Pathomorphological changes in sheep paramphistomatosis

Bokhodir Kuliyev[1*], *Gayrat* Eshmatov[1], *Eshmukhammad* Bobonazarov[1], *Bakhtiyor* Mukhtarov[1], and *Sunnat* Akhmedov[1]

[1]Samarkand State University of Veterinary Medicine, Livestock and Biotechnologies, Samarkand, Uzbekistan

Abstract. The scientific article provides information on the pathomorphological changes caused by paramphistomes that develop in the digestive organs. The results of pathologoanatomical, pathogistological and histochemical changes in paramphistomatous digestive organs and parenchymatous organs are also written in detail. Pathomorphological changes are clearly explained in colourful pictures. Mutsicarmine, mucopolysaccharides, glycogen, polysaccharides and various pathomorphophysiological characteristics of adhesion were determined in epithelial cells and perivascular tissues by histochemical methods. The scientific article provides practical conclusions as well.

1 Introduction

Statement of the Problem. Today, in most countries of the world, great attention is paid to the development of the food industry and the development of sheep farming in order to provide the population with quality food products. Among other things, under the influence of various environmental and anthropogenic factors, some parasitic diseases, including paramphistamatosis, are occurring among small horned animals, as well as the occurrence of new outbreaks. As a result of infestation of small horned animals with trematodes, the quality of wool products is reduced by 10-25 percent, and the quality of meat products is observed to decrease by 15-35 percent [1, 5, 7, 9].

Therefore, it is important to study the pathanatomical, pathogistological and histochemical changes of this parasitic disease in the body. In different regions of the world, some unfavorable environmental factors have a negative effect on the resistance of the livestock organism and allow the adaptation and wide spread of disease-causing parasites. Based on this, it is highly effective to take into account pathanatomical and pathogistological changes in determining the spread of diseases, countermeasures and treatment measures. Pathanatomical, pathogistological and histochemical examination of sheep infected with paramphistomatosis is an important urgent task [3-4, 8].

Extensive measures are being implemented in our republic aimed at reducing trematodosis infection of livestock, especially sheep, their spread, prevention and combating. Cases of death of small horned animals due to various infectious, non-infectious

* Corresponding author: sunnat.axmedov.88@mail.ru

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

and invasive diseases among livestock, retardation of their development, sharp decrease in productivity and forced slaughter due to severe disease are being prevented. Determining the prevalence of sheep paramphistamatosis in different geographical regions, correct diagnosis, pathanatomical, pathogistological and histochemical examinations of the organs of spontaneously infected and dead animals are not paid enough attention [2, 6, 10].

The pathomorphology of this disease in sheep has not been studied at all, although this disease is widespread among sheep today.

2 Materials and methods

Scientific research work was carried out in the laboratory of the Department of Animal Anatomy, Histology and Pathological Anatomy of Samarkand state university of veterinary medicine, livestock and biotechnologies. The tests were carried out on internal organs of sheep spontaneously infected with paramphistomatosis. The main direction was the pathomorphological and histochemical examination of the organs of sheep that died from the disease and infected and forcibly slaughtered sheep. We studied the litter materials obtained from dead, infected and forcibly slaughtered sheep from paramphistomatosis in the conditions of farmers and private farms in several districts. Together with the larvae removed from the abdomen, samples - slices were cut and placed in formalin liquid. We studied samples taken from naturally infected sheep using pathomorphological methods. Paramphistome causative agents were isolated from the large stomach, stomach, stomach, rennet, duodenum and stored in 3% formalin liquid. Internal organs were studied by pathanatomical methods.

We determined sections cut from paraffin blocks, mucicarmine in mucous membranes by Mayera's method, fibrin and collagen fibers by Mallory's method, elastic and reticular fibers by Hartou's method, mucopolysaccharides and glycogen by Mc-Manus' method, RNK by Brashe's method, polysaccharides and bronchial epithelium by Schick's reaction method.

We studied acidic and alkaline phosphatase in frozen sections cut in a cryostat by Gomori's method.

3 Results and Discussion

Sheep infected with paramphistomosis and those that died from the disease were first examined for the external appearance of the body.

Pathanatomical changes: the external appearance of the body - the wool is wrinkled, the skin is dry, elasticity is not preserved, the nostrils are loose, there is a mucous discharge, the mucous membranes of the mouth and gums are gray in color, the eyes are sunken, the mucous membranes are light-red, the ear holes are empty, dry, the back there is a liquid mucous discharge in the discharge hole, the wool around it is dirty. The following pathanatomical changes were detected in the bodies of sheep that died from paramphistomatosis: sharp emaciation, atrophy of the subcutaneous tissue, muscles and fat layer, curvature of the spine, paleness of mucous membranes (anemia), diarrhea, swelling under the lower jaw and in the chest in some sheep. 1.5-3 liters of watery, shiny, light-red liquid has accumulated in the chest and abdominal cavities.

Subcutaneous tissue - dry, fat depot in a state of atrophy, hyperemia in veins, partial blood leakage. Lymph nodes are enlarged, the cut surface is wet, light gray.

Lungs - partially swollen diaphragm and middle lobes, denser consistency, opaque edges, dark red in color, serous mucus fluid flows from the cut surface.

Liver - the surface is yellowish-brown in color, the size is increased, the edges are opaque, the cut surface is uneven, granular protein dystrophy, the consistency is thickened, the gallbladder is swollen, filled with stretchy yellow bile fluid, venous hyperemia has developed.

Spleen is hard consistency, wrinkled, dry, its surface is gray in diseased sheep, and the cut surface is brown-red in the state of hyperplasia.

Kidneys - the consistency is firm, fluid, increased in size, the shell is slightly separated, there is no fat around the organ, the fat is replaced by a cystic infiltrate.

Heart - muscle fibers are swollen, granular protein dystrophy in the myocardium, blood vessels are filled with blood (hyperemia) and dilated. Under the pericardium of the heart, 300-500 ml of bright light red serum fluid was collected, the heart was enlarged, the muscles of the right ventricle were thickened in the ratio of 1:3 compared to the left ventricle. Under the epicardium, small point-like and spot-like hemorrhages (petechiae) are visible, hyperemia of blood vessels around the valves in the endocardium, atrophy of adipose tissue, and in its place, a sarcoid-like infiltrate has formed.

Large abdomen - different numbers of trematodes are firmly attached to the microsuckers of the large abdomen, mucous membranes are mechanically injured, the abdominal wall is thinned, blood vessels are anemic.

The net abdomen is filled with food mass, the food has a liquid porridge-like consistency, the mucous membranes are swollen, partially displaced, the veins are filled with blood.

Solid - food mass mixed with mucous fluid has accumulated, red spots have formed on mucous membranes, blood vessels have expanded.

In the chronic form of the disease, the main characteristic changes were detected in the large and reticulated abdomen, adult paramphistome helminths adhere to the mucous membranes of the large and reticulated abdomen, 4-12 mm long parasites located close to each other throughout the mucous membrane hang on the abdominal mucosa. A parasite that is not firmly attached can be easily separated. Hyperkeratosis, sclerosis, atrophy of the nipples, thinning of the mucous membrane, 500-786 copies of parasites were detected in the mucous membrane of the large and net abdomen.

Duodenum intestine - mucous membranes are swollen, serous-catarrhal inflammation, mucous mass, folds are formed in the intestinal food and on the surface of the mucous membranes, there are small point hemorrhages.

Lymph nodes are partially enlarged, their surface is uneven, their consistency is weakly thickened, serum fluid is collected on the cut surface.

Pathohistological changes: in the mucous membranes of the duodenum intestine and the mucous membranes of the small intestine, basal membranes are exposed, epithelial cells are detached from their walls, erosions are formed, the number of packed cells is increased. Suckers are short, needle-like in some places. Lymphoid, plasmatic cells, histiocytes, eosinophils are always collected in the submucosa layer, dilation of capillary vessels and hemorrhages are seen. Swelling of the private layer, fragmentation of elastic fibers, and swelling of collagen fibers were found. They are weakly stained, the muscle fibers are not uniformly stained, in some places the parasite larvae are invisible, in the form of a tumor.

The follicles of the mesenteric lymph nodes are without outline, the reactive center is developed. Peripheral and central sinuses are expanded, and lymphocytes, neutrophilic leukocytes and plasma cells are collected in large numbers. It was found that lymphocytes, eosinophils and plasma cells were scattered and concentrated in the capsule and trabeculae.

On the mucous membranes of the duodenum intestine, fragments of paramphistome larvae cut in different directions are visible. Similar parasite fragments are also present among suckers, which are also located in the deep parts of the intestinal mucosa. Suckers of the larva tightly adhering to the surface of the intestinal mucosa were detected. As a result

of proliferation, there are many epithelial cells and papillae in these places. A large number of lymphoid cells, histiocytes, and eosinophilic leukocytes are seen infiltrating the connective tissue of the stroma. Paramphistome larvae, located deep in the mucous membranes, pinch the secretory glands and their pathways. As a result, accumulation of secretions occurs and cysts are formed. In other sheep, hemorrhages, desquamation of the teat epithelium and small necrotic foci were detected at the beginning of the duodenum and jejinum intestines. On the surface of the mucous membranes, young paramphistome larvae are visible in large numbers in transverse and longitudinal sections. They are located in the submucosa and even in some parts of the muscle layer. Capillaries in the submucosal layer were expanded, filled with blood, hemorrhages, necrosis of epithelial cells, deformation of the nipples, and disruption of the glandular tissue structure were found. Collagen fibers in the connective tissue are swollen. Cell infiltration consists of lymphocytes, histiocytes, plasmocytes, neutrophilic leukocytes and eosinophils: Eosinophilic leukocytes are found in all internal organs, mainly in intestinal wall, liver, spleen, and lymph nodes. The glands in the submucosal layer are swollen, the structure is broken, and cysts of various sizes are formed. Desquamation of the epithelium has developed in the ducts of the glands. Muscle fibers are swollen and split.

In abomasum - vascular hyperemia, homogeneous foci were detected. An increase in the number of lymphoid follicles in the cortex layer of Charvi's lymph nodes, and an increase in lymphocyte and plasma cells in the medullary layer, swollen sinusoids, formation of dark nets, accumulation of small and medium lymphocytes, eosinophils.

Hyperemia of vessels, desquamation of epithelial cells, edema of the submucosal layer are characteristic in the mucous membrane of the esophagus, esophagus and large stomach. This reports that the parasite larvae have fallen on these mucous membranes later. It is located in 2-3 rows in the bark layer of the sheep's lymph nodes. It can be seen that lymphocyte follicles are quantitatively increased, lymphocyte and plasmatic cells are located at the edges of the nucleus.

In the myocardium - fat cells accumulated around its blood vessels, and cell infiltration in other parts. In the connective tissue layers, collagen fibers are swollen and the number of fat cells is increased, desquamation of vascular endothelial cells is characteristic.

In the thyroid gland - the follicles are filled with dark colloid. This change is caused by hypofunction of the thyroid gland.

In the spleen - hyperplasia of lymphoid follicles was seen. Histosections of the spleen clearly show white and red pulp, secondary follicles are increased. The reactive centers in the lymphatic follicles of the white pulp are large, and in the red pulp, lymphocytes, neutrophils, and eosinophils are collected in large quantities. Endothelial cells of trabecular arteries and small vessels are eroded (necrosis). Lymphatic follicles with reactive centers are also developed in the spleen, in which the central arteries have increased by 2-3 units. Histochemical method revealed an increase in the amount of hemosiderin when stained with Perlsud.

In the liver - granular protein dystrophy of hepatocytes, proliferation of histiocytes and lymphoid cells in interlobular connective tissue. Hyperemia of venous vessels, disorder of beams developed. Protein granules are seen in the cytoplasm of hepatocytes, and eosinophils are seen in the intercellular connective tissue.

Kidney - curved tubules are enlarged to the size of epithelial cells, nuclei are divided into small chromatin bundles, blood vessels in the core part of the kidney are hyperemic.

In other organs, vascular hyperemia and partially accumulated cellular elements in the stroma of the organ were detected.

Histochemical changes: When the sections prepared from the intestine were stained by the Schick-reaction method, the absence of glycogen granules was found in the epithelial papillae of the intestine. In contrast to the epithelial cells lining the teats, the epithelia of the

crypt glands were found to be highly sensitive to Shik-reagents. It was revealed that the crypt epithelial cells were intensively stained in the Shik-reaction, which indicates the accumulation of mucoid secretion in the crypt epithelium.

In the histochemical examination, it was found that the amount of protein was high in the mammary epithelial cells of the internal mucous membranes and in the intestinal crypts. A combination of mucopolysaccharides with protein was seen in green-blue color in the mucous membranes. This situation reduces the protective activity of the body. Carbohydrate components composed of neutral muco and glycoproteins were detected in fragments of sheep small intestinal teats in Shik-reaction. The phenomenon of metachramasia is high in the papillae in the epithelial layer of the mucous membranes. This is due to the accumulation of chromotropic substances in the main intermediate. Metachromasia refers to the association with hyaluronic acid, which provides cell permeability. The tegument of Paramphistome is also well stained. This refers to stimulus response adaptation. Weak purple staining of epitheliocytes, large-sized alcyanophilic bodies were detected in the apical parts of sour mucopolysaccharides in the cell. Adhesion detection was achieved in paramphistomatosis. Adhesions of different variants in solid contact between Paramphistoma and epithelial cells of intestinal mucosa: 1. Absorption of helminth in leafy suckers; 2. Interaction between the parasite tegument and the mammary epithelial cell.

Various morphophysiological characteristics of adhesion were determined, which of course depend on the level of interaction between the parasite and the organism.

The first stage of the adhesive process is the approach of the parasite to the host tissue. In the formation of adhesion, two glycocalyx layers: the paramphistome tegument and the surface of intestinal epithelial cells. The two glycocalyx layers of the parasite and the host body form a tight contact, through which exchange processes take place between the helminth and its endostocia. This is transepithelial contact adhesion.

Histochemical reactivity of the tissues of the parasite and the host organism shows that alcyanophilia in transepithelial adhesion is similar to the glycocalyx, and metachromasia was detected in the adhesion zone. The Schick reaction is positive in the parasite tegument and intestinal epithelium. In the surface version of the adhesion, it confirms the accumulation of protein in this zone, that is, bromphenolophilia.

The second stage is deep contact, in which the growth of connective tissue in the paramphistome tegument and intestinal wall tissue was determined. In this contact, the body of the helminth separates from the epithelium of the intestinal tract, desquamation occurs and the parasite adheres to the tegument. In this case, a mechanical injury of the intestinal walls is seen, because the adhesive bonds are broken, which is characteristic of the distant mechanism between the parasite and the host organism.

Intrafibrillar type of deep adhesion differs from transepithelial adhesion in histochemical reactivity of parasite and host tissue. Alcyanophilia is weakly developed in the adhesion zone, and there is no metachromatic effect. Shik-reaction staining decreases due to adhesion of paramphistome tegument and intestinal epithelial connective tissue. Adhesion types were clearly identified in the parasite-host intestinal system by histochemical method.

Depending on the mechanism of the pathological process, the results of histochemical examination also differed. The most characteristic change reaction was alkaline phosphatase in epithelial cells. Different activities of enzymes were determined in different tissues. An acute positive reaction is in the focus of inflammation, in which the normal structure of these tissues is partially disturbed.

Alkaline phosphatase was detected in the epithelial cells of the large abdominal walls. That is, alkaline phosphatase activity was also seen in the fluid (exudate) of the large abdominal wall.

Alkaline phosphatase was detected in perivascular connective tissue. The development of inflammation in the large intestine was accompanied by a relative change of sour and neutral mucopolysaccharides, that is, sour mucopolysaccharides were abundant in epithelial cells, and glycogen was sharply reduced. Their amount increases with the increase of pathological processes. This situation was observed especially in catarrhal-purulent inflammation. In intestinal tracts with advanced exudative inflammation, lipid-storing cells accumulate in perivascular connective tissue. A positive reaction of lipids is characteristic in catarrhal-purulent inflammation. This is certainly related to the accumulation and destruction of macrophages and leukocytes in the inflammatory process.

Pyronychophilic cells were detected in small amount in the connective tissue by the Brashet method.

It was found that the reaction was developed in the follicles in the lymph nodes in the intestinal mesentery during RNA staining by the Brashe method. Plasma cells are focally collected around the central artery of splenic follicles. The reaction was weakly developed in the epithelium of kidney malpighian bundles. DNA-storing cells were detected in a large number in the epithelium of the mucous membrane of the large and net stomach, and in a small amount in the muscle layer.

An increase in the amount of glycogen is seen in interstitial tissue, and lipids in epithelial cells and connective tissue.

When examined by the Futu method, it was seen that the fibers were dissolved in the centers of necrosis and around it. The shape of collagen fibers is developed.

The analysis of histochemical changes in the organs shows that alkaline and acid phosphatases accumulate in the epithelial cells of the large and net stomach.

4 Conclusion

- Sheep infected with paramphistomatosis were characterized by severe shedding, atrophy of skeletal muscle tissue, paleness of mucous membranes, dystrophy of parenchymatous organs and hyperemia of blood vessels.
- In paramphistomatosis of sheep, the most characteristic pathanatomical changes were in the wall of large and net stomachs - atrophy, necrosis, mucoid and fibrinoid dystrophy, hyperplasia, hyperkeratosis, sclerosis developed in the intestines. Desquamation of epithelial cells, infiltration of lymphoid, plasma cells, histiocytes, eosinophils in the mucous membranes of the stomach and intestinum duodenum was characteristic.
- In the histochemical examination, it was found that the amount of protein was high in the mammary epithelial cells of the internal mucous membranes and in the intestinal crypts.
- The histochemical reactivity of the tissues of the parasite and the host organism showed that alcyanophilia in transepithelial adhesion was similar to the glycocalyx.
- Intrafibrillar type of deep adhesion differed from transepithelial adhesion in histochemical reactivity of parasite and host tissue.
- Depending on the mechanism of the pathological process, the results of histochemical examination also differed. The most characteristic change reaction was alkaline phosphatase in epithelial cells.

References

1. Sh.D. Avezimbetov, Bioecological and epizootological features of koramols and koi tremotodoses in the Republic of Korakalpogistan, Vet. science. name science level diss..abstract written to get Samarkand, SamKHI, 19 (2007)

2. D. A. Azimov, Recommendations on measures against trematodosis of farm animals and poultry, Tashkent, 5-45 (2006)
3. O.I. Bibik, Morphofunctional characteristics of organs and tissues of the parasite and host during trematodes after chemotherapy with anthelmintics: Dis. ...doc. biol. Sci., Moscow, 308-311 (2012)
4. O.I. Bobik, Micromorphological changes in organs and tissues of Paramphistomum cervi after tetraxychol, Proceedings of the All-Russian Institute of Helminthology named after K.I. Scriabin, Moscow, **44**, 53-57 (2006)
5. A.M. Bukanov, Pathomorphological changes in the digestive organs of cattle and sheep with paramphistomosis, dissertation of the Can. Veterinary Sciences, UFA, 48-63 (1999)
6. E.A. Vasileva, Epizootology of trematodosis in cattle and improvement of the system of anti-trematodosis measures in the Altai Republic, Author's abstract, Dissertations of Candidate of Veterinary Sciences, Tyumen, 17-19 (2010)
7. A.S. Daminov, Epizootological and immunological features of coramollar trematodes in different biogeocenos of the Republic, Doctoral dissertation, Samarkand, 167-200 (2016)
8. S.A. Shemyakova, Trematodes in cattle (epizootology, pathogenesis, diagnosis) and measures to combat them in the central region of the Russian Federation, Author's abstract. Dissertations of Doctor of Veterinary Sciences, Moscow, 35-39 (2018)