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RESEARCH ARTICLE

Morphological changes in sheep organs caused by *Bunostomum trigonocephalum* infestation

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Strongylatoses are the most common diseases of the ungulates globally and in Ukraine, caused by extremely pathogenic helminths that provoke diseases with noticeable clinical signs and high mortality rate. The humid climate of Western Ukraine's mountainous regions, the lack of veterinary specialists in the highlands, and the resistance of larval forms and helminth eggs to the environmental conditions contribute to the spread of strongyles among sheep. In sheep invasion by the *Bunostomum trigonocephalum* nematode, the main clinical manifestations were characterized by depression, tachycardia, tachypnea, anemia, cachexia, and recurrent diarrhea. The following signs represent characteristic pathological changes: edema of subcutaneous tissue in the neck and chest area, anemia of the mucous membranes, congestive hyperemia and pulmonary edema, serous lymphadenitis and hydropericarditis, dystrophic changes in the liver and kidney, and catarrhal enteritis. Histologically, an inflammatory reaction and edema with thickening of the interstitial lung tissue in the areas of mass accumulation of *Bunostomum trigonocephalum* larvae and cellular infiltration were discovered in the alveoli and bronchi. Edema of the mucous membrane plate was detected in the jejunum and the proliferation of lymphocytes, histiocytes, plasma cells and macrophages, dystrophic and necrobiotic changes in endocrinocytes with pyknosis and lysis of the nuclei. Circulatory disorders with dystrophy and diffuse necrosis of hepatocytes were found in the liver, and hyaline-drop dystrophy of the nephrothelium of the nephron cortical tubules was found in the kidney.

Keywords: *Bunostomum trigonocephalum*; clinical signs; pathological changes; pneumonia; parenchymal hepatitis; proliferation; delimphatization

Introduction

In terms of the private farms' revival, sheep breeding is of significant social and economic importance. To a certain extent, the emergence of farms with many sheep and goats in Western Ukraine depends on natural and climatic conditions and feed resources. For the Carpathian region, where sheep breeding is a traditional activity, large mountain pastures prioritize. They provide animals with green fodder for five months. Besides, green tourism and the availability of folk crafts in this region result in high demand for milk, dairy products, sheep wool, and meat (Vdovychenko et al., 2013; Kovalenko, & Kovalenko, 2017, Fernandada et al., 2018).

Modern intensive sheep breeding largely depends on the observance of comprehensive preventive antiparasitic measures, which aim to prevent the ingress of low-quality products to the consumer and fully benefit from the genetic potential of animals. One of the restraining factors that lead to a decrease in the selection of sheep and their economic qualities are invasions caused by the endoparasites, namely, strongyles (Goossens et al., 2005; Abebe et al., 2010; Boyko, 2015; Eisa et al., 2017; Melnychuk, & Antipov, 2019).

Strongylatoses are the most common diseases of the ungulates globally and in Ukraine (Nwosu et al., 2007; Khan et al., 2010; Kuzmina, 2012; Owusu et al., 2016; Dugassa et al., 2018). A large group of nematodes causes disease from the suborder *Strongylata*, which are considered the most pathogenic animal helminths are causing diseases with noticeable clinical signs and high mortality rate (Kuzmina et al., 2011; Aga et al., 2013; Asmare et al., 2016; Antipov et al., 2019).

Bunostomosis is a widespread invasive disease of sheep caused by *Bunostomum trigonocephalum* nematode. It is frequently manifested in combination with other strongyloides and protozoan diseases of the gastrointestinal tract, amplifying the pathogenic impact of Bunostoma on the affected animals' organisms. Parasites cause severe pathological changes in animals up to their death, especially during their preimaginal development stage. Both helminths' larval and imaginal stages are pathogenic for the organism of sheep. Mature forms of Bunostoma are hematophagous with a very well-developed oral capsule with two cutting plates, which are firmly attached to the intestinal mucosa. The ulcers lead to significant blood loss with the further advent of anemia and digestive disorders. Also, helminths secrete toxins, causing decreased appetite and reduced feed conversion (Awizer et al., 2014; Kovalenko, 2016; Satish et al., 2017; Gunathilaka et al., 2018; Baihaqi et al., 2019).

Migration of the Bunostoma larvae in the alveoli and bronchi damages the walls of blood vessels and causes numerous hemorrhages in the lung tissue. In case of complications provoked by pathogenic microorganisms like E. Coli, Streptococcus, Staphylococcus, Mycoplasmaspp., lobar pneumonia at various stages may develop (Hoste et al., 2016).

The humid climate of the mountainous regions in Western Ukraine promotes the development of strongyles, which can survive in the environment for quite a long time, i.e., from March to November. Besides, the lack of infrastructure and veterinary specialists in

the highlands, resistance of larval forms and helminth eggs to the environmental conditions, and many modern chemotherapeutics and anthelmintic drugs contribute significantly to the spread of the disease among sheep.

Our study aimed to conduct helminthoscopic and helminthoovoscopic examinations to detect and identify the strongyles of the sheep's gastrointestinal tract, describing the main pathological and histopathological changes in some organs of sheep during the acute invasion by *Bunostomum trigonocephalum* nematode.

Materials and Methods

Material for parasitological and histopathological examinations was selected from sheep of the Ukrainian Carpathian mountain breed, two months of age, which were kept at a private farm in Mostyska district, Lviv region. The mass death of sheep began in autumn and winter (November-December), accompanied by cachexia, diarrhea, and anemia.

The pathological autopsy was performed in the prosectorium of the Department of Normal and Pathological Morphology and Forensic Veterinary Medicine (Lviv National University of Veterinary Medicine and Biotechnology named after S.Z. Gzhytsky). The conventional methods confirmed parasitological diagnosis. Upon opening the small intestine, mature nematodes were selected and afterward put into Barbagallo fluid. Feces from the animals were collected individually from the rectum. Helminthoovoscopy of fecal samples was performed utilizing the flotation method using zinc sulfate (Taylor et al., 2016). The species of nematodes were defined according to the qualifier (Zajac et al., 2011).

Fragments of internal organs were taken for histological examination and fixed in a 10% aqueous neutral formalin solution. The tissues fixed in the formalin solution were washed and dehydrated in an ascending row of alcohols, followed by pouring into paraffin according to the conventional method. Histological cuts with a thickness of 7 μm were made from paraffin blocks using a sled microtome MS-2. To perform the light optical microscopy, paraffin sections were stained with Mayer's hematoxylin and eosin. Light microscopy and macrophotography of the obtained histological preparations were performed using the Leica DM-2500 microscope and the Leica DFC 450C camera.

Results and Discussion

Clinical signs of the disease in sheep were manifested by depression, weakness, pallor of the visible mucous membranes, tachycardia, tachypnea, and recurrent diarrhea. The animals lay more frequently, subcutaneous edema appeared in the area of the chest and neck. Still, the appetite was preserved. Sheep died of general exhaustion with the development of degenerative changes in the organism's organs and tissues.

The pathological autopsy revealed swelling of the subcutaneous tissue, anemia of the mucous membranes, the presence of foamy fluid in the trachea and bronchi (Figure 1), congestive hyperemia and pulmonary edema, serous lymphadenitis, and hydropericarditis (Figures 2 and 3). The liver and kidney were dystrophically altered. The small intestine's mucous membrane was slightly thickened with the mucous contained in the state of catarrhal inflammation, revealing a significant number of mature helminths, which were identified as *Bunostomum trigonocephalum* (Figures 4 and 5). Conducted coproovoscopic studies revealed strongyle-type eggs at the morula stage (Figure 6).

Figure 1. Foamy fluid in the bronchi. Pulmonary edema.

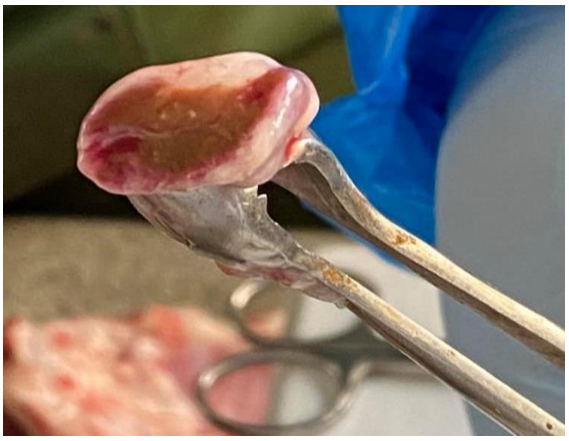

Figure 2. Serous lymphadenitis of mesenteric lymph nodes.

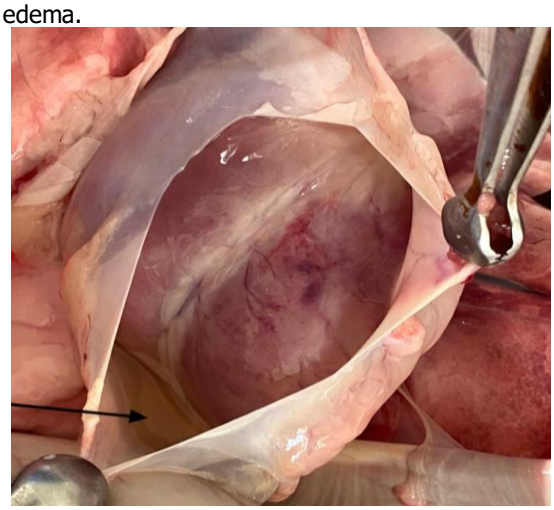

Figure 3. Transudate in the pericardial cavity - hydropericardium (arrow).

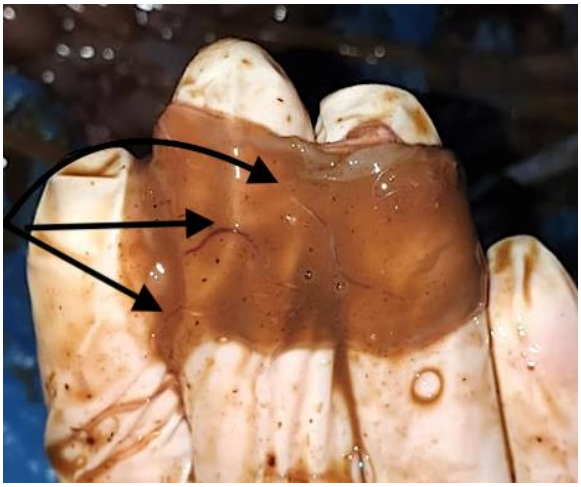

Figure 4. Small intestine. Mature nematodes *Bunostomum trigonocephalum* (arrows).

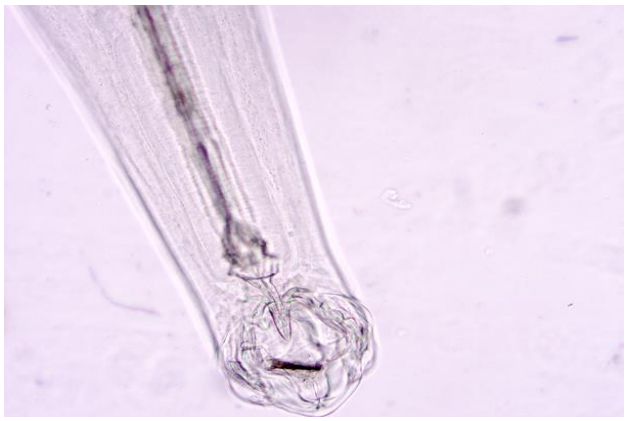

Figure 5. The primary end of *Bunostomum trigonocephalum.*

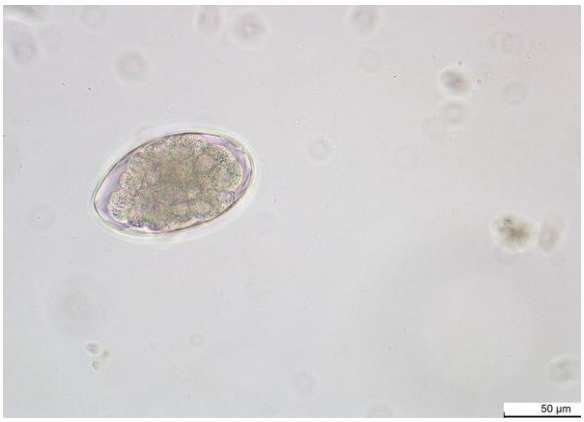

Figure 6. Strongyle-type egg. x400

Migrating with the blood flow from the intestines to the lungs, the larvae damaged the walls of blood vessels, destroyed the alveoli, shed and exited the trachea, were coughed with mucus, swallowed again, and entered the intestine. Some authors believe that morphological changes in the lungs, namely the development of inflammation, play a key role in the etiology of mass pneumonia in lambs, which is usually complicated by saprophytic microorganisms. Mass accumulation of *Bunostomum trigonocephalum* larvae was detected optically in the alveoli and bronchi (Figures 7-9). There is a cellular infiltration of lymphocytes, histiocytes, and macrophages in the peribronchial connective tissue (Figure 7). Delimphatization is characteristic of the peribronchial lymph nodes. It was on the route of larvae migration to the bronchi that an inflammatory reaction was established. There was edema with the subsequent thickening of the lungs' interstitial tissue: the alveoli' lumen is filled with an edematous fluid of pale pink color (Figure 10). Changes in other parts of the lungs represent emphysema's characteristic features, i.e., alveoli are excessively stretched by air, alveolar septa are thin, sometimes destroyed.

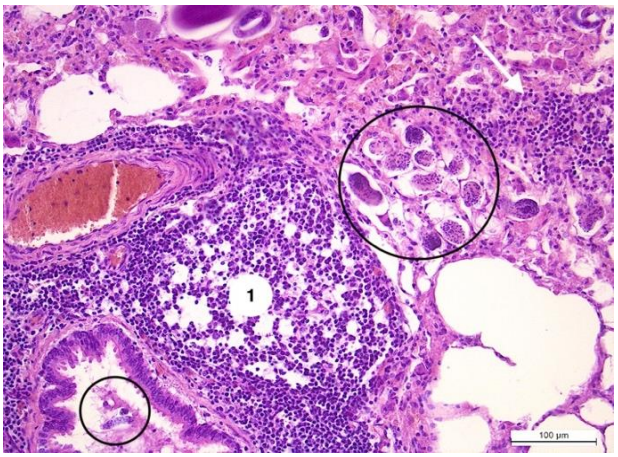

Figure 7. Lungs. Larvae of *Bunostomum trigonocephalum* in the lumen of the bronchi and alveoli. Peribronchial lymph node (1), cellular infiltration (arrow). Hematoxylin and eosin, x200.

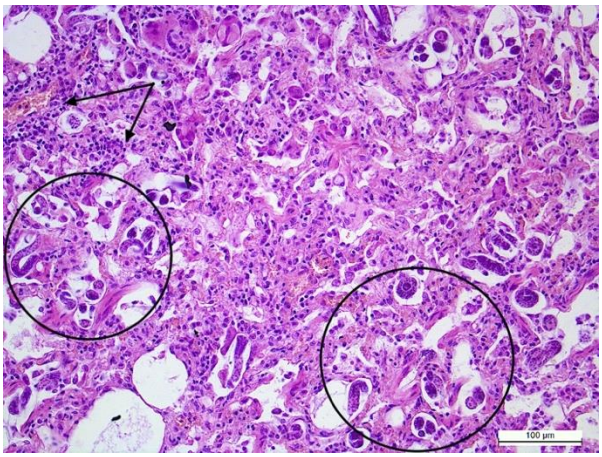

Figure 8. Lungs. Cross and longitudinal section of *Bunostomum trigonocephalum* larvae in the lumen of the alveoli. Cellular reaction (arrows). Hematoxylin and eosin, x200.

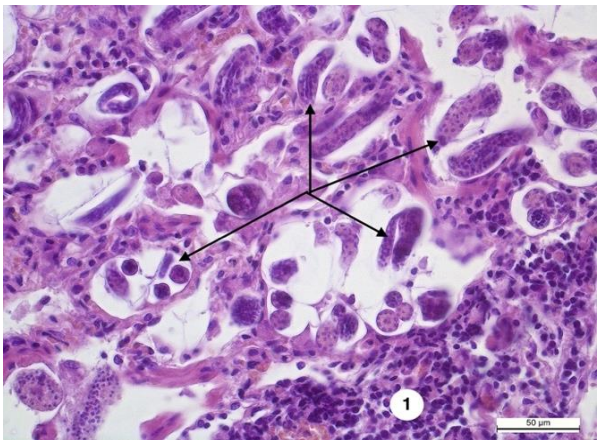

Figure 9. Lungs. Cell proliferation of lymphocytes, histiocytes, plasma cells, and macrophages (1) around alveoli with *Bunostomum trigonocephalum* larvae (arrows). Hematoxylin and eosin, x400.

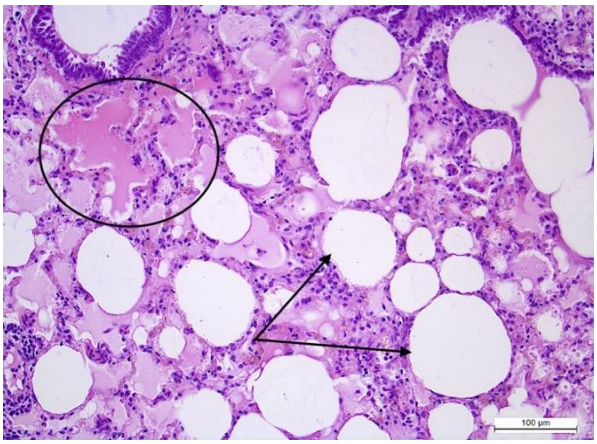

Figure 10. Pulmonary edema. Areas of emphysema (arrows). Hematoxylin and eosin, x200.

The pathogenic effect of helminths on the organism of sheep usually depends on the invasion intensity. It is associated with the changes in the absorption processes of amino acids, vitamins, proteins, and other essential nutrients, which leads to severe depletion of animals. The products of parasitic activity are toxic to the organism of sheep, cause violations in the organism's immune reactivity, and lead to general toxemia. Morphologically, perivascular edema was revealed in the lymph nodes; the organ's parenchyma is without a clear division into the paracortical, cortical and cerebral substances. Also, delymphatization of lymph nodes with reticular skeletal exposure and stroma edema, as well as a sharp decrease in lymphoblastic cells and reactive centers in secondary nodules, was noted (Figures 11 and 12).

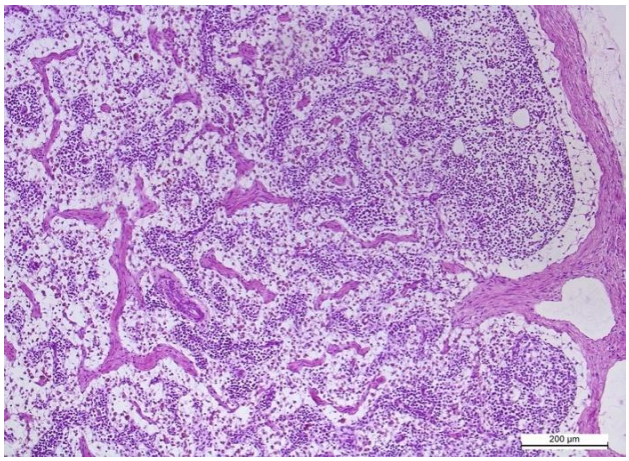

Figure 11. Lymph node. Delimphatization with reticular skeleton exposure. Hematoxylin and eosin, x100.

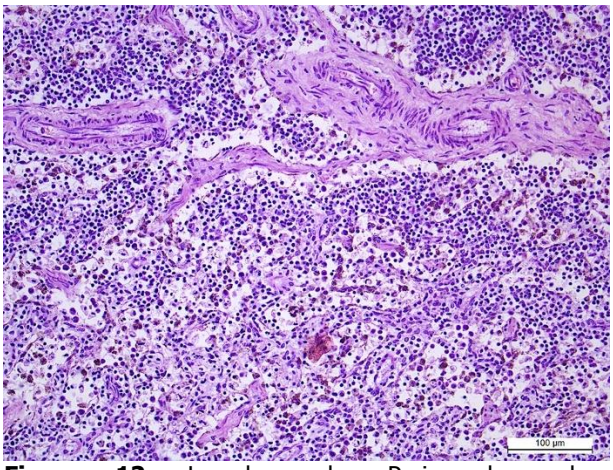

Figure 12. Lymph node. Perivascular edema. Hematoxylin and eosin, x200.

During the histological examination of the jejunum, morphological changes manifested themselves in the form of the mucosal plate's edema, dystrophic and necrobiotic changes in endocrinocytes pyknosis and lysis of the nuclei, as well as narrowing of intestinal crypts (Figure 13). Cylindrical villi epithelial cells are desquamated and necrotized. In the mucous membrane's connective tissue base, the proliferation of lymphocytes, histiocytes, plasma cells, and macrophages is observed (Figure 14). The fringed epithelial cells lost microvilli and their shape, which significantly affected the absorption of nutrients. In turn, cells' activity increased along with the amount of exocrinocyte mucus (Figure 15). The epithelial layer's basement membrane retained its morphological structure, while the muscular layer was slightly thickened. The mature bunostomosis is hematophagous and damaged the intestinal mucosa with the chitinous plates available in the oral cavity, causing diffuse capillary hemorrhage.

Parasites that entered the liver hematogenously or from the intestine caused the development of focal parenchymal hepatitis. Light red strands oriented in different directions were well visualized in the liver lobes. They indicated the routes of larvae migration, which caused damage to intraparticle capillaries and interparticle vessels with hemorrhage and perivascular edema and necrosis of hepatocytes (Figure 16-18).

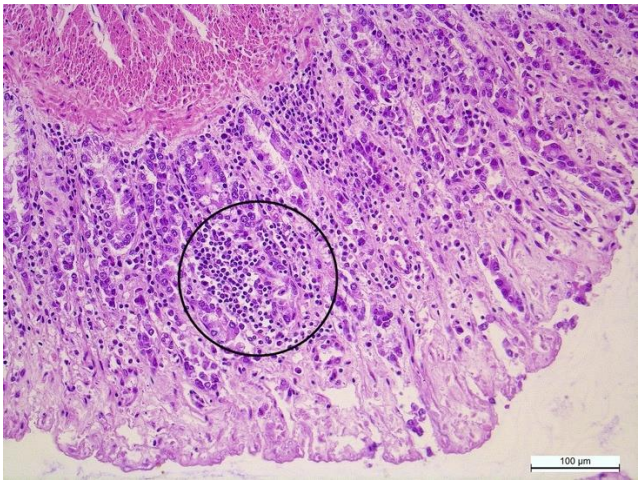

Figure 13. Empty intestine. Cellular infiltration of the mucous membrane plate by lymphocytes, histiocytes, plasma cells, macrophages, x 200.

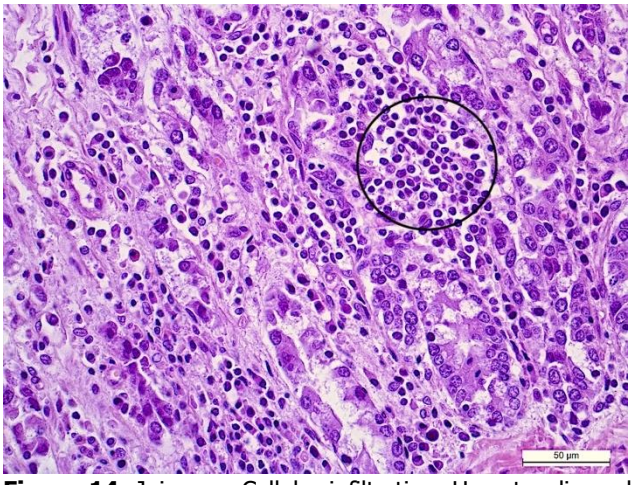

Figure 14. Jejunum. Cellular infiltration. Hematoxylin and eosin, x 400.

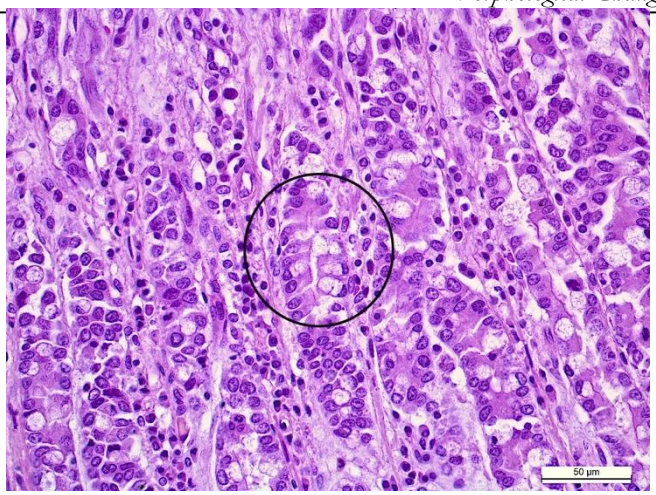

Figure 15. Jejunum. Increased activity of goblet exocrinocytes, x400.

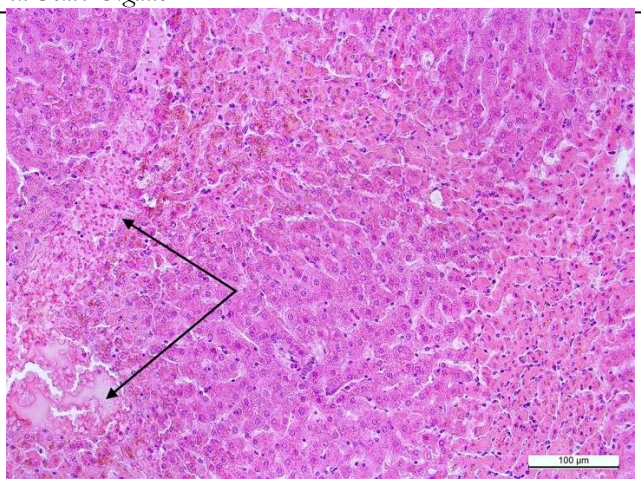

Figure 16. Liver. Massive hemorrhage (arrows). Hematoxylin and eosin, x200.

As a result of circulatory disorders and toxic effects of helminthic products, granular dystrophy developed in hepatocytes. These hepatocytes with dense oxyphilic cytoplasm, altered forms, and pyknotic nuclei were clearly distinguished on larval migration routes. Cellular infiltration composed of lymphocytes, eosinophils, and plasma cells is available around the central veins and bile ducts.

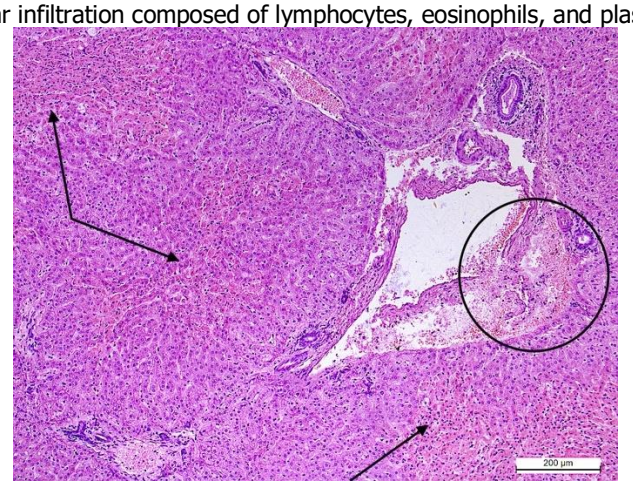

Figure 17. Liver. Focal necrosis of hepatocytes (arrows), perivascular edema. Hematoxylin and eosin, x200.

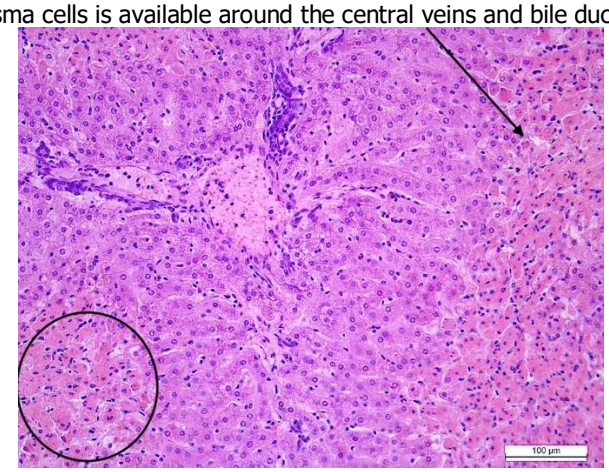

Figure 18. Liver. Necrosis of hepatocytes on the migration route of parasite larvae (arrow). Hematoxylin and eosin, x200.

Morphologically, the development of hyaline-drop dystrophy was noted in the renal tubules. The tubules were filled with eosinophilic protein cylinders, which contained desquamated nephrocytes, nephrocyte nuclei with the signs of karyopyknosis and karyorrhexis (Figures 19 and 20). In some proximal tortuous tubules, the nuclei of nephrocytes are enlarged and hyperchromic, while the cytoplasm is somewhat compacted and foamy in some cells. The nephron loop is lined with compacted elongated cells with a weak eosinophilic cytoplasm. In some cases, the epitheliocytes occurred that have undergone necrotic changes and desquamated into the tubules' lumen. The distal convoluted tubules' cells are with light or somewhat basophilic cytoplasm; the nucleus is displaced in the apical part of the cell, the brush border is absent. Necrotic changes of the epithelium of the distal convoluted tubules were detected in some locations. The Shumlyansky-Bowman capsule is stretched and filled with protein masses (Figure 19).

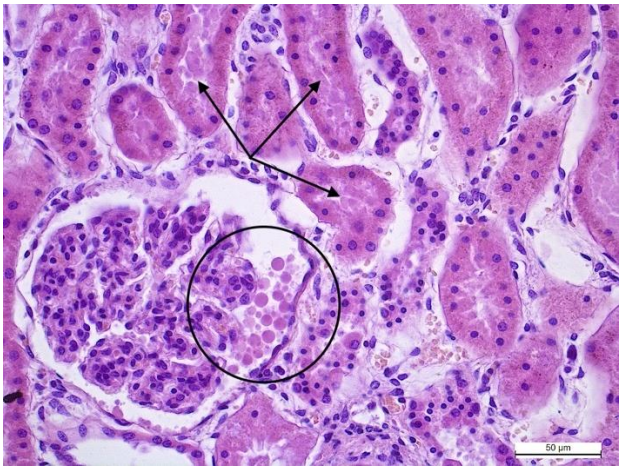

Figure 19. Kidney. Protein masses in the lumen of the tubules (arrows) and glomerulus. Hematoxylin and eosin, x400.

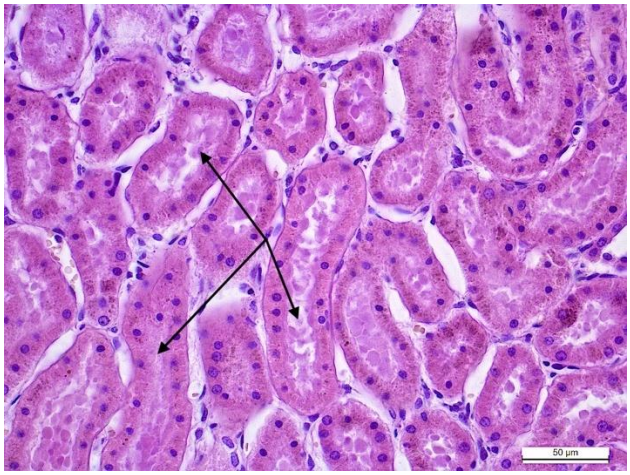

Figure 20. Kidney. Tubules filled with eosinophilic protein masses. Hematoxylin and eosin, x400.

Conclusions

The results of helminthoscopy and helminthoovoscopy revealed strongyle-type eggs and identified the adult helminths *Bunostomum trigonocephalum*.

The main clinical signs in sheep with acute helminthic Bunostoma invasion were represented by the manifestations of allergic reactions in the form of the subcutaneous tissue edema in the neck and chest area, pulmonary and heart failure, gastrointestinal tract disorders. General anemia is associated with the helminths' hematophagy. As a result of *Bunostomum trigonocephalum* larvae migration, a sensitization of an organism of sheep by its metabolism products occurred. It caused the violations in metabolic processes, pulmonary edema and thickening of interstitial fabric in larvae localization, dystrophic and necrobiotic changes in small intestine epithelium, delymphatization of the lymph nodules, and reduction of reactive centers in secondary nodes. Additionally, one of organ pathology factors under helminth infestation is inflammatory mediators' activation, resulting in increased vascular permeability, perivascular edema, and circulatory disorders. *Bunostomum trigonocephalum* larvae in sheep, passing the hepatopulmonary migration route, mechanically damaged the liver parenchyma, caused dystrophy and diffuse necrosis hepatocytes. The development of hyaline-drop dystrophy of the tubular nephrothelium in the nephron's cortical substance manifests secondary kidney damage.

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