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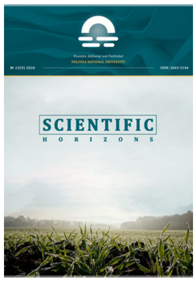

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A sporadic case of small ruminant plague in sheep: morphological and functional manifestation of cellular and humoral immunodeficiency

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Abstract. The threat of the spread of small ruminant plague to livestock in a number of countries makes the study of the mechanisms of development and prevention of this disease a priority area of research. Therefore, the purpose of the study was to investigate the changes in the blood system and organs of the immune system in small ruminant plague, which led to the development of immunodeficiency in the body of infected animals. Haematological and histological methods were used in the study. As a result of the work carried out, a decrease in the total number of leukocytes in the

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peripheral blood from 12.4 to 7.3×109/l was detected in 3 sheep that died from small ruminant plague. There was also a shift in the normal ratio of granulocytes to agranulocytes from 1:1 to 2.5:1 towards granulocytes, which was provoked by a sharp decrease in the number of lymphocytes from 49% to 25% in the leukocyte blood count. Such changes in the blood system were provoked by significant degenerative changes in the lymphoid tissue of immunocompetent organs – lymph nodes and spleen. Histological examination of the parenchyma of the above organs revealed atrophy of T- and B-dependent zones and apoptosis of lymphocytes. Taken together, these signs – haematological and histological – allow for the conclusion that both the humoral and cellular immune response systems in the body are reduced. In the affected areas of the lungs, an inflammatory reaction is observed with active migration of neutrophils and macrophages to the inflammation zone. But the active multiplication of the virus in the lung cells indicates a lack of immunity. Thus, the study revealed the mechanisms of the development of humoral and cellular immunodeficiency in small ruminant plague (SRP), which would allow developing more effective methods of combating this disease

Keywords: lymphoid tissue; histological and haematological studies; lymph node; spleen; lung tissue; differential leukocyte count

INTRODUCTION

Small ruminant plague is a dangerous transboundary disease of sheep and goats, characteristic of southern countries, and according to the Food and Agriculture Organisation of the United Nations (FAO), it poses a significant threat to agricultural and farming enterprises in more than 65 endemic countries in Africa, the Middle East, and Asia (Peste des petits…, 2024). This disease is dangerous not only for farm animals, but according to J.T. Amanova (2023), is also the cause of the death of wild, susceptible animals and threatens to reduce the biodiversity of the country's fauna. Based on such dangerous consequences of the development and spread of the disease in the Asian region, the research aimed at finding mechanisms for the protection and prevention of plague in small ruminants is an urgent area of scientific research.

According to K. Zhumakanov and A. Abdurasulov (2022), no cases of small ruminant plague have been detected in Kyrgyzstan in recent years. However, a number of researchers indicate cases of animal plague in neighbouring countries. Thus, M. Munibullah *et al.* (2022) indicate that cases of disease in sheep and goats are regularly detected in the border areas of Kazakhstan. M. Amirbekov *et al.* (2020) reported animal morbidity in Tajikistan, and S. Gao *et al.* (2021) – in China.

The outbreak of the disease among farm animals, according to J. Amanova *et al.* (2020), leads to significant economic losses, which are associated with the death or forced slaughter of infected animals, a decrease in their productivity, losses on veterinary, sanitary, and quarantine measures. Therefore, the most common method of preventing this viral disease is active immunisation of all susceptible livestock (Bayantassova *et al.*, 2023). The materials provided by M. Legnardi *et al.* (2022) indicated that USD 10.1 million was spent on animal vaccination in Kyrgyzstan only for preventive work in the period from 2017 to 2020. According to the same researchers, mass vaccination is carried out mainly in the southern regions of Kyrgyzstan and in buffer zones established on the borders with China, Kazakhstan, Tajikistan, and Uzbekistan. Since 2016, only young animals of the same year of birth have been vaccinated; to date, the vaccination coverage of the country's livestock is about 60%. All these approaches should support animal immunity and prevent the possibility of disease in the country. But a number of factors, indicated by W. Niedbalski *et al.* (2022), make it impossible to guarantee this even with this investment in prevention. These include the possibility of long term persistence of the virus in bovine cattle, migratory processes during grazing and atypical clinical signs of the disease, which complicate the diagnosis.

During the period of active replication of the virus in the body of animals, according to S. Begum *et al.* (2021), mainly the organs of the immune system are affected, which is associated with the tropism of the pathogen. The researchers point out that most of the changes concerned lymph nodes and the mucous membrane of the gastrointestinal tract with signs of immunodeficiency, due to the active breakdown of leukocytes and damage to lymphoid tissue. The development of immunosuppression in infected animals is also indicated by other researchers, in particular, O. Tenuche *et al.* (2023), but all researchers focus only on changes in regional immune organs (pharyngeal submandibular and other lymph nodes of the head and neck, mesentery), while ignoring the processes taking place in other lymph nodes and lymphoid organs. Ultimately, a decrease in the number of lymphocytes in the peripheral blood is a sign of a systemic pathological process.

Therefore, the purpose of this study was to conduct haematological studies, to investigate pathomorphological changes in parenchymal organs and organs of the immune system in sheep that died from small ruminant plague (SRP), which led to the development of an immunodeficiency condition in the body. To achieve the intended goal, the following tasks were set: determination of the total number of leukocytes and derivation of the differential leukocyte count, morphohistological studies of pulmonary and broncho-associated lymphoid tissue; investigation of the morphofunctional state of T- and B-dependent zones of lymph nodes and spleen in the body of sheep that died from the plague.

LITERATURE REVIEW

SRP is a viral disease that is caused by an RNA-containing virus belonging to the species *Morbillivirus,* family *Paramyxoviridae*. According to B. Vinayagamurthy *et al.* (2020), there are four strains of the virus in the world that differ in the nucleoprotein (N) gene, and according to R. Eloiflin *et al.* (2022), have different virulence and different degrees of clinical signs and internal organ damage. The danger of this disease is also manifested in the possibility of prolonged persistence or subclinical course of the disease in the body of non-susceptible animals – camels (Abdalla *et al*., 2021), bovine cattle and other animals (Rahman *et al*., 2020), which maintains a high incidence rate. Among susceptible livestock, the small ruminant plague virus maintains high virulence and mortality among infected animals. These indicators in the goat population were 51.1 and 73.9% (Shanmugavadivu *et al*., 2023), in sheep – 88.2 and 26.7% (Abd-Elfatah *et al*., 2022), respectively. Moreover, P. Rath *et al.* (2020) indicate that the younger the age of the affected animals, the higher the mortality rate. Thus, the incidence among weaners was 63%, and among the stock from 6 months to a year old only about 42%. Similar case results were obtained by K. Ramakrishna and P. Ramadevi (2024), noting that there were two waves of mortality, the first caused by the small ruminant plague virus and the second by the development of immunosuppression and secondary infectious agents such as pasteurellosis and enterotoxaemia. N. Sobhy *et al.* (2023) attribute a significant role in the development of immunosuppression in ruminant plague to the association with other viruses: small ruminant retrovirus (SRR), enzootic nasal tumour virus (ENTV), and Jaagsiekte sheep retrovirus (JSRV).

Authors S. Begum *et al.* (2021) indicate that, regardless of age, primary pathomorphological changes occur in the mucous membrane of the oral and nasal cavities, and the lymph nodes of the head and neck. Subsequently, according to Z. Bamouh *et al.* (2023), depending on the age of the infected animal, the disease develops in young sheep and goats with the development of respiratory symptoms and subsequent diarrhoea, and in adults – mainly mild respiratory symptoms. A. Jarad *et al.* (2022) indicate inflammatory infiltration of the surface layers of the intestinal wall with further desquamation of the epithelium and the development of necrosis. Further, G. Alwan and K. Al Saad (2023) report the development of haemorrhagic enteritis and thickening of intestinal villi walls. However, the studies by T. Jelsma *et al.* (2021) indicated that the maximum accumulation of viral particles in the intestine was observed in the mucous membrane – 4.6 $\log^{10}$ $TCID_{50}$-eq/g, and in the submucosal, muscular, and serous layer, the viral load was 3.5, 2.8 and 1.7 $\log^{10}$ $TCID_{50}$-eq/g, respectively.

But the main changes noted by such authors as A. Chota *et al.* (2020) occurred in the organs of the respiratory system. At the same time, all researchers noted histological disorders such as consolidated pneumonia in 38.1% of cases, lung congestion – 28.6%, serous fibrinous pleurisy – 28.6% on day 7 after infection. Among other pathologies, generalised lymphadenopathy with necrosis and hyperaemia of the lymph nodes was noted. The histological pattern of the lungs was characterised by infiltration of the lumen of the alveoli and bronchioles by white blood cells – neutrophils and monocytes. But the main changes occurred in the lymph nodes. According to V. Balamurugan *et al.* (2023), the main processes occur in the lymphoid tissue of mediastinal and mesenteric lymph nodes.

The tropism of the plague virus of small ruminants to lymphoid tissues was confirmed in the study by D. Adwitiya *et al.* (2022), which indicates that the main replication of viral agents occurs in regional lymph nodes. Due to intensive multiplication there is necrosis of tissues inside the lymph node and destruction of leukocytes, which leads to the development of immunosuppression. Similar conclusions were obtained by K. Schmitz *et al.* (2023), who indicate that massive infection of lymphoid tissues causes immune suppression. This leads to increased susceptibility to secondary bacterial infections. This explains the dependence of the intensity of the infectious process on the virulence of the virus strain that caused the disease, or the amount of virus that infected the animal (Niyogi *et al*., 2023). According to A. Fayyad *et al.* (2020), if the detection of the virus in the oral cavity is taken as 100%, then from other organs the virus was isolated from lymph nodes in 100% of cases, from intestine in 30-70%, and from lung tissue in 70-100%. In addition, in recent years, a number of researchers have reported the detection of the virus from other organs – the spleen, brain, liver, and other internal organs (Sahoo *et al*., 2020; Singh *et al*., 2021).

Thus, the main histological studies of pathomorphological processes during the infection of animals with small ruminant plague virus were paid to the mucous membranes and lymph nodes of the head and neck, respiratory system, and gastrointestinal tract. However, the systemic action of the virus on the immune system organs of infected sheep and goats has not been given due attention.

MATERIALS AND METHODS

The research was conducted at the Faculty of Veterinary Medicine of the Kyrgyz National Agrarian University named after K.I. Skryabin The subject of the study was selected pathological material of the lungs, spleen, and lymph nodes from fallen animals and samples of relevant organs obtained from healthy sheep to investigate histomorphological changes caused by the development of viral infection. Samples of internal organs were selected for the manufacture of histological preparations and the study of pathological changes. For this purpose, the cadavers of 3 dead sheep with characteristic clinical signs of small ruminant plague were autopsied. A preliminary diagnosis for the small ruminant plague was made based on the results of a pathological autopsy of sheep. The final diagnosis was established after conducting histological studies of the affected parenchymal organs and organs of the immune system and comparing them with the characteristic pathoanatomical changes in small ruminant plague and the results of virological examination. Slices of lungs, lymph nodes, and spleen from both dead animals and 3 healthy animal analogues were selected for the study to conduct a comparative analysis. All the procedures of this study were carried out in accordance with the Regulations of the Kyrgyz National Agrarian University named after K.I. Skryabin on bioethics and were approved at the meeting of this commission.

The selection of pathological material from dead and healthy animals for histological examination was carried out in compliance with the necessary approaches to prevent the spread of pathogens in the environment and microbial contamination of feed and animal care tools. Pathological material was sampled from the spleen and lungs of the dead animals at the border of diseased and normal tissue, and lymph nodes were sampled completely followed by fixation in 10% neutral formalin solution, according to the techniques described in L.P. Gartner's (2020) manual of pathohistological techniques for delivery to the pathological anatomy laboratory. Further processing of pathological material to obtain histological preparations was carried out according to the technique with its dehydration in increasing concentration of ethyl alcohol and pouring into paraffin. Slices 5-6 µm thick were prepared from paraffin blocks on a MC-2 sledge microtome. For histological examination, histological sections were selected at the border between the affected and unaffected tissue, so that all structures of the organ under study were represented. Cross sections were made from the lymph nodes so that the capsule, cortical, and cerebral zones of the organ were represented in the preparations. Cross-cut pieces were also taken from the spleen so that the red and white pulp and the capsule were visible in the preparations. In addition, histological preparations from healthy organs – lungs, analogous lymph nodes and spleen – were prepared in the same way from the control group of sheep (3 animals).

Histological preparations were stained with a mixture of organic dyes – haematoxylin and eosin, followed by analysis under a PZO (Warszawa) light microscope with a camera and software that enables, in addition to displaying images on the screen, to keep a research journal with an archive of images for each sample. The study of histological changes in organs was carried out with both weak and large magnification. Moreover, simultaneously with the selection of pathological material, venous blood was taken from the pulmonary veins for haematological studies. Blood was collected using a sterile disposable syringe and transferred to vacuum tubes with EDTA. Blood was collected from healthy animals at slaughter in a similar manner. Haematological examinations included determination of total leukocyte count and derivation of differential leukocyte count. The result was statistically calculated in the Excel software suite from Microsoft 365.

RESULTS

Studies of the immunosuppressive effect of the small ruminant plague virus on the organism of sheep were carried out in two areas: the direct effect on the organs of the immune system – lymph nodes, spleen, and interstitial lung tissue, and the indirect effect on the level of white blood cells in the organism of animals associated with the immune response. This approach has its advantages, since it allows forming a versatile perspective on the causes and mechanism of development of the immunodeficiency state of the body in infected animals.

Haematological changes in blood in infected sheep. One of the most effective and rapid responses of the body to infection is an increase in the number of leukocytes in the bloodstream, or the body's standard immune response. This condition occurs in almost all infectious diseases, with the exception of a small part of infections in which the cycle of reproduction of infectious agents associated with immunocompetent cells of the bone marrow or immune system. It is these pathogens that include the SRP virus. The simplest and most effective method of detecting the immunodeficiency state of a sick animal is to determine the number of immunocompetent cells and, first of all, different classes of leukocytes. Therefore, to identify the degree of immunosuppression in this disease, the first stage was to compare the number of leukocytes and their main classes (differential leukocyte count) in the blood of infected and healthy sheep. The results of a comparative analysis of these indicators are presented in Table 1.

***Table 1**. Haematological parameters of the blood system in healthy and SRP virus-infected sheep*

No.	Status	Number of leukocytes, 10^9/l	Granulocytes, %	Agranulocytes, %
1	Infected	7.9	68	32
2	Infected	7.6	71	29
3	Infected	6.3	76	24
Average for infected animals		7.3±0.49	71.7±2.33	28.3±2.33

Table 1. Continued

No.	Status	Number of leukocytes, 10^9/l	Granulocytes, %	Agranulocytes, %
4	Healthy	11.9	52	48
5	Healthy	12.7	54	46
6	Healthy	12.5	52	48
Average for healthy animals		12.4±0.24	52.7±0.67	47.3±0.67

Source: *compiled by the authors*

The concentration of leukocytes in the blood of infected animals was significantly lower than their level in healthy animals. Notably, the leukocyte content in the group of healthy animals corresponded to the reference values for this type of animal. The difference in the concentration of the total number of leukocytes between animals with different status was 41.1%, which already indicates inhibition of leukocyte production in infected animals. Even despite the small statistical sample of animals, the difference between the groups was highly significant (P = 0.0007). In addition to a decrease in the total number of leukocytes in the peripheral blood, a decrease in the ratio of granulocytes to agranulocytes was also noted. Thus, in healthy animals, the ratio of these groups of leukocytes was approximately 1:1. In animals infected with the SRP virus this ratio was shifted towards granulocytes. It is an indirect confirmation of the malfunction of lymphoid tissue in the body of an infected animal. However, the work of the bone marrow was not disrupted. This can be confirmed by the fact that the number and age of neutrophils in healthy and infected animals did not change, but only their concentration in infected sheep increased due to a decrease in the number of agranulocytes, whose reproduction cycle is not associated with the bone marrow. The difference between healthy and sick animals for this indicator also turned out to be significant with a level of P = 0.001. For a more detailed understanding of the processes occurring in the white blood system in the body of sheep infected with SRP, the differential leukocyte count was performed in Table 2.

Table 2. *Differential leukocyte count in healthy and sick sheep with small ruminant plague, %*

No.	Status	Leukocyte classes							
		Eosinophils	Basophils	Neutrophils				Lymphocytes	Monocytes
				M	J	B	S		
1	Infected	1	2	0	0	13	58	24	2
2	Infected	0	1	0	2	18	47	31	1
3	Infected	2	2	0	0	13	61	20	2
Average for infected animals		1±1	1.6±0.6	0±0	0.7±1.2	14.7±2.9	55.3±7.4	25±5.6	1.7±0.6
4	Healthy	0	6	0	0	3	39	49	3
5	Healthy	1	8	0	0	6	33	50	2
6	Healthy	1	7	0	1	3	36	48	4
Average for healthy animals		0.7±0.8	7±1	0±0	0.3±0.6	4±1.7	36±3	49±1	3±1

Note: *neutrophil classes: M – myelocyte; J – juvenile neutrophil; B – band neutrophil; S – segmented neutrophil*
Source: *compiled by the authors*

When analysing the indicators of the differential leukocyte count, a decrease in the percentage of lymphocytes by almost 2 times compared to healthy animals, and an increase in the number of neutrophils, are clearly visible. This increase in the number of segmented and band neutrophils was relative and was not associated with intensive synthesis of granulocytes, since the increase was observed only in the population of mature cell structures, and juvenile forms were absent. Therefore, such an increase in the concentration of neutrophils was associated not with their active reproduction, but with a relative increase in their proportion against the background of an active decrease in the level of lymphocytes, which became one of the main reasons for the development of an immunodeficiency condition.

Thus, changes in quantitative and qualitative blood parameters indicate possible functional changes in the lymphoid tissue of the immune system, therefore, further studies were aimed at investigating histological changes in the tissue of lymph nodes, spleen, and lungs of infected animals. Externally, stagnation and signs of spot bleeding under the capsule were found in the lungs and lymph nodes of sheep infected with the SRP virus. The spleen is enlarged in size, of flabby consistency with obtuse edges. But such macroscopic changes are also characteristic of many other infectious diseases, including viral diseases. Therefore, histological studies were performed in these organs as the most likely sites of localisation of the source of infection.

Pathohistological changes in the lungs. Histological examination of the SRP-affected lung revealed pathological changes in both the alveolar and bronchial parenchyma systems of the organ. The main changes were concentrated in the affected areas of the lung. They had signs characteristic of the development and progression of an inflammatory reaction. In contrast to the histological structure of the lungs in healthy animals, morphohistological signs of bronchopneumonia development were observed in infected sheep, which manifested by signs of filling the alveoli with serous fibrinous fluid, purulent necrotic tissue residues, and inflammatory cells. The signs of thickening of the interalveolar septa were also observed. Around the bronchial system, the bronchoassociated lymphoid tissue was weakly expressed and there were no secondary lymphoid follicles (Fig. 1).

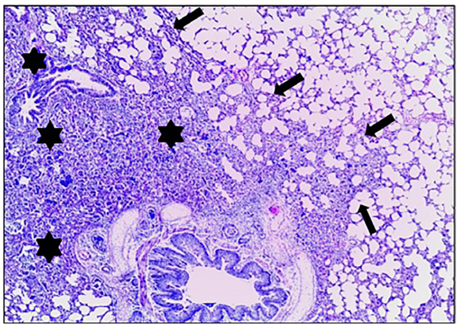

Figure 1. *Histological section of a sheep lung with SRP Serous fibrinous and interstitial bronchopneumonia*
Note: *alveoli are filled with serous fibrinous fluid (asterisks) and cells; alveolar septa are thickened (arrows). Broncho-associated lymphoid tissue is almost not expressed around the bronchus. Haematoxylin and eosin. ×25*
Source: *compiled by the authors*

When examining histological sections at high magnification (250 times or more), deeper signs of lung tissue damage were observed. They were manifested by the following morphological changes in the affected areas of the lungs: the lumen of the alveoli and bronchioles were filled with neutrophils, macrophages, desquamated epithelial cells, and necrotic mass. Among these masses, there were individual syntial cells with inclusion bodies. In contrast to the state of epithelial cells in the bronchi and lungs of healthy animals, sheep infected with SRP had numerous abnormalities at the level of cellular structure. Thus, in epithelial cells of bronchioles in infected lung sections, large eosinophilic intracytoplasmic viral inclusions were observed, whereas intranuclear eosinophilic inclusions were relatively rare. The presence of a small number of plasma cells, macrophages, lymphocytes, and neutrophils was noted around the bronchioles and vessels in the affected areas. Syntial cells with several nuclei in the cytoplasm are found in the alveoli (Fig. 2).

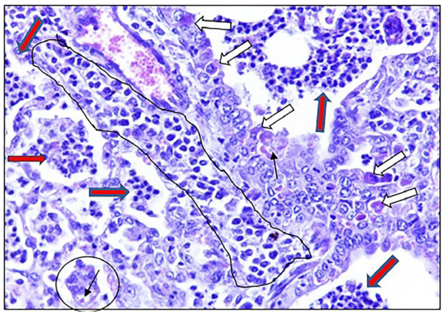

Figure 2. *Histological section of a sheep lung with SRP Bronchopneumonia*
Note: *lumen of the alveoli and bronchioles are filled with neutrophils, macrophages, exfoliated epithelial cells of the alveoli and bronchioles, and necrotic mass (red arrows). Syncytial cells with inclusion cells (inside the circle) are found in the alveoli. Numerous epithelial cells of bronchioles contained large eosinophilic viral inclusion bodies, mainly intracytoplasmic (white arrows), but rarely intracellular (black arrows). There are not many plasma cells, macrophages, lymphocytes, and neutrophils around bronchioles and blood vessels (inside the black line). Hematoxylin and eosin. ×250*
Source: *compiled by the authors*

In addition, at higher magnification, in pathologically changed areas in the epithelium of bronchi and bronchioles their hyperplasia, and the presence of intracellular eosinophilic viral inclusions were noted. These inclusions were localised both in the cytoplasm and in the nuclear structures of the cell (Fig. 3). In the bronchial lymph nodes, minor haemorrhages in the subcortical layer were observed, and atrophic changes in the cortical and cerebral ball of the node. The degree of manifestation of pathological changes in the lung tissue was proportional to the period of the disease.

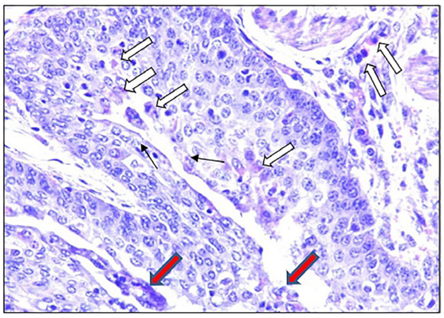

Figure 3. *Histological section of a sheep lung with SRP*
Note: *bronchial epithelial cell hyperplasia. Numerous epithelial cells placed around large bronchi contain relatively large eosinophilic viral inclusion cells, mostly intracytoplasmic (white arrows) and rarely intranuclear (black arrows). There are not many plasma cells, macrophages, lymphocytes and neutrophils around bronchioles and blood vessels. There is a necrotic mass in the lumen of the bronchi (red arrows). Hematoxylin and eosin. ×250*
Source: *compiled by the authors*

Thus, pathohistological changes in the lungs indicate the development of an inflammatory reaction in alveolar-bronchial tissues and active proliferation of cellular elements into the inflammatory zone. There is an active replication of viral particles – eosinophilic intracellular particles, which indicates a deficiency of the immune system.

Pathohistological changes in regional lymph nodes. When analysing the differences in histological changes in the tissues of the regional lymph nodes affected by the SRP virus, in comparison with those obtained from healthy animals, the presence of significant areas of lymphoid tissue atrophy in the paracortical or T-zone, and the destruction of secondary lymphoid follicles associated with the B-dependent zone was noted in the affected tissues at low magnification (Fig. 4). When analysing histological preparations obtained from lymph nodes from infected animals at high magnification, the following changes were found: progressive atrophy of lymphoid follicles in the B-zone of the cortical layer of the node, which was confirmed by the absence of signs of mitosis of lymphoblasts in their light centre, the presence of apoptoses as a result of the development of apoptosis of lymphocytes (Fig. 5). The development of lymphocyte destruction process was confirmed on histological preparations by the presence of significant atrophy areas in the paracortical zone of lymph nodes (Fig. 6). In the brain matter, the histological structures of the medullary thrust and brain matter are partially erased. In this layer of the lymph node affected by the SRP virus, areas of lymphocyte decay are also noted, identified by the presence of apoptoses (Fig. 7); in some preparations, diffuse and focal haemorrhages were found in the medulla layer of the substance (Fig. 8).

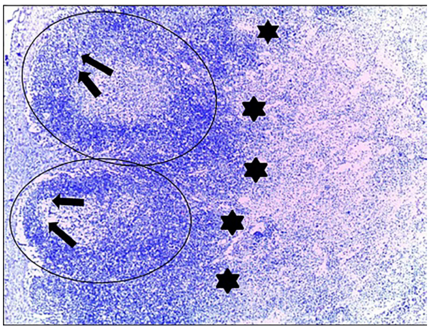

***Figure 4**. Transverse histological section of the regional lymph node*
***Note**: atrophy of the T-zone (asterisks) and disintegration of secondary lymphoid follicles (inside the oval and arrow). Haematoxylin and eosin. ×25*
***Source:** compiled by the authors*

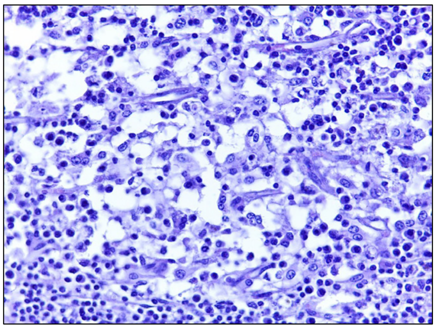

***Figure 5**. Transverse histological section of the regional lymph node*
***Note**: atrophy of lymphoid follicles (B-zones), in the light centre there is no mitosis of lymphoblastic cells, apoptosis (decay) of lymphocytes and apoptosomes. Hematoxylin and eosin. ×250*
***Source:** compiled by the authors*

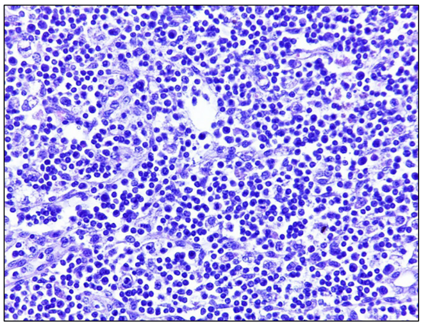

***Figure 6**. Transverse histological section of the regional lymph node*
***Note**: atrophy of the paracortical zone (T-zone), apoptosis (decay) of lymphocytes and apoptosomes. Hematoxylin and eosin. ×250*
***Source:** compiled by the authors*

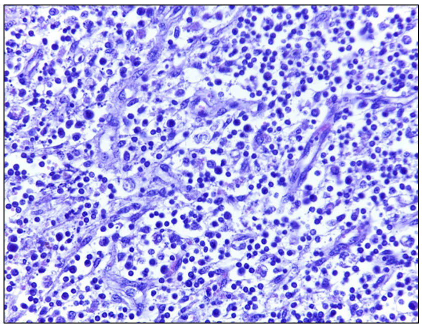

***Figure 7**. Transverse histological section of the regional lymph node*
***Note**: change in the histological picture of the brain matter and inability to distinguish between cerebral tracts and cerebral sinuses. Apoptosis of lymphocytes and apoptosomes in the brain matter. Hematoxylin and eosin. ×250*
***Source:** compiled by the authors*

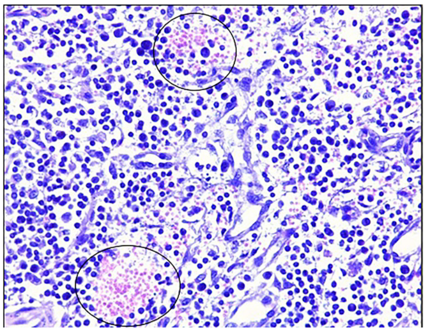

***Figure 8**. Transverse histological section of the regional lymph node*
***Note**: haemorrhage in the brain matter (inside the circle). Hematoxylin and eosin. ×250*
***Source:** compiled by the authors*

Thus, in small ruminant plague, the main morphohistological changes in regional lymph nodes are partial atrophy of T- and B-dependent zones of lymphocyte reproduction, which is probably the main reason for the decrease in the level of cellular and humoral immune response to infectious agents. Thus, significant apoptosis of lymphocytes in the lymph nodes, and atrophy of lymphoid tissue, are the main reasons for the decrease in the level of leukocytes in the blood of animals and the associated development of an immunodeficiency condition.

Pathohistological changes in the spleen. In the spleen, as in other organs of the lymphoid system, for example, in regional lymph nodes, foci of diffuse atrophy of the internal structures of the organ are noted on histological preparations. In particular, degenerative processes in the pulp of the spleen, namely in the T- and B-zones, are noted in infected animals. The white pulp contains a small number of lymphocytes, which may be the result of a decrease in their concentration in the peripheral blood, and there are also no signs of lymphoblast proliferation. There are no lymphoid structures (follicles) in the spleen tissue. It is difficult to distinguish the white pulp from the red pulp on the preparations (Fig. 9). Diffuse and focal areas with capsule haemorrhages were revealed on histological preparations at high magnification. The red pulp shows the presence of erythrocytes, lymphocytes, plasma cells, macrophages, eosinophils and haemosiderin in small numbers (Fig. 10).

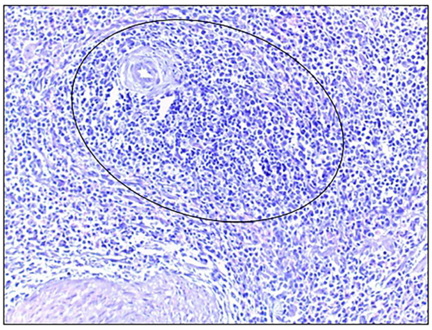

***Figure 9**. Histological section of the spleen Atrophy of the white pulp of the spleen*
***Note**: atrophy of the T- and B-zones of the white pulp (inside the oval). No proliferation of lymphoblasts is observed. Lymphoid follicles are absent. Hematoxylin and eosin. ×62.5*
***Source:** compiled by the authors*

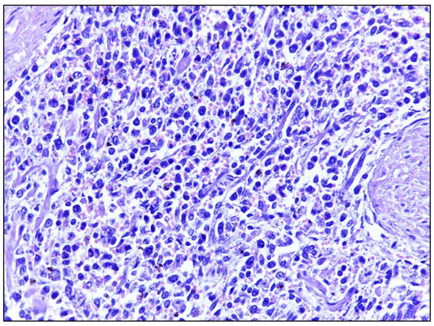

***Figure 10**. Histological section of the spleen*
***Note**: red pulp. Trabeculae (arrows), erythrocytes, lymphocytes, plasma cells, macrophages, eosinophils, and haemosiderin are visible. Hematoxylin and eosin. ×250*
***Source:** compiled by the authors*

Thus, in the spleen affected by the small ruminant plague virus, and in other organs of the lymphoid system, atrophic changes are noted, which reduce the immune potential of the organism. At the histological level, this was manifested in the smoothing of the boundary between the T- and B-zones of the white pulp, responsible for the formation of cellular and humoral reactions in the body of sheep, respectively. Summing up the results of the studies and histological changes occurring in the body of small ruminants infected with plague, it should be noted specific morphofunctional and immunological changes in lymphoid tissue that cause a decrease in the number of lymphocytes and the development of an immunodeficiency state of the body. The main histological changes in lymphoid tissue cells are diffuse eosinophilic granularity in the cytoplasm and nucleus, which is associated with the active reproduction of the virus. Similar changes are characteristic of the cytoplasm and nuclei of the alveolar and bronchial epithelium, alveolar macrophages, and syncytial lung cells.

DISCUSSION

Given that small ruminant plague is characterised by the progressive development of an immunodeficiency state in infected and re-infected animals, this disease is a significant threat to sheep production. The possibility of virus persistence in immune animals and the use of vaccines on sheep and goats leads to the development of atypical forms of the disease. One of the key signs of the disease remains the development of progressive immunosuppression, which subsequently leads to secondary infection with the development of a symptom complex of signs characteristic of secondary infection (Turmagambetova *et al.*, 2017). In such cases, the primary disease is mitigated and overshadowed, and the only way to determine the presence of the plague virus is through characteristic pathohistological changes in the body of diseased animals. In addition, even in vaccinated animals, when exposed to a pathogenic strain of the SRP virus, signs of infection develop in immunocompetent organs. This, according to I. Saeed *et al.* (2022), is the cause of weakening of the immune system in some cases.

The simplest way to detect the development of an immunodeficiency condition is to estimate the total number of leukocytes and the differential leukocyte count (Turgenbayev *et al.*, 2023). The conducted studies clearly show a significant decrease in the total number of leukocytes. Their level is only 40% of the reference value. Thus, a decrease in the number of lymphocytes, which are associated with the work of the humoral part of the immune system, namely the production of antibodies, leads to the development of immunodeficiency and a decrease in the body's protective response (Namouchi, 2022). In addition, a shift in the ratio of granulocytes to agranulocytes was observed in the blood of infected animals. Similar changes in the blood system of infected goats and sheep were also observed by M. Uddin *et al.* (2022), and S. Khaliq *et al.* (2020). However, S. Maina *et al.* (2015) found no decrease in the total number of leukocytes in the blood of infected goats. Perhaps this could be due to the low virulence of the virus used in the experimental infection of animals in the experiment, since a laboratory strain was used. But despite these differences in the number of leukocytes in the peripheral blood of infected animals, all the above-mentioned researchers and experiments conducted within the framework of this study agree that when goats and sheep are infected with the SRP virus, there is a decrease in the number of lymphocytes. Considering that the processes of reproduction and differentiation of this fraction of white blood cells occur in the lymphoid tissue of the immune system, unlike other fractions of leukocytes, the development cycle of which is associated with the bone marrow, it can be concluded about the tropism of the causative agent of the small ruminant plague. The absence of changes in the number of neutrophils, eosinophils, and basophils in the differential leukocyte count from infected animals in the conducted studies indicates that the functions of the bone marrow, as the main organ of reproduction of blood cells, are not pathologically affected. Similar results were also obtained by A. Jarad *et al.* (2022). In addition, this is confirmed by the almost constant number of other blood cells, the development of which occurs in the bone marrow – red blood cells and platelets. Thus, further work was aimed at studying histological changes in the lymphoid tissue of immunocompetent organs, in particular interstitial lung tissue, regional lymph nodes and spleen, which could lead to a decrease in the number of lymphocytes in the blood.

During the pathological and anatomical autopsy of animals fallen from the small ruminant plague, it was noted that the main changes were found in the organs of the respiratory system, gastrointestinal tract, and other internal organs. However, morphofunctional changes in the lungs, lymph nodes, and spleen were the most pronounced, which is why these organs were selected for further histological studies.

During microscopy of histological preparations of the lungs, a significant part of the pathomorphological changes in tissues was characteristic of the development of an inflammatory reaction. Through this, in the affected tissue areas, the alveoli and bronchioles were filled with exudate with significant inclusion of cellular elements including neutrophils, macrophages, and other cells of the cellular immune response system. The active work of cellular immunity is confirmed in the study by S. Gautam *et al.* (2021), which indicate that there is an active accumulation of CD163+ and M2c macrophages in the affected areas of the lungs, which are involved in inflammatory reactions. This explains the development of syntial structures in the affected areas of the lungs. These histological symptoms indicate that this part of the body's protective function is working normally and adequate reactions to the ingress of an infectious agent are taking place. However, active reproduction of viral particles is observed in the epithelium of the respiratory tract and alveoli, which is diagnosed by the presence of eosinophilic inclusions, which are the centres of assembly of viruses in the cytoplasm and nuclear structures of cells (Busol *et al.*, 2023). The changes occurring in certain areas of the lungs show a different intensity of pathological processes. This is probably due to the elongation in time of infection of certain areas of organ tissue. A similar conclusion regarding the manifestation of pathological changes depending on the time after infection was made by M. Islam *et al.* (2023) when dissecting 36 fallen goats at different stages of the disease. The researcher noted a different degree of manifestation of pathohistological changes in the structures of alveolar-bronchial tissue in animals with different intensity of disease progression and the period of time elapsed between the manifestation of clinical signs and the death of animals.

However, the most characteristic changes occurred in the lymphoid tissue of the lymph nodes, where the development of lymphocytes takes place. Among the histological signs of structural abnormalities, there were point and diffuse haemorrhages in the lymph node tissue, a decrease in the clarity of the boundary between the cerebral and cortical layers, but the main changes concerned atrophy of morphological structures responsible for the differentiation of immunocompetent cells and apoptosis of already formed lymphocytes. Such changes were typical for almost all the studied lymph nodes and degenerative changes occurred in both T- and B-dependent zones of lymphocyte development with different intensity in the controlled animals. That is, as in the case of pathological processes in the lungs, the degree of manifestation of the pathological picture in the lymph nodes depended on the duration of the disease in animals (Radsikhovskiy *et al.*, 2023). The exception was the characteristic of histomorphological changes in the spleen. Regardless of the condition of the animals at the time of their death on histological preparations of the spleen, the intensity of the processes was almost at the same level – these were diffuse haemorrhages into the capsule of the spleen, significant foci of lymphoid tissue atrophy with damage to T- and B-dependent lymphocyte formation centres, haemosiderin residues. Perhaps this is due to early infection by viral particles of spleen tissues through the circulatory system during the incubation period, and at the time of manifestation of clinical signs in animals, pathological changes provoked by the SRP virus have already occurred in the spleen. Thus, given the tropism of the virus to lymphoid tissue, the damage to the organs of the immune system depends on the time interval from infection to testing. Similar findings were obtained by M. Islam *et al.* (2023).

Summing up the work performed to investigate the influence of the causes of immunodeficiency state development from pathomorphological changes in the lymphoid tissue of immunocompetent organs, it should be noted that these processes were the reason for the decrease in the number of lymphocytes in the peripheral blood, and the decrease in the humoral and cellular immune response of the organism. A significant concentration of macrophages and neutrophils was noted in the zone of inflammatory reaction in the lungs. Thus, immunodeficiency in small ruminant plague is a consequence of the destruction of lymphoid tissues of the immune system of the organism of infected animals.

CONCLUSIONS

Based on the received literature information and the results of studies on the difference between leukocytes and their fractions in the blood of healthy and infected animals, considering morphofunctional changes in the lymphoid tissue of the immune system organs of fallen animals from the small ruminant plague, the following conclusions can be drawn. In all fallen animals, a decrease in the number of lymphocytes in the blood was observed, by more than 40%, in comparison with the reference indicators characteristic of this type of animal. In addition, the ratio of granulocytes and agranulocytes shifted from haematological parameters towards cells with granules present in the cytoplasm – neutrophils, eosinophils, and basophils. Such changes in the blood system were also manifested by changes in the differential leukocyte count, where, in addition to a decrease in lymphocytes (by almost 50%), a relative increase in the fractions of rod-core and segmented neutrophils was observed.

Histological studies have shown systemic disorders in the lymphoid tissue of the organs of the immune system – lymph nodes and spleen, which manifested themselves in atrophy of T- and B-dependent zones of lymphocyte generation, and as apoptosis of already developed lymphocytes in the parenchyma of these organs. Focal and diffuse haemorrhages also indicate a violation of the functional state in the studied organs. Such morphofunctional changes in the organs of the immune system led to a decrease in the level of lymphocytes in the peripheral blood, and thus caused the development of an immunodeficiency state of the animal body.

In the alveolar-bronchial lung tissue, signs of the development of an inflammatory reaction were found, which is characterised by cellular proliferation of tissues by neutrophils and macrophages. But the active reproduction of viral particles in the epithelial cells of the bronchi and alveoli, which is confirmed by the presence of eosinophilic inclusions in their cytoplasm, indicates the insufficiency of this mechanism of immune response. These conclusions are based on the results of the study of only three fallen animals, which does not allow them to be extrapolated to all cases of the disease. Therefore, such studies should be continued with the involvement of a large number of cases and a more detailed analysis of the mechanisms of development of the immunodeficiency state in the body of sheep infected with SRP.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Спорадичний випадок чуми овець: морфофункціональний прояв клітинного та гуморального імунодефіциту

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Анотація. Небезпека поширення чуми дрібної рогатої худоби для тваринництва низки країн робить вивчення механізмів розвитку та профілактики цього захворювання пріоритетним напрямом наукових досліджень. Тому метою роботи стало вивчення змін у системі крові та органів імунної системи за чуми дрібних жуйних, які призвели до розвитку імунодефіцитного стану організму хворих тварин. При проведенні роботи були використані гематологічні та гістологічні методи дослідження. У результаті проведеної роботи у 3 овець, які померли від чуми дрібної рогатої худоби, було виявлено зниження загальної кількості лейкоцитів у периферичній крові з 12,4 до 7,3х109/л. При цьому також спостерігалося зміщення нормального співвідношення гранулоцитів до агранулоцитів з 1:1 до 2.5:1 у бік гранулоцитів, що було спровоковано різким зниженням кількості фракції лімфоцитів з 49 % до 25 % у лейкоцитарній формулі крові. Такі зміни в системі крові були спровоковані значними дегенеративними змінами в лімфоїдній тканині імунокомпетентних органів – лімфатичних вузлах і селезінці. При гістологічному дослідженні в паренхімі вище зазначених органів було виявлено атрофію Т- і В-залежних зон і апоптоз уже лімфоцитів. У сукупності ці ознаки – гематологічні та гістологічні – дають змогу зробити висновок про зниження як гуморальної, так і клітинної системи імунної відповіді в організмі. В уражених ділянках легень спостерігається розвиток запальної реакції з активною міграцією в зону запалення нейтрофілів і макрофагів. Але активне розмноження вірусу в клітинах легень вказує на недостатність імунітету. Таким чином проведені дослідження розкривають механізми розвитку гуморального та клітинного імунодефіциту за чуми дрібних жуйних тварин (ЧМР Тварин), що дасть змогу розробити більш ефективні методи боротьби з цим захворюванням

Ключові слова: лімфоїдна тканина; гістологічні та гематологічні дослідження; лімфатичний вузол; селезінка; легенева тканина; лейкоцитарна формула