 times a day, 7 days
2 experimental group	"Triazavirin"	internally	1 capsule per day per head, 7 days
	"Lexoflon"	intramuscularly	2 ml, once a day, per head, 7 days
	"Mukaltin"	internally	1 tablet, 3 times a day, 7 days
3 experimental group	Control	-	Not treated

Before and after the experiment, blood was taken from the jugular vein of the experimental calves. Blood was tested in the "Interfaculty Educational and Scientific Laboratory of Biotechnology" of the Izhevsk State Agricultural Academy for hematological and immunological parameters, the analysis was carried out using generally accepted methods.

Cellular immunity is characterized by the following indicators: the content of T-lymphocytes (E-ROL) in the spontaneous rosette formation reaction using ram erythrocytes. Phagocytic activity of neutrophils is studied by the opsonophagocytic reaction method using E. coli culture. Humoral immunity is characterized by the level of B-lymphocytes (M-ROL), the spontaneous rosette formation method using mouse erythrocytes is used. Index T/B - the ratio of T- and B-lymphocytes is calculated.

Smears were fixed and stained using the Romanovsky-Giemsa method. The reaction study was carried out using a Motic binocular light microscope (China).

The following parameters were taken into account: phagocytic index, 100 neutrophils were counted in each smear, the percentage was derived from the number of cells that captured microbial bodies and the number of particles absorbed by them.

Statistical processing of the results was performed using the Microsoft Office Excel and Statistica 8.0 application software packages. The reliability of differences in the groups was determined using Student's t-test.

3 Results and Discussion

From year to year, animals regularly suffer from respiratory diseases of various etiologies - viral, bacterial. Enterprises increase economic costs associated with the purchase of drugs, losses in animal productivity, reduced growth as a result of diseases, changes in the immune status of animals after illnesses. Timely diagnosis and treatment, monitoring of the immune system and its support when taking various drugs will increase the effectiveness of treatment.

During the experiment on the use of antiviral drugs for the treatment of calves with symptoms of respiratory system damage, hematological and immunological data were obtained.

Analysis of hematological parameters revealed a decrease in the level of leukocytes by 84.79% during the treatment of calves in the first experimental group and corresponds to the average level of physiological parameters, in the second group the level of leukocytes

decreased by 82.44%, and in the third - by 10.4%. The level of lymphocytes also changed, in the first group a decrease of 46.2% is noted, in the second - by 42.95%, in the third - by 14.8%. Monocytes increase by 157.45%, 44.68%, 77.66%, granulocytes decrease by 13.07%, 40.52%, 40.03% in the first, second and third groups, respectively (Table 2).

Table 2. Dynamics of hematological parameters of blood of calves of experimental groups.

Index	Before the experiment	Group 1	Group 2	Group 3
Leukocytes, *109/l	52.4±10.9	7.97±3.09***	9.2±3.78***	46.95±1.75
Lymphocytes, %	64.5±2.15	34.7±6.105**	36.8±6.801***	54.95±1.527***
Monocytes, %	4.7±1.3	12.1±1.869***	6.8±2.6	8.35±1.25*
Granulocytes, %	61.2±1.6	53.2±3.62*	36.4±7.04***	36.7±6.991***

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

When assessing the immunoglobulins in the blood of experimental animals, conclusions can be made about the immunological status of the body during the disease and treatment (Table 3). Immunoglobulin A (Ig A) is responsible for protecting the mucous membranes of the respiratory organs of the body. Sick calves have a low level of Ig A before the experiment, in the group using triazavirin, in the control group, and only in calves when taking ingavirin does Ig A 0.88 g / l appear in the blood serum. Immunoglobulin M (Ig M) is produced at the earliest stages and provides an immune response to the introduction of a pathogenic agent. In the first experimental group, we observe a decrease in the level of Ig M by 1.39%, in the second group there is an increase of 31.7%, in the third - 66.7%. T-helper lymphocytes do not participate in the synthesis of Ig M, and their half-life is 5 days. Immunoglobulin G (Ig G) is involved in long-term protection of the body from viral and bacterial agents. An increase in the level in the first group by 20% is noted. In the second and third groups, a decrease in the amount of Ig M by 72.5% and 65.5%, respectively, is noted.

Table 3. The level of immunoglobulins in the blood serum of calves of experimental groups.

Indicator	Before the experiment	Group 1	Group 2	3 group control
Ig A, g/l (mg/dl)	-	0.88±0.21	-	-
Ig M, g/l (mg/dl)	0.18±0.02	0.1775±0.031	0.237±0.02*	0.3±0.05*
Ig G, g/l (mg/dl)	0.2±0.006	0.24±0.014**	0.055±0.07*	0.069±0.05**

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

condition of the body. IgA consists of serum and secretory forms. A decrease in the concentration of IgA indicates a deficiency of humoral and local immunity, a violation of the synthesis or increased catabolism of IgA, as well as its adsorption on immune complexes [1-2, 4, 6, 9-10].

IgM are antibodies of the acute period of the immune response, which are synthesized by plasma cells upon first contact with a certain pathological agent. IgM has 10 antigen binding centers at once, which is especially important during the acute period of infection, when there is a need for rapid recognition and destruction of a large amount of pathogen. This requirement is also met by the strongest among all immunoglobulins ability of IgM to activate complement, which ensures the implementation of complement-dependent cytotoxicity. High concentrations of specific IgM are recorded from the 6th to the 7th day after infection, later the level of IgM decreases significantly against the background of an increase in the content of IgG, i.e. a switch from the synthesis of IgM to IgG occurs. The diagnostic value of high levels of specific IgM consists in the possibility of establishing the

fact of acute infection, in which primary infection with a certain pathogen took place [1-2, 4, 6, 9-10].

IgG are late-phase antibodies of the immune response that begin to be synthesized after a period of IgM dominance. IgG is a more specific antibody than IgM. High levels of specific IgG are recorded during periods of regression of clinical manifestations and convalescence in acute inflammatory processes. Specific IgG can be produced and circulate in the blood for a long time after recovery, since IgG is synthesized by immune memory cells. After an infection, either a stable concentration of specific IgG or a gradual decrease in their titers can be ensured. An increase in specific IgG titers over a long period after an acute disease does not indicate the maintenance of immune memory, but an incomplete cure and the transition to a chronic form of infection, since IgG is an antibody of the secondary immune response, which is realized upon contact with an already familiar antigen. Thus, in case of repeated acute infection or exacerbation of chronic infection, the phase of IgM dominance is absent, since IgG is immediately synthesized [1-2, 4, 6, 9-10].

In the experimental groups, IgA is not detected in sick calves during the analysis both before and after treatment, only when taking ingavirin is its level increased. IgA has a low concentration in young animals, and its concentration increases after the disease. Long-term infection is characterized by a rapid increase in the level of IgA and IgG antibodies. For a complete assessment of the state, it is necessary to determine IgA and IgG antibodies simultaneously. If the IgA result is unclear, confirmation of the disease is carried out by an additional IgM analysis.

IgA in the blood of sick animals appears 10-14 days after the penetration of viruses. The level of IgA antibodies begins to decrease 2-4 months after infection and disease, even after successful treatment. With repeated infection, the level of IgA antibodies will increase again. After treatment, the level of IgA should decrease, if this does not happen, then this is a sign of chronic infection. When treating calves with triazavirin, an increase in IgM and a decrease in IgG is observed. IgM antibodies are detected in the blood as early as 5 days after the onset of the disease and reach a maximum of 1 - 4 weeks, then decrease to an insignificant level in several months. An increase in the level of IgM antibodies may indicate the presence of an acute form of the disease.

The functions of IgG are long-term protection of the body from bacteria and viruses. Their formation occurs slowly, but lasts for a long time, compared to IgM. The level of IgG antibodies increases slowly (15-20 days after the onset of the disease), but remains elevated longer than IgM. Therefore, they are markers of a long-term infection in the absence of IgM. Immunoglobulins G decrease their concentration in the blood over time, but with repeated introduction of a similar antigen, their level quickly increases. Cellular immunity plays a significant role in the fight against viral infections. Monitoring the indicators - phagocytic activity of leukocytes, phagocytic number, phagocytic index will allow you to study the immune status of calves during treatment (Table 4). The physiological norm of the phagocytic activity of leukocytes is considered to be an indicator of 60 - 80%.

Table 4. Immunological parameters of calves.

Index	**Experimental group 1**		**Experimental group 2**		**Control group**	
	Before the experience	**After the experience**	**Before the experience**	**After the experience**	**Before the experience**	**After the experience**
PAL %	65.3±1.83	77.2±1.22	66.4±1.71	88.5±1.82	67.1±1.74	61.2±1.33
PN %	5.8±0.44	7.6±0.53	5.2±0.51	8.3±0.55	5.6±0.54	4.8±0.31
PI average number	6.8±0.92	7.6±0.21	6.4±0.64	7.9±0.5	6.7±0.48	5.9±0.34

As a result of the conducted research activities in sick animals in the experimental groups and clinical observations, it was established that the phagocytic activity of blood

leukocytes in animals before taking medications was at the lower limit of the physiological norm, below the control group by 2.7% and 1.04% in the first and second groups, respectively. Absorption capacity - in the first group is higher by 3.57%, in the second group below the control by 7.14%, phagocytic index - higher by 1.5% and lower by 4.5% in the groups, respectively.

But the most noticeable positive changes in the cellular link of immunity were noted after the implementation of therapeutic measures.

In the first experimental group, with the complex treatment of calves and the use of the drugs "Ingavirin", "Azitronit", "Ambroxol", we note an increase in the phagocytic activity of leukocytes - by 18.22%, phagocytic number - by 31.04%, phagocytic index - by 11.77%. In the second experimental group, with the complex treatment of calves and the use of the drugs "Triazavirin", "Lexoflon", "Mukaltin", we note an increase in the phagocytic activity of leukocytes - by 33.28%, phagocytic number - by 59.62%, phagocytic index - by 23.44%. In the third control group, we note a decrease in the indicators of phagocytic activity of leukocytes, phagocytic number, phagocytic index by 8.79%, 14.3%, 11.94%, respectively. In relation to the control, the phagocytic activity of leukocytes changed significantly in the first and second experimental groups, an increase in this indicator by 26.14% and 44.61% is noted. In turn, the absorption capacity also actively increases, the phagocytic number increases by 58.33% and 72.92%, the phagocytic index is higher by 28.81% and 33.9% in groups, respectively.

In a comparative analysis of the experimental groups with different treatment regimens, it was noted that in the second group, when taking the antiviral drug "Triazavirin" and the antibacterial drug "Lexoflon", the indicators of phagocytic activity of leukocytes, phagocytic number, phagocytic index are higher by 1.05%, 7.69%, 4.69%, respectively.

The immunological tests used in the experiment in sick animals, before the treatment measures, allow us to conclude that the immune system of calves is in a stressful state, there is suppression of non-specific immunity, the ability to phagocytose protective cells is reduced, and the phagocytic reaction is incomplete.

The absorptive capacity of leukocytes is impaired in a number of acute and chronic infectious diseases, autoimmune processes and a number of other diseases [3, 5, 7, 11-13]. After using drugs to treat calves with respiratory syndrome, non-specific immunity indicators - phagocytic activity of leukocytes, phagocytic number, phagocytic index - began to improve, actively increase, the percentage of incomplete phagocytosis decreases. The use of therapeutic therapy has a positive effect on the growth of immunological indicators in calves.

4 Conclusion

Thus, the data from the study of immunological parameters of blood of calves with respiratory syndrome indicate immune deficiency in the development of respiratory diseases, accompanied by a decrease in the level of cellular immunity.

When using various drugs and treatment regimens, including triazavirin, we observe the activation of protective components and an increase in immune activity.

The maximum quantitative value for phagocytic activity and leukocyte absorption capacity was noted in the second experimental group, with the use of the antiviral drug "Triazavirin" and the antibacterial drug "Lexoflon". Antiviral therapy should be included in the combined treatment regimen for animals with respiratory diseases.

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