

The experimental invasion of rats with *Eustrongylides excisus* (Nematoda, Dioctophymatidae) larvae during the acute course of infection

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Ten laboratory rats were experimentally invaded per os with the *Eustrongylides excisus* (Jägerskiöld, 1909) nematode larvae (L₃–L₄) received from *Perca fluviatilis* (Linnaeus, 1758), caught in the water area of the Dnipro-Buh Estuary, Ukraine. The aim of the experimental study was to measure the pathogenic effect of the given parasite on the mammals' organisms by means of the histological analysis of the affected organs of the infected rats. The researched animals were divided into two groups. The experiment lasted 15 days; the second group was a control group. The histological analysis showed purulent-serous and purulent-fibrinous peritonitis, acute circulatory disorders in brain tissues (signs of hypoxic-ischemic lesions of the brainstem in the form of neuronal changes) and the heart (partial spasm of arteries and edema of interstitial myocardial tissue); pathological changes in the lungs (acute interstitial purulent pneumonia, acute focal emphysema of the lungs), liver (acute exudative hepatitis), kidneys (signs of shock reaction) and intestines (acute serous-purulent enteritis). Formation of non-specific granulomas was noticed, which contained the fragments of the parasitic larvae. The acute course of the purulent-serous and purulent-fibrinous inflammatory processes in the organs of the invaded rats was noticed; signs of systemic inflammatory response syndrome with pronounced distributive vascular leukocytosis in the studied organs.

Keywords: eustrongylidosis; parasitic diseases; zoonotic parasite; histological analysis; acute course.

Introduction

The trophic connection of two organisms of different types, during which the nutrition of one of them depends on the other one is considered a classic parasitism, as the host is considered an environment containing all the necessary resources for the parasite (Boyko & Brygadyrenko, 2017, 2019). This type of connection leads to pathophysiological processes and the corresponding immune response, which requires the host's organism to regenerate the damaged organs and tissues (McDougald, 2019). Dioctophymatidae nematode larvae, more specifically *Eustrongylides excisus* (Jägerskiöld, 1909) are the parasites of this kind (Jägerskiöld, 1909).

Eustrongylides excisus nematode larvae are potentially threatening for human health (Ljubojevic et al., 2015). The species was initially described by Jägerskiöld in 1909 while studying the nematodes found in the glandular stomach of researched cormorants (Jägerskiöld, 1909).

Eustrongylides spp. is considered a zoonotic parasite, meaning that it is potentially dangerous for humans. The invasion is transmitted through consumption of insufficiently heat-treated fish and fish products (Witner et al., 1989; Narr et al., 1996).

This nematode species is widespread in both natural and artificial aquatic areas of Europe, North and South America, etc. *Eustrongylides excisus* has been registered in Serbia, Romania, Turkey, Brazil, the USA, Italy, Iran, Azerbaijan, Czech Republic, Russia and Ukraine (Lichtenfels & Stroup, 1985; Pazooki et al., 2007; Novakov et al., 2013; Soylu, 2013; Fedorov et al., 2014; Melo et al., 2015; Noei et al., 2015; Branciani et al., 2016; Goncharov et al., 2018). The nematode *Eu. excisus* is a typical biohelminth with a complicated development cycle. Fish-eating water birds from such genera as Ciconiiformes, Anseriformes, Gaviiformes and

Pelecaniformes mostly serve as the main definitive hosts (Novakov et al., 2013). Bridging hosts are usually oligochaetes of the Tubificidae and Lumbriculidae bloodlines, in the organisms of which the parasites go through the first and the second larval stages of their development (Karmanova, 1968; Lichtenfels & Stroup, 1985; Spalding et al., 1993).

Regarding absence of any scientific data on histological analysis of pathogenic material samples from animals infected in the laboratory with the eustrongylidosis agent and suffering the acute course of the disease, we conducted an experimental invasion of the rats with the *Eu. excisus* nematode larvae with an aim of conducting a detailed analysis of the pathological impact of the parasite, which is extremely widespread in the world and potentially dangerous for human health.

Material and methods

The research was conducted in accordance with the Council of Europe's Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18 March 1986, Directive 2010/63 the European Parliament and the Council of Europe of 22 September 2010 on the Protection of Animals used for Scientific Purposes and the Law of Ukraine of February 21, 2006 No. 3447-IV (as amended on June 22, 2017 No. 2120-VIII) "On protection of animals from cruel treatment". The given protocol development is approved by the bioethics commission of the National University of Life and Environmental Sciences of Ukraine (the conclusion of the bioethics commission No. 143/21 of April 5, 2021). The experimental analysis was conducted on ten non-lineal albino laboratory rats (*Rattus rattus*) of the same age (3.5 months) with a body weight of 190–230 g. The animals were kept

separately in cages with perforated bottoms to avoid coprophagy. The access to food and water was ad libitum. Before the experimental invasion the animals were subjected to the clinical and coprological analyses.

Ten animals were invaded per os with the *Eu. excisus* nematode larvae (L₃-L₄), 10 samples for each, using the neotological feeding catheter of the CH08 type connected to a 2 mL injector. The parasites were taken from perch (*Perca fluviatilis* (Linnaeus, 1758), caught in the waters of the Dnipro-Buh Estuary (Ukraine). 10% of the selected nematodes were fixed in a special solution and prepared for further analysis in order to define their taxonomy (Karmanova, 1968). Three rats were kept for control.

Observations lasted 15 days and when the experimental term expired the animals were subjected to euthanasia by intraperitoneal injection of the sodium thiopental solution (0,015 g for 1 kg of the body weight) followed by a pathological anatomical autopsy.

The internal organs of the rats subjected to euthanasia (brain, heart, lungs, liver, gastrointestinal tract organs) were fixed in 10% neutral formalin, then fragmented into pieces, subjected to alcohols of increasing concentration and filled with paraffin in accordance with the commonly used method. Paraffin sections with a thickness of 5–7 μm were made using a Leica SM 2000 R microtome and coloured with hematoxylin and eosin. Microscopic examinations were performed using an Olympus BX41 microscope (x40 lens, x10 eyepiece).

Results

Observation of the cerebellum with the area of the brain stem showed that the cerebellum structure was damaged, most vessels contained no blood, particular vessels of the cerebellum were characterized with irregular blood supply. The walls of the blood vessels and their perivascular spaces were slightly permeated with small clusters of erythrocytes. Cerebellar substance was unevenly loosened, pear-shaped neurons were swollen, some pericellular spaces were extended (Fig. 1).

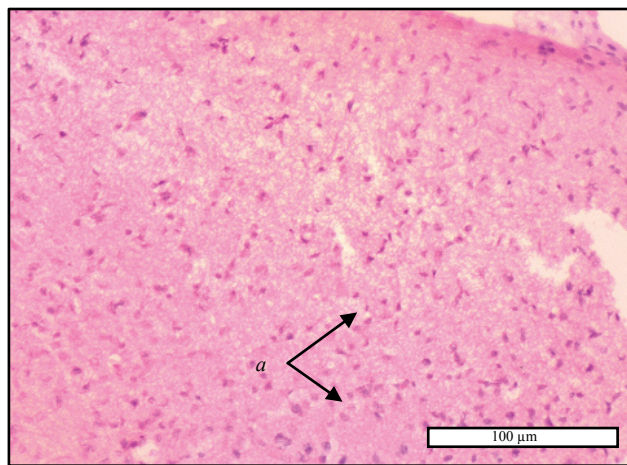


Fig. 1. Uneven loosening of the brain neuroglia substance (a)

Soft cerebellar membranes were partially preserved in the form of small pieces, the regular membrane vessels did not contain blood. The brainstem contained vessels with irregular blood supply, some vessels contained groups of leukocytes. The substance of the brain was unevenly loosened, the neurons were swollen; groups of neurons were in the form of shadow cells. Neuronophagia, glia activation, sharp loosening of subpial marginal fragments of brainstem substance were fixed from time to time. Thus, morphological signs of hypoxic-ischemic lesions of the brainstem in the form of neuron changes (neuronophagia, swelling of neurons, shadow cells) and edema of microneuroglia, as well as cerebellum swelling, were found (Fig. 2).

Histological analysis of the heart showed that the epicardium was loose and swollen, particular veins and arteries contained groups of clumped erythrocytes. Often the epicardium was infiltrated by lymphocytes, single macrophages, groups of leukocytes, single plasma cells. Myocardium vessels were characterized with irregular blood supply, groups of leukocytes were found in some cases. The walls of some parti-

cular myocardial veins and their perivascular spaces were unevenly full of loose and dense clusters of erythrocytes, intermediate and perivascular tissues were unevenly loosened. Particular arteries of the myocardium were partially spasmed, the cores in their walls were reoriented. Cardiomyocytes of normal size and uneven colouring were found, in some cases the groups of cardiomyocytes were fragmented. In some points of observation the groups of cardiomyocytes with homogenized cytoplasm were found. They were not diametrically segmented, their cores were not visualized or very pale, in a shadow form. It means that in case of the experimental invasion of the laboratory rats with the eustrongylidosis agent it was possible to notice morphological signs of acute cardiovascular insufficiency in the form of acute ischemic changes in the myocardium; acute circulatory disorders in the vessels of the myocardium in the form of partial spasm of the myocardial arteries, edema of the interstitial tissue of the myocardium; focal fragmentation of cardiomyocytes; acute purulent inflammation of the epicardium (acute purulent pericarditis) (Fig. 3).

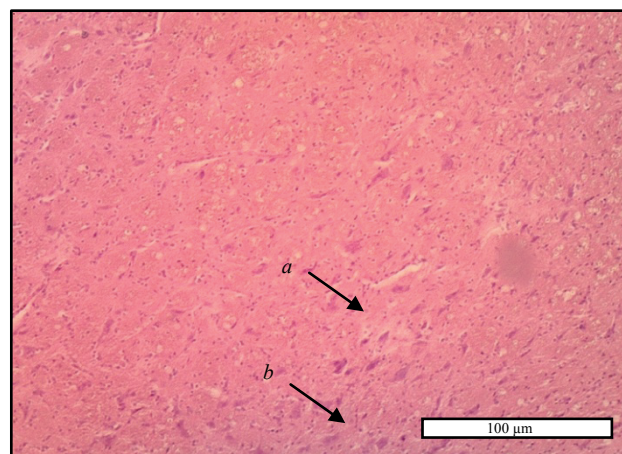


Fig. 2. Swelling of brain neurons (a) and neurobiosis (b)

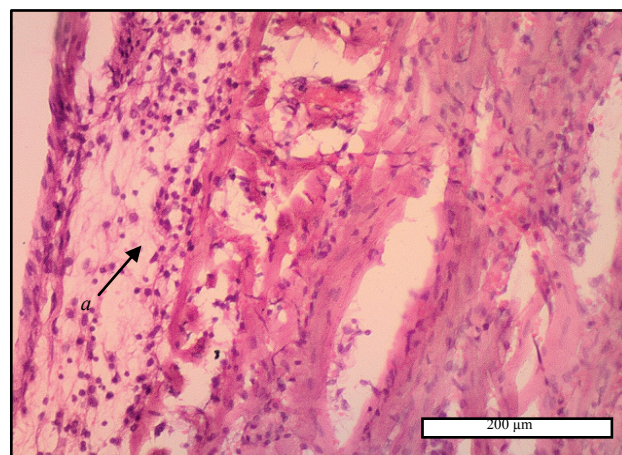


Fig. 3. Symptoms of the acute purulent endocarditis: heart tissues are significantly infiltrated with leukocytes (a)

Observation of eustrongylidosis affecting the lungs found that the vessels of the stroma and interalveolar segments were characterized with irregular blood supply, the vessel wall and their perivascular spaces were slightly permeated with loose clusters of erythrocytes in some cases. The walls of single arteries, stroma and perivascular spaces were filled with fibrin (fibrinoid swelling of vascular walls). Groups of leukocytes found in the stroma vessels, interstitium of the lungs were characterized with signs of uneven leukocyte infiltration. A significant loosening of the perivascular and peribronchial stroma was noted. Histological section analysis showed dysteclastasis of some alveoli, while other alveoli were significantly emphysematically extended, up to the interalveolar membranes being torn apart with small hemorrhages in the places of damage. A small group of alveoli was in condition of decrease (atelectasis). Some little bronchi were in spasm, in some part of them the epithelium was

desquamated. In the lumen of single small bronchi pink mucus was found. In particular points of observation hemorrhages were shown, characterized with tight groups of erythrocytes with no cellular reaction. The middle bronchi epithelium was partly desquamated, bronchi were partly in spasm, the peribronchial stroma was unevenly infiltrated with lymphocytes, leukocyte groups, single macrophages, and plasma cells. Thus, the following disorders were registered with eustrongylidosis in rats: acute interstitial purulent pneumonia, acute circulatory disorders in the pulmonary vessels with peribronchial and intraalveolar hemorrhages, acute focal emphysema of the lungs, small focal atelectasis of the alveoli and edema of the particular stroma vessels (Fig. 4, 5).



Fig. 4. The symptoms of the acute interstitial pneumonia and purulent pleurisy: lung tissues are significantly infiltrated with leukocytes (a)



Fig. 5. The symptoms of the acute interstitial pneumonia: conglomeration of a significant amount of leukocytes is noted in the lung vessels (a)

As for kidneys of rats with eustrongylidosis, the capsule remained as usual, with no atypical characteristic. Most cortex vessels contained no blood, only in some of them could groups of erythrocytes be found. The mesangial glomeruli capillaries were characterized with irregular blood supply. Medullary vessels were characterized with irregular blood supply as well as shunting of vessels of a juxtamedullary site, with small perivascular hemorrhages. Groups of leukocytes were found in particular arteries of the juxtamedullary area. The cortical nephrothelium was unevenly swollen, mostly with granular cytoplasm, the cores were unchanged. It was found that in some tubules of the juxtamedullary area the nephrothelium cytoplasm contained vacuoles; the cores of some cells of the epithelium of the tubules were either not visualized or shadow-formed. Morphological signs of shock reaction in the kidneys in the form of shunting of vessels of the juxtamedullary area, anemia of the vessels of the cortical layer and necrotic changes of the epithelium of the tubules of the juxtamedullary area were registered in the research animals with eustrongylidosis (Fig. 6).



Fig. 6. Vessel shunting in the juxtamedullary area of the kidneys (a)

Histological analysis of the liver showed that the capsule remained as usual. The veins of portal tracts and triads, as well as sinusoidal capillaries, were characterized by irregular blood supply. Groups of leukocytes were found in some particular veins and sinusoidal capillaries. In particular veins of triads the blood was separated, meaning that the liquid part of the blood was separate from the erythrocytes. Perisinusoidal space (Disse's space) was extended. The hepatic lamina structure was not damaged, hepatocytes were granular, their cores were unchanged. Some groups of hepatocytes were swollen with no clearly defined counters. Their membranes and partly their cytoplasm had signs of necrotic changes; their cores were large and light, containing chromatin and clearly visualized nucleoli. Therefore, in case of eustrongylidosis the following conditions were found: acute exudative hepatitis; necrotic changes in the cytoplasm of hepatocyte groups, as well as edema of the liver parenchyma (Fig. 7).

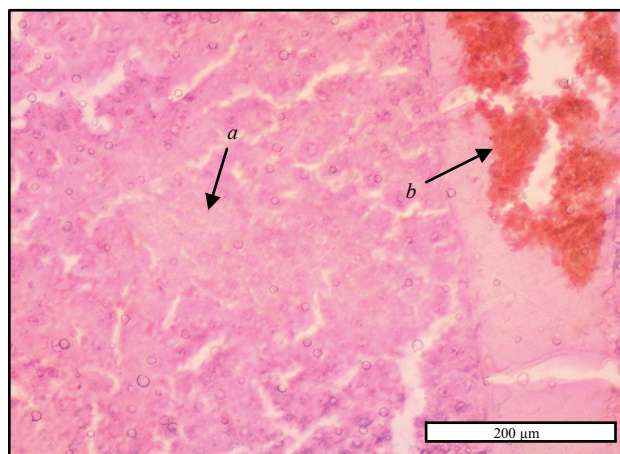


Fig. 7. Symptoms of the hepatocyte necrosis (a) and blood dissociation in the liver vessels (b)

Histological analysis of the small intestine showed that all of its layers were kept in place. The vessels of the submucosa and lamina propria mucosae were filled with blood unevenly. The epithelium of the glands was significantly swollen, with unevenly coloured granular cytoplasm having unchanged or pale-coloured cores. The upper parts of the mucous layer had necrotic changes and were partly desquamated. The interstitium of the mucous layer showed an uneven infiltration with groups of leukocytes, lymphocytes, single macrophages and eosinophils, as well as groups of plasma cells. There were no atypical characteristics in the muscular layer. The vessels of the muscular layer and the serous membrane contained no blood. The serous membrane was unevenly infiltrated with leukocytes, lymphocytes and plasma cells. The adipose tissue close to the serous layer contained particular vessels of irregular blood supply, the tissue was unevenly infiltrated with lymphocytes, leukocytes, plasma cells, single eosinophils. Fibrin deposition was observed in places perivascularly, and vessel walls were full of fibrin threads. Most of the vessels did not contain fat. Thus, acute serous-purulent enteritis, acute purulent inflammation of the mesentery and serous membrane of the small intestine

(acute purulent peritonitis) were observed in rats experimentally invaded with the eustrongylidosis agent (Fig. 8, 9).

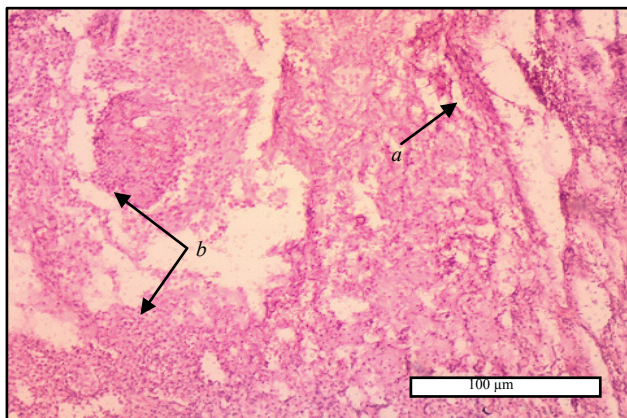


Fig. 8. Symptoms of the purulent peritonitis: fibrin deposition (a) and significant leukocyte infiltration of the mesentery of the small intestine (b)



Fig. 9. Purulent inflammation of the serous membrane of the small intestine (a) and of the mesentery tissue (b): symptoms of the purulent peritonitis

Also, abscesses were noted in the soft tissues of the abdominal wall, from the majority of the observation points they were not observed to have capsules. The abscesses were shown in the form of dense clusters of leukocytes, cores of destroyed leukocytes and fibrin threads. In some observation points of the perifocal abscess sites it was possible to notice the capsule formation in the form of gentle accumulations of fibroblasts.

Discussion

Mammals, including humans, are usually accidental hosts for the *Eustrongylides* nematode larvae (Karmanova, 1968). Studying the pathological impact of the *Eustrongylides* spp. (*E. ignotus* (L₄) nematode larvae on experimentally invaded rabbits, Shirazian et al. (1984) and Barros et al. (2004) noted the inflammatory processes in the organs of the abdominal cavity area: peritonitis and granuloma formation on the liver surface. The authors report hyperemia of the gastric mucosa with areas of necrosis in the center; abscesses in the abdominal cavity and parasites in the chest cavity. Histological analysis showed an intensive inflammatory reaction in the stomach wall with predominance of eosinophils in the tissues, damage to the mucosal and submucosal layer, hemorrhages, necrosis and inflammatory infiltrate in the muscle layer. In the thickness of the abdominal wall, mixed inflammatory reactions with a predominance of mononuclear cells, necrosis and abscesses were noted (Shirazian et al., 1984; Barros et al., 2004). It should be noted that the studies of Shirazian et al. (1984) established the pathological changes in rabbits invaded with *E. ignotus* nematode larvae, but neither noticeable clinical symptoms nor death of the animals was registered (Shirazian et al., 1984). Meanwhile Barros et al. (2004), conducting analogical research, noticed clinical symptoms and deaths of the rabbits affected by eustrongylidosis (Barros et al., 2004).

In our previous studies on experimental infestation of laboratory rats with larvae of the nematode *Eu. excisus*, we recorded changes in the clinical condition of laboratory rats: decreased appetite and motility, suppression of the general condition, tachypnea, abdominal wall pain. At pathological and anatomical autopsy serous-fibrinous and purulent-fibrinous peritonitis, perforations of the wall of the gastrointestinal tract, inflammatory phenomena of the wall of the stomach and intestines were noted. The presence of pathology of the liver, kidneys and thoracic organs was also noted. Larvae, both living and having no vital signs, were found in the intestinal lumen and directly in the abdominal cavity (Honcharov, 2020).

Inflammation is a key protective mechanism of the host's organism in the case of the parasitic invasion and development of the pathological process (Guseva et al., 2019). Histological analysis of the biological material taken from the infected rats showed pathological changes in organs and tissues with signs of general inflammatory response syndrome. This fact is defined by the peculiarity of the pathogenesis in the case of eustrongylidosis.

When the eustrongylidosis agent gets into the definitive host's organism the parasitic larva reaches the wall of the gastrointestinal tract. During the invasion the parasite significantly mechanically damages tissues and perforates the walls of the hollow organs causing hemorrhages and inflammatory processes in the peritoneum (Karmanova, 1968). Under the natural conditions such processes were commonly described among fish-eating birds. In particular, during the pathological and anatomical autopsy of cormorants suffering from eustrongylidosis, the nematodes were found into the wall of the glandular stomach of birds. The invasion caused hemorrhages and ulceration. Inflammatory reactions with granulomatosis of different levels could be viewed microscopically. Degenerated parasites were found deep in the wall of the gastric tract, surrounded by the fibrous structures of the connective tissue (El-Dakhly et al., 2012). The fish-eating birds with the high invasion rates had the gastric tract perforations and the symptoms of peritonitis, which were often lethal. The signs of the intestinal obstruction with this type of parasites were noted as well (Franson & Cluste, 1994). Cole (1999) describes the gastrointestinal tract wall perforations and as a result of it – damage to the hollow organ (peritonitis). The researcher also notes that there were fibrinous granulomas with a necrotic center and nematodes inside (Cole, 1999).

Our study shows that the pathological impact of the eustrongylidosis agent is achieved mainly by means of perforation of the gastrointestinal tract, which leads to the intestinal content and the parasites penetrating into the abdominal cavity, mechanic damage to the parenchymal tissues and severe toxicity. All of the above leads to the acute pathological conditions, in part: serous-fibrinous and purulent-fibrinous inflammation of the peritoneum with a significant necrotic component. A severe toxic condition which occurred and developed in the organisms of the rats during the course of the disease has a general impact on the organs and tissues of the animals, which in part can be confirmed by the pathological changes in the cells of the liver, the heart, the kidneys etc. (necrotic changes of the cytoplasm, cell membrane loss, cell fragmentation, reorientation of the cores).

Inflammation is a general protective and adaptive process, evolutionarily created as a response to the damage of different nature aimed at localization and elimination of the damaging factor with a following regeneration or reparation of the damaged tissues (Guseva et al., 2019).

Observing eustrongylidosis in laboratory rats we described the formation of nonspecific granulomas localized in the tissues of the abdominal cavity. The study of the granulomas showed the severely destructed parasitic larvae inside them.

Some researchers believe that the living strategy of the parasites is defined by affecting different physiological aspects in the host's body in order to activate different immunological factors (Rohlenová et al., 2011). Formation of capsules and granulomas is a result of the mutual adaptation of the immune systems of the host and the parasite and the element of the strategic compromise between the two organisms for their survival (Bruschii & Chiumiento, 2011).

Formation of parasitic capsules and granulomas is a typical symptom of eustrongylidosis, which is especially common for the second bridging hosts of this parasitic species – fishes. The pathological-anatomical analysis of the fish infected with *Eustrongylides* spp., nematode larvae, accor-

ding to Goncharov et al. (2018), showed signs of inflammation around encapsulated larvae: redness, compaction of surrounding tissues and edema. The inflammatory processes were significantly severe in the tissues of the mesentery and muscles (Goncharov et al., 2018). Histological analysis of the muscular tissues of *Perca fluviatilis* (Linnaeus, 1758) showed that the nematode larvae were encapsulated. The centers of necrosis were noted directly at the parasitic penetration spots. The muscular tissue was degenerated. The ends of spindle-shaped capsules were characterized by the massive macrophage aggregation, while formation of multifocal fibro-epithelial granulomas was observed in the capsule thickness (Dezfuli et al., 2015). Histological analysis of the tissues of *Galaxias maculatus* (Jenyns, 1842) invaded with *Eustrongylides* spp., nematode larvae showed the symptoms of the compressive tissue atrophy and replacement of the muscles of the connective tissue element. Chronic inflammatory process with fibrous capsule formation and intensive melanization of surrounding tissues was noted (Guagliardo et al., 2019). Histological analysis of the liver (hepatopancreas) of the *Gymnotus* fishes showed significant destructive changes in hepatocytes, which are typical for the chronic course of the disease, alternating areas of intense iron accumulation with areas of unaltered tissue were registered, as well as the apoptosis and necrosis areas (Ventura et al., 2016).

Cases of the invasion of humans by eustrongylidosis agents were also registered when people consumed fish and fish products infected by the agent that were not preliminary subjected to heating at the necessary level (Guerin et al., 1982; Gunby, 1982; Eberhard et al., 1989; Wittner et al., 1989; Narr et al., 1996). These invasions were characterized by gastritis, intestinal wall perforations and the formation of nonspecific subcutaneous granulomas (Panesar & Beaver, 1979; Deardorff & Overstreet, 1991; Eberhard & Ruiz-Tiben, 2014).

Considering that fact that the *E. excisus* nematode is able to invade many species as hosts (including humans), that the parasite is very widespread around the world and there is insufficient scientific data concerning the histological pathological analysis of mammals invaded with *Eu. excisus* nematode larvae, the given paper is relevant and it is of a significant scientific interest. The conducted study allows us to value the pathological impact of the *Eu. excisus* nematode larvae on the organism in the case of invasion and the mechanism of the disease development considering the biological characteristics of the agent. In part during the migration process the parasite mechanically damages the tissues and the organs of the abdominal cavity and perforates the walls of the organs, which leads to severe pathological conditions, such as hemorrhages, peritonitis and septicemia.

Regarding all of the above it is possible to conclude that the laboratory rats affected by eustrongylidosis show the morphological signs of the systemic inflammatory response syndrome, as evidenced, in particular, by distributive vascular leukocytosis of the internal organs of animals.

Conclusion

Histological analysis of the pathological material of the laboratory rats experimentally invaded with *Eu. excisus* nematode larvae showed the signs of systemic inflammatory response syndrome with severe distributive vascular leukocytosis of the studied organs. The pathological changes in the organism of mammals (laboratory rats) invaded with *Eu. excisus* nematode larvae – a parasite which is typically zoonotic, meaning that it is potentially dangerous for human health – were described for the first time. The changes were characterized by purulent-serous and purulent-fibrinous type of inflammatory process, acute circulatory disorders in parenchymal organs and the formation of nonspecific granulomas. The development of intoxication and septicemia in rats led to pathological changes in the brain, heart, organs of the gastrointestinal tract and respiratory tract, liver, kidneys, etc.

The authors declare the absence of any conflict of interest.

References

Barros, L. A., Tortelly, R., Pinto, R. M., & Gomes, D. C. (2004). Effects of experimental infections with larvae of *Eustrongylides ignotus* Jägerskiöld, 1909 and

- Contraecum multipapillatum* (Drasche, 1882) Baylis, 1920 in rabbits. *Arquivos Brasileiros de Medicina Veterinária e Zootecnia*, 56, 325–332.
- Boyko, A. A., & Brygadyrenko, V. V. (2017). Changes in the viability of the eggs of *Ascaris suum* under the influence of flavourings and source materials approved for use in and on foods. *Biosystems Diversity*, 25(2), 162–166.
- Boyko, O. O., & Brygadyrenko, V. V. (2019). Nematocidal activity of aqueous solutions of plants of the families Cupressaceae, Rosaceae, Asteraceae, Fabaceae, Cannabaceae and Apiaceae. *Biosystems Diversity*, 27(3), 227–232.
- Branciarì, R., Ranucci, D., Miraglia, D., Valiani, A., Veronesi, F., & Urbani, E. (2016). Occurrence of parasites of the genus *Eustrongylides* spp. (Nematoda: Dioctophymatidae) in fish caught in Trasimeno Lake, Italy. *Italian Journal of Food Safety*, 6130, 206–209.
- Bruschi, F., & Chiumiento, L. (2011). *Trichinella* inflammatory myopathy: Host or parasite strategy? *Parasit and Vectors*, 4, 42.
- Cole, R. A. (1999). Eustrongylidosis. In: *Field manual of wildlife diseases: General field procedures and diseases of birds*. U.S. Department of the interior fish and wildlife service, Washington. Pp. 223–228.
- Deardorff, T. L., & Overstreet, R. M. (1991). Sea food transmitted zoonoses in the United States: The fishes, the dishes, and the worms. In: Ward, D. R., & Hackney, C. R. (Eds.). *Microbiology of marine food products*. Van Nostrand Reinhold, New York. Pp. 211–265.
- Dezfuli, B. S., Manera, M., Lorenzoni, M., Pironi, F., Shinn, A. P., & Giari, L. (2015). Histopathology and the inflammatory response of European perch, *Perca fluviatilis* muscle infected with *Eustrongylides* sp. (Nematoda). *Parasit and Vectors*, 8, 227.
- Eberhard, M. L., & Ruiz-Tiben, E. (2014). Cutaneous emergence of *Eustrongylides* in two persons from South Sudan. *American Journal of Tropical Medicine and Hygiene*, 90, 315–317.
- Eberhard, M. L., Hurwitz, H., Sun, A. M., & Coletta, D. (1989). Intestinal perforation caused by larval *Eustrongylides* (Nematoda: Dioctophymatoidea) in New Jersey. *American Journal of Tropical Medicine and Hygiene*, 40, 648–650.
- El-Dakhly, K. M., El-Nahass, E., Uni, S., Tuji, H., Sakai, H., & Yanai, T. (2012). Levels of infection of gastric nematodes in a flock of great cormorants (*Phalacrocorax carbo*) from Lake Biwa, Japan. *Journal of Helminthology*, 86, 54–63.
- Fedorov, N. M., Firsov, N. F., & Solovyov, N. A. (2014). Veterinary and sanitary examination of river perch with eustrongylidosis [Veterinarno-sanitarnaya ekspertiza rechnogo okunya pri eustrongilidoze]. *Veterinarnaya Patologiya*, 3–4, 68–73 (in Russian).
- Franson, J. C., & Cluste, T. W. (1994). Eustrongylidosis in wading birds from colonies in California, Texas, and Rhode Island, USA. *Colonial Waterbirds*, 17(2), 168–172.
- Goncharov, S. L., Soroka, N. M., Pashkevich, I. Y., Dubovy, A. I., & Bondar, A. O. (2018). Infection of predatory fish with larvae of *Eustrongylides excisus* (Nematoda, Dioctophymatidae) in the delta of the Dnipro River and the Dnipro-Buh Estuary in Southern Ukraine. *Vestnik Zoologii*, 52(2), 137–144.
- Guagliardo, S., Viozzi, G., & Norma, B. (2019). Pathology associated with larval *Eustrongylides* sp. (Nematoda: Dioctophymatoidea) infection in *Galaxias maculatus* (Actinopterygii: Galaxiidae) from Patagonia, Argentina. *International Journal for Parasitology: Parasites and Wildlife*, 10, 113–116.
- Guerin, P. F., Marapendi, S., & Grail, S. L. (1982). Intestinal perforation caused by larval *Eustrongylides* – Maryland. *Morbidity and Mortality Weekly Report*, 31, 383–384.
- Gunby, P. (1982). One worm in the minnow equals too many in the gut. *Journal American Medical Association*, 248, 163.
- Guseva, E. Y., Zhuravleva, Y. A., & Zotova, N. V. (2019). Correlation of immunity evolution and inflammation in vertebrates [Korrelatsiya evolyucii immuniteta i vospaleniya u pozvonochnyh]. *Uspehi Sovremennoj Biologii*, 139(1), 1–16 (in Russian).
- Honcharov, S. L. (2020). Pathological anatomic changes among laboratory rats in case of experimental infection with the larvae of the nematode *Eustrongylides excisus* (Nematoda: Dioctophymatidae). *Animal Biology*, 22(1), 30–37.
- Jägerskiöld, L. A. (1909). Zur Kenntnis der Nematoden-Gattungen *Eustrongylides* und *Hystrichis*. *Nova Acta Regiae Societatis Scientiarum Upsaliensis*, 4(2), 3 (in German).
- Karmanova, E. M. (1968). Dioctophymidea of animals and man and the diseases caused by them [Dioktiffimidei zhivotnyh i cheloveka i vyzyvaemye imi zabolevaniya]. *Nauka, Moscow* (in Russian).
- Lichtenfels, J. R., & Stroup, C. F. (1985). *Eustrongylides* sp. (Nematoda: Dioctophymatoidea): First report of an invertebrate host (Oligochaeta: Tubificidae) in North America. *Proceedings of the Helminthological Society of Washington*, 52(2), 320–323.
- Ljubojevica, D., Novakov, N., Djordjevic, V., Radosavljevic, V., & Pelica, M. (2015). Potential parasitic hazards for humans in fish meat. *Procedia Food Science*, 5, 172–175.
- McDougald, L. R. (2019). Internal parasites. In: Swayne, D. E. et al. (Eds.). *Diseases of poultry*. Wiley. Pp. 1157–1191.
- Melo, F. T., Melo, C. S., & Nascimento, L. C. (2015). Morphological characterization of *Eustrongylides* ssp. larvae (Nematoda, Dioctophymatoidea) parasite of

- Rhinella marina* (Amphibia: Bufonidae) from Eastern Amazonia. *Brazilian Journal of Veterinary Parasitology*, 25(2), 7–12.
- Narr, L. L., O'Donnell, J. G., Libster, B., Alessi, P., & Abraham, D. (1996). Eustrongylidiasis – a parasitic infection acquired by eating live minnows. *Journal of the American Osteopathic Association*, 96, 400–402.
- Noei, M. R., Ibrahimov, S., & Sattari, M. (2015). Parasitic worms of the Persian sturgeon, *Acipenser persicus* Borodin, 1897 from the southwestern shores of the Caspian Sea. *Iranian Journal of Ichthyology*, 2, 287–295.
- Novakov, N., Bjelic-Cabrilo, O., Circovic, M., Jubejevik, D., & Lujic, J. (2013). Eustrongylidosis of european catfish (*Silurus glanis*). *Bulgarian Journal of Agricultural Science*, 1, 72–76.
- Panesar, T. S., & Beaver, P. C. (1979). Morphology of the advanced-stage larva of *Eustrongylides wenrichi* Canavan 1929, occurring encapsulated in the tissues of amphiuma in Louisiana. *Journal of Parasitology*, 65(1), 96–104.
- Pazooki, J., Masoumian, M., Yahyazadeh, M., & Abbasi, J. (2007). Metazoan parasites from freshwater fishes of northwest Iran. *Journal of Agriculture, Science and Technology*, 9, 25–33.
- Rohlenová, K., Morand, S., Hyršl, P., Tolarová, S., Flajšhans, M., & Šimková, A. (2011). Are fishimmune systems really affected by parasites? An immunoeological study of common carp (*Cyprinus carpio*). *Parasit and Vectors*, 4, 120.
- Shirazian, D., Schller, E. L., Glaser, C. A., & Vondeerfecht, S. L. (1984). Pathology of larval *Eustrongylides* in the rabbit. *Journal of Parasitology*, 70(5), 803–806.
- Soylu, E. (2013). Metazoan parasites of perch *Perca fluviatilis* L. From Lake Siğircı, Ipsala, Turkey. *Pakistan Journal of Zoology*, 45(1), 47–52.
- Spalding, M. G., Bancroft, G. T., & Forrester, D. J. (1993). The epizootiology of eustrongylidosis in wading birds (Ciconiiformes) in Florida. *Journal of Wildlife Diseases*, 29, 237–249.
- Ventura, A. S., Ishikawa, M. M., Gabriel, A. M. A., Silbiger, H. L. N., Cavichiolo, F., & Takemoto, R. M. (2016). Histopathology from liver of tuvira (*Gymnotus* spp.) parasitized by larvae of nematodes. *Ciencia Rural*, 47, 1233–1239.
- Wittner, M., Tumer, J. W., Jacquette, G., Ash, R. L., Salgo, M. P., & Tanowitz, H. B. (1989). Eustrongylidiasis – a parasitic infection acquired by eating sushi. *The England Journal of Medicine*, 320, 1124–1126.