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HISTOPATHOLOGICAL CHANGES IN THE ORGANISM OF RATS UNDER THE CHRONIC COURSE OF EXPERIMENTAL INFECTION WITH LARVAE OF *EUSTRONGYLIDES EXCISUS* (NEMATODA : DIOCTOPHYMATIDAE)

S. L. Honcharov [1], N. M. Soroka [1], M. V. Galat [1], I. Yu. Pashkevich [1], R. O. Slobodian [1], A. I. Dubovyi [2], O. P. Lytvynenko [3]

[1] National University of Life and Environmental Sciences of Ukraine, the Ministry of Education and Science of Ukraine, 16, Polkovnyka Potekhina Str., Kyiv, Ukraine, 03041

[2] Department of Molecular Medicine and Pathology, the University of Auckland, 85 Park Road, Auckland, Grafton 1023, New Zealand

[3] State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, State Service of Ukraine on Food Safety and Consumer Protection, 30, Donetska Str, Kyiv, 03151 Ukraine

E-mail: sergii.honcharov@gmail.com, 5278823@ukr.net, maryna.galat@gmail.com, pashk_ira@ukr.net, sunraissa@gmail.com, dr.dubovoy.andrey@gmail.com, litvinenkoop@gmail.com*

Orcid: https://orcid.org/0000-0001-7464-6689, https://orcid.org/0000-0003-4559-6666, https://orcid.org/0000-0001-8881-0865, https://orcid.org/0000-0003-0673-6249, https://orcid.org/0000-0001-8446-3851, https://orcid.org/0000-0003-1979-9163, https://orcid.org/0009-0003-0682-8917

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Aim. To evaluate the pathological effect of *Eustrongylides excisus* nematode larvae (L3–L4) on the organism of laboratory rats, the latter were infected *per os* by these parasites. The larvae of *Eustrongylides excisus* were obtained from perch *(Perca fluviatialis,* Linnaeus, 1758), caught in the Dnipro-Buh estuary – 46°37'48.45" N, 31°51'43.72" E, near the village of Dniprovske, Mykolaiv region (Ukraine). **Methods.** The experimental studies, which lasted for 30 days, were conducted using 15 non-linear male laboratory rats, which composed the experimental group by the analogy principle, three other animals were used as control. The intensity of the pathological process under the chronic course of eustrongylidosis was evaluated by the histological testing of the damaged organs of animals under investigation, which were euthanized after the completion of the waiting period. **Results.** The histological testing found pathological changes in the tissues of the brain, lungs, spleen, and organs of the gastrointestinal tract. There was a notable formation of non-specific granulomas (i.e. the ones, which were not found in the control group of animals), which consisted of adipose and connective tissue of mesostenium, mesocolon, and mesogaster. These granulomas had vivid exudative-proliferative inflammation and contained the elements of parasitic larvae (remnants of larvae bodies: fragments of cephalic and caudal ends, cuticles), which were subjected to considerable destructive changes. The walls of these granulomas consisted of fibrin and necrotic detritus. It was found that the animals of the group (the waiting period of 30 days) had mostly fibrino-purulent type of inflammatory reaction with the prevailing exudative-proliferative processes and the formation of non-specific granulomas. **Conclusions.** The results of scientific studies demonstrated necrotic and hypoxic-ischemic changes in the brain tissues; signs of cardiovascular deficiency, dystrophic and hypertrophic changes in the myocardium tissues; purulent inflammation of pleura and interstitial tissue of the lungs; changes in kidney tissues in the form of balloon dystrophy and granular degeneration of tubular epithelium; acute exudative-proliferative inflammation of the liver capsule and parenchyma; proliferation of white pulp with necrotic changes in lymphoid follicles; the thickness of the walls of gastrointestinal organs was considerably infiltrated with eosinophils, plasma cells, and macrophages. The phenomena of tissue hypertrophy and sclerosis as a pathomorphological response of the organism to the chronic course of the disease were determined.

Key words: *Eustrongylides excisus,* rats, experimental infection, histological testing, chronic course.

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INTRODUCTION

The Dioctophymatidae family consists of three independent genera, two of which (*Eustrongylides* and *Hystrichis*) are mainly found in large granulomas of gastric walls in fish-eating birds and one species (*Dioctophyma renale* Goeze, 1782) mainly parasitizes in the kidneys of animals from the families *Canidae* and *Mustelidae* (Eberhard et al, 2014). The nematodes of the genus *Eustrongylides* pose a potential threat to human health. This genus also contains the nematode of *Eustrongylides excisus* Jägerskiöld, 1909 (Ljubojevica et al, 2015).

The nematode of *E. excisus* Jägerskiöld, 1909 has a complicated development cycle in which the main definitive hosts are fish-eating waterbirds from the line of *Ciconiiformes*, *Anseriformes*, *Gaviiformes* and *Pelecaniformes*, which spread the eggs along with feces in the water bodies (Novakov et al, 2013). The first intermediate hosts for this parasite are oligochaetes from the families *Tubificidae* and *Lumbriculidae* (*Limnodrilus* spp.), which swallow the eggs of *E. excisus* and in which parasites develop during the first and second larvae periods (Karmanova, 1968). The additional or second intermediate hosts are predatory, planktivorous, and the species of fish and benthos feeders which get infected while swallowing infected oligochaetes (Measures, 1988; Novakov et al, 2013; Goncharov et al, 2018). The agent of eustrongylidosis also poses a threat to fish farming, using fish tanks; for instance, there were reports about the infection of *Danio rerio* Hamilton, 1822 (Fusco et al, 2023). The definitive hosts – fish-eating birds – get infected when fed with fish, infected with nematode larvae of *Eustrongylides*.

The nematode of *E. excisus* can use some amphibia and reptiles as an accidental (final) host: marsh frog (*Rana ridibunda* Pallas, 1771), cane toad (*Rhinella marina* Linnaeus, 1758), Eurasian marsh frog (*Pelophylax ridibundus* Pallas, 1771), and tesselated snake (*Natrix tasselat* Laurenti, 1768) (Sloboda et al, 2010; Melo et al, 2015; Yermolenko et al, 2022). People who consume fish and fish products may also get infected with the eustrongylidosis agent (Deardorff et al, 1991).

This species of parasites is rather widely common. The registration of *E. excisus* was reported in Serbia, Romania, Turkey, Brazil, the USA, Italy, Iran, Azerbaijan, Czech Republic, and Ukraine (Karmanova, 1968; Lichtenfels et al, 1985; Pazooki et al, 2007; Novakov et al, 2013; Soylu, 2013; Ljubojevica et al, 2015; Branciari et al, 2016; Goncharov et al, 2018; Guardone et al, 2021).

According to some scientific data, the occurrence of *Eustrongylides* nematodes in water basins is a bioindicator of water quality (Biswas et al, 2023).

In Ukraine, *E. excisus* nematodes are mainly registered among the representatives of ichthyofauna. For instance, eustrongylidosis was registered in the Zaporizhzhia dam pond in the populations of perch (*Perca fluviatilis* Linnaeus, 1758), wels catfish (*Silurus glanis* Linnaeus, 1758), zander (*Sander lucioperca* Linnaeus, 1758) (Sinyaeva, 2014). This species of helminths was also registered in gobiids of the Black Sea and the Sea of Azov (Korniychuk et al, 2008), and in the Dnipro-Buh estuary, in the populations of perch (*P. fluviatilis* Linnaeus, 1758), zander (*S. lucioperca* Linnaeus, 1758), pike (*Esox lucius* Linnaeus, 1758), and roach (*Rutilus rutilus* Linnaeus, 1758) (Goncharov et al, 2018, Honcharov, 2020c).

Considering that the available scientific publications did not contain any information about histological changes in the organism of rats, infected with *E. excisus* nematode larvae, specifically under the chronic course, we conducted the experimental infection of laboratory rats with eustrongylidosis agent.

Previously we received representative data about pathohistological changes in the organism of experimental rats under the acute course of eustrongylidosis (Honcharov et al, 2022). Still, there is a need to highlight rather significant differences in the course of pathological processes in the organism of sick animals on the cellular and tissue levels.

MATERIALS AND METHODS

The study involved the use of eighteen non-linear laboratory albino rats (*Rattus rattus* Linnaeus, 1758) of the same age (3.5 months), a bodyweight of 190–230 g. The animals were kept separately, in cages with meshy bottoms to prevent coprophagy. The access to feeds and water was *ad libitum*. Prior to the experimental infection, the animals were subjected to clinical and coprological testing.

Fifteen animals were infected *per os* with *E. excisus* nematode larvae (Jägerskiöld, 1909) (L3-L4), 10 per each animal, using the feeding catheter, type CH08, connected to the 2 ml syringe. The parasites were obtained from perch (*P. fluviatilis* Linnaeus, 1758), caught in the Dnipro-Buh estuary, Ukraine. Ten percent of the selected nematodes were fixed in a special solution and processed for the study to determine the taxonomic relations (Karmanova, 1968). Three rats were kept as control.

The animals were observed for 30 days. When the waiting period was over, euthanasia was administered by an intraabdominal injection of thiopental sodium solution in the dose of 0.015 g/kg of the bodyweight of the animal, and the post-mortem examination was conducted.

All the studies were conducted in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, dated March 18, 1986, the Directive of the European Parliament and the EU Council, 2010/63/EU dated September 22, 20101, "On Protection of Animals, Used for Scientific Purposes" and the Law of Ukraine dated February 21, 2006, No. 3447-IV (with amendments as of June 22, 2017 No. 2120-VIII) "On Protection of Animals from Cruelty".

The internal organs of the euthanized rats (brain, lungs, heart, spleen, liver, stomach, intestines) were extracted and fixed in 10 % neutral formalin, then they were cut into pieces, processed with a series of alcohols of increasing concentration and poured with paraffin by the common method. The sections of paraffin blocks, 5–7 μm wide, were done on the microtome Leica SM 2000 R and stained with hematoxylin and eosin. The microscopic studies were conducted using Olympus BX41 microscope (object-lense x40, ocular lens x10).

This protocol was approved by the bioethics committee of the National University of Life and Environmental Sciences of Ukraine (the conclusion of the bioethics committee No. 23/22 dated April 05, 2022).

RESULTS

The histological testing of the pathological material samples, obtained from the laboratory rats, infected with *E. excisus* nematode larvae, Jägerskiöld, 1909), yielded the following results.

The investigation of the brain structure found that most vessels of the brain and cerebellum did not contain blood; only some vessels contained erythrocytes. The cerebellum matter had a notable expansion of perivascular and pericellular spaces; some cells of the granular layer were necrotically altered. The process was characterized by uneven disintegration of the brain axis matter, swelling of some neurons, and expansion of perivascular and pericellular spaces. There is some proliferation of ependyma in cerebral ventricles. In some places, subependymally, neuroglia were intensely disintegrated in the form of mesh structures. Some single vessels of the vascular net contained erythrocytes, some – groups of leukocytes.

Thus, there were morphological features of hypoxic-ischemic damage to the cerebellum matter in the form of necrotic changes in the granular layer cells, swelling of the cerebellum matter, hypoxic-ischemic damage to the brain axis matter in the form of the swelling of microneuroglia and brain axis matter (**Fig. 1**).

The investigation of the heart found mixed knots in the left and right ventricles. Single chordae tendineae of the left ventricle were thickened and fibrous. The thickness of the wall of the left ventricle (in its thickest part) exceeded that of the right ventricle (in the thinnest part) four times. The epicardium was preserved, unevenly infiltrated with leukocytes, and the visible vessels of the epicardium were unevenly filled with blood; the vascular walls and their perivascular spaces were unevenly soaked with disintegrated accumulations of erythrocytes. The myocardium walls did not have the even blood flow; some vessels had dissociation (separation) of blood, the myocardium arteries were mostly spasmodic, did not contain any blood, the nuclei in their walls were reoriented. There was uneven disintegration of the intermediary and perivascular tissue. The disarray was noted in the myocardium in rare fields of vision (**Fig. 2**).

The cardiomyocytes were unevenly stained, some cardiomyocytes of the left ventricle were enlarged, in some places, the groups of cardiomyocytes were fragmented.

Thus, we determined the signs of acute cardiovascular deficiency in the form of acute impairment of the blood circulation in the epicardium vessels, the spasms of myocardium arteries, uneven swelling of the interstitial tissue in the myocardium, and focal fragmentation of cardiomyocytes. This pathological process was also characterized by morphological signs of hypertrophic cardiomyopathy in the form of uneven hypertrophy of the myocardium of the left ventricle and uneven cardiosclerosis.

The histological testing of the lungs demonstrated that the pleura was preserved, disintegrated, and unevenly infiltrated with lymphocytes, leukocytes, rare eosinophils, macrophages, and plasma cells. The stroma vessels and the vessels of the interalveolar septa had uneven blood flow, the walls of some vessels and their perivascular spaces were unevenly soaked with disintegrated accumulations of erythrocytes up to the falling of the latter into the lumen of alveoli groups. Blood dissociation or mixed knots and accumulations of leukocytes were registered in some vessels. The bronchi were partially spasmodic, the epithelium of small bron-

chi was preserved. There was some disintegration of perivascular stroma and groups of leukocytes, lymphocytes, eosinophils, rare macrophages, and plasma cells in the interstitium of the lungs. The dystelectasis was noted in some alveoli; other alveoli were sharply emphysematously dilated up to the rupture of interalveolar septa, with small, disintegrated hemorrhages in rare rupture points (**Fig. 3, 4**).

Some alveoli had pink liquid and air vacuoles. In some places along the bronchial trunk, there were registered lymph nodes with impaired stratification; the cortical and cerebral layers were not distinguished, lymphoid follicles of the cortical layer were absent, and there was slight necrosis in some parts of the lymph nodes. Some vessels of lymph nodes had uneven blood flow, small diapedetic hemorrhages, and accumulations of leukocytes.

Thus, under experimental eustrongylidosis, the experimental animals had morphological features of acute purulent pleurisy, acute purulent interstitial pneumonia, and acute pulmonary patchy emphysema. We also registered acute impairment of blood circulation in pulmonary vessels with the formation of patchy intraalveolar hemorrhages, and uneven swelling in the interstitial pulmonary tissue.

The investigation of kidneys demonstrated that the capsule was partially preserved, unevenly thickened, and there were fibrosis signs in the thickened areas. There were fibrin layers on the surface of the capsule in some fields of the vision, the leukocytic infiltration was uneven: there were rare eosinophils and macrophages, groups of plasma cells. Most renal vessels did not contain any blood, and erythrocytes were only in some vessels of the juxtamedullary area. The capillaries of the mesangium of glomeruli contained erythrocytes. There was shunting of the vessels in the juxtamedullary area with the formation of small perivascular (diapedetic) hemorrhages. The nephrothelium of the cortical matter was unevenly swollen and stained, with granular cytoplasm, the nuclei were unaltered (**Fig. 5**).

Epithelium with vacuolated cytoplasm was registered in some parts of subcapsular tubuli, There were granular or hyaline casts in some tubuli of the juxtamedullary area. Therefore, under eustrongylidosis of rats, there were registered morphological features of shock reaction in kidneys in the form of shunting the vessels of the juxtamedullary area and insufficient blood flow in the cortical and cerebral matter of kidneys. The pathological changes in renal tissue were determined in the form of present hyaline and granular casts in some tubuli of the juxtamedullary area and the balloon dystrophy of the epithelium of the tubuli of subcapsular parts.

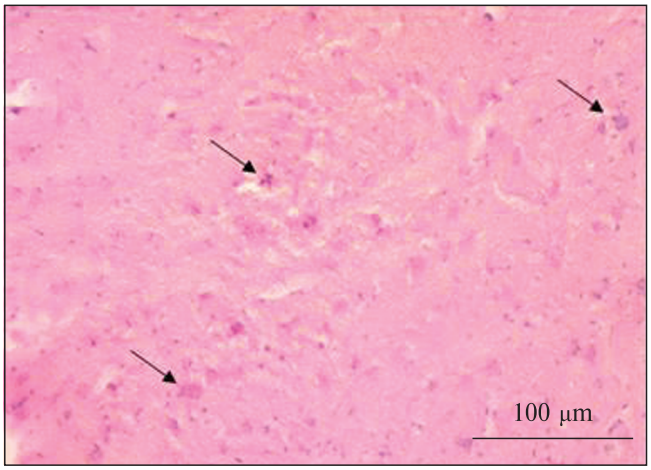

Fig. 1. The swelling of brain neurons and their neurobiosis (magnification ×400). There were rare instances of neuron enlarging

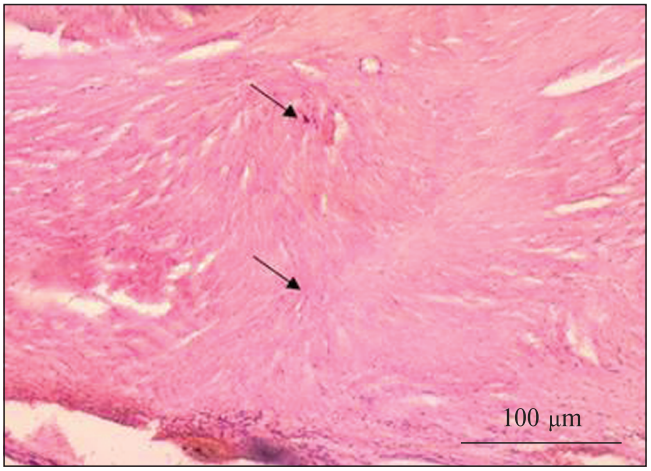

Fig. 2. The disarray figures in the ventricular septum of the heart (magnification ×400)

Under eustrongylidosis, the liver was characterized by a preserved, thickened capsule, unevenly infiltrated with lymphocytes and accumulation of leukocytes, some fields of vision on the surface of the capsule had noted layers of fibrin, and single eosinophils, macrophages, and plasma cells. Some fields of vision contained subcapsular granulomas, containing multinuclear cells, in the area, perifocal from granulomas – hemorrhages, presented with small dense accumulations of erythrocytes, without any cellular reaction. In the liver, subcapsularly, hepatocytes were noted with a necrotically changed cytoplasm, the membranes of hepatocytes were absent. Some veins of portal tracts, triades, and some sinusoid capillaries had uneven blood flow, there was accumulation of leukocytes in the

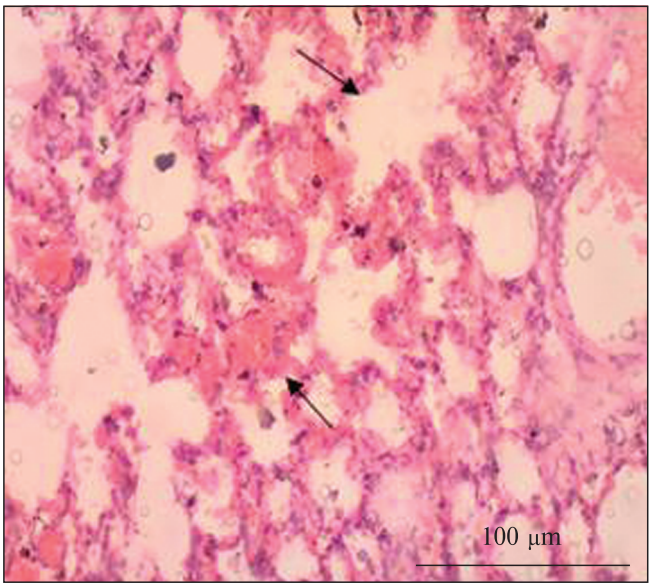

Fig. 3. The signs of acute impairment of blood circulation in the lungs of the rat (magnification ×400)

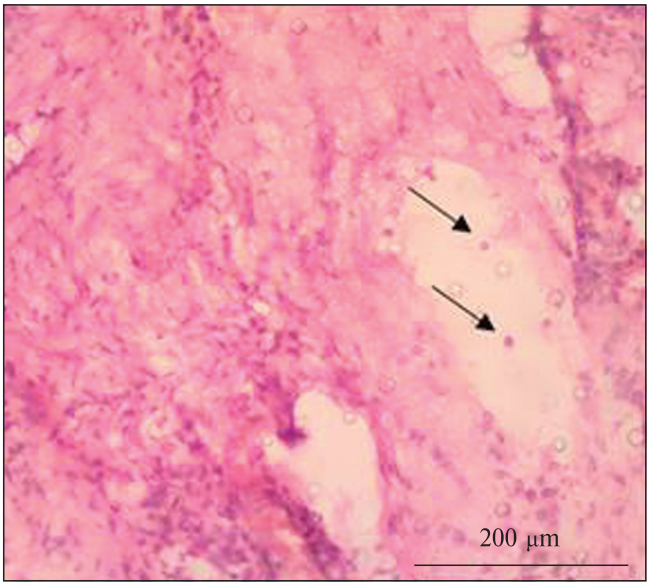

Fig. 4. Eosinophils in the interstitium of the lungs of the rat (magnification ×400)

lumen of some single veins. The structure of hepatic plates was not impaired, hepatocytes had granular cytoplasm, the nuclei were unaltered. There was uneven expansion of perisinusoid Disse's spaces, where single eosinophils, lymphocytes, leukocytes, and plasma cells were localized (**Fig. 6**).

Centrolobularly, hepatocytes were rapidly swollen, with lighter granular cytoplasm, the nuclei of hepatocytes were swollen, vacuolated, and the nucleoli were visualized in the nuclei. In some sections, the adipose cellular tissue with hemorrhages and newly formed granulomas was adjacent to the hepatic capsule. The hemorrhages contained lumps of formalin pigment.

Thus, under this parasitosis, there were morphological signs of acute exudative-proliferative hepatitis with necrotic changes in hepatocytes, the formation of non-specific granulomas, swelling of the interstitial hepatic tissue, and acute exudative-proliferative inflammation of the hepatic capsule.

The histological testing of the spleen noted the preservation of the capsule. The red pulp had insignificant blood flow. There was a proliferation of white pulp with necrotic changes in lymphoid follicles (**Fig. 7**).

There were changes in the pancreas, which had signs of expressed autolysis.

All the layers of the gastric wall were preserved. The mucous layer and the very plate of the mucosa, submucous, and muscle layers did not have any pathological changes. The serous coat of the stomach and small intestine were often infiltrated with leukocytes, mostly eosinophils, macrophages, and plasma cells. In some places, the elements of mesentery in the form of adipose tissue were adjacent to the serous coat, the vessels had uneven blood flow, and some vessels had dissociation (separation) of blood. The mesentery was infiltrated with leukocytes, eosinophils, lymphocytes, single plasma cells; some fibrin strands were also noted.

The mucous coat of small intestines was autolytically altered. There were some parts of the wall of small intestine, where all the layers were preserved, except for the serous coat; the mucosa layer was unevenly infiltrated with lymphocytes, leukocytes, accumulation of eosinophils, plasma cells, and rare macrophages (**Fig. 8**).

The vessels of the mucosa layer and the muscle coat did not contain any blood. The vessels of the submucous coat had uneven blood flow. The serous coat of the small intestine was often infiltrated with leukocytes, mostly eosinophils, macrophages, and plasma cells.

The formation of a conglomerate in the form of necrotic detritus (exudative-proliferative inflammation) with admixtures of the masses of leukocytes, nuclei of destroyed leukocytes, sediments of fibrin, accumulation of eosinophils were registered between the loops of the small intestine and the gastric wall. The formation of granulomas was noted here as well. The centers of single granulomas had holes of irregular form, the walls of which were coated with necrotic detritus, fibrin, groups of leukocytes, and nuclei of destroyed leukocytes. Single multinuclear cells with fluffy, bright

pink cytoplasm were visualized perifocally from granulomas.

The testing of the conglomerate, formed due to the joining of soft tissues of the abdominal cavity, found expressed exudative-proliferative inflammation, manifested by vivid cellular infiltration of soft tissues (leukocytes, lymphocytes, eosinophils, plasma cells, single macrophages) with the formation of non-specific granulomas. The central parts of single granulomas had elements of connective tissue fibrils, and small, disintegrated hemorrhages. Some granulomas had the inclusion of helminths of round shape, red-brown color, and granular structure. Single vessels of soft tissues had uneven blood flow, and small, disintegrated hemorrhages were found in some areas of the conglomerate stroma. Most other vessels of soft tissues did not contain blood.

The testing of the large intestine found that all the layers were preserved. The mucous coat in basal areas was unevenly infiltrated with single lymphocytes, leukocytes, and eosinophils, the vessels of basal areas had full blood flow. It was sometimes noted that the mucous coat was autolytically altered, the superficial areas were partially desquamated, and goblet cells were clearly visualized in the glands. The submucous layer was sharply disintegrated, and infiltrated with the accumulation of plasma cells, single macrophages, and eosinophils. The vessels of the submucous layer had uneven blood flow, with small perivascular hemorrhages, there were fibrin clots in single vessels, some vessels contained the accumulations of leukocytes. The muscle layer did not have any changes. Most vessels of the muscle layer did not contain blood. The serous coat was unevenly disintegrated, unevenly infiltrated with leukocytes, lymphocytes, eosinophils, plasma cells, and macrophages. There were focal hemorrhages in the form of fluffy and dense accumulations of erythrocytes in the serous coat. The adipose tissues, adjacent to the serous coat, were unevenly infiltrated with the accumulations of lymphocytes, plasma cells, and single monocytes. The visible vessels of the adipose tissue did not contain blood.

Between the loops of the intestine, there was adipose and connective tissue (intestinal mesentery) with expressed exudative-proliferative inflammation (leukocytes, lymphocytes, eosinophils, plasma cells, macrophages, and non-specific granulomas). In the central area of one granuloma, there were areas of necrotic detritus; in the granuloma wall there were elements of the parasite larva under considerable destruction, which looked like a single red-brown sediment with a granular

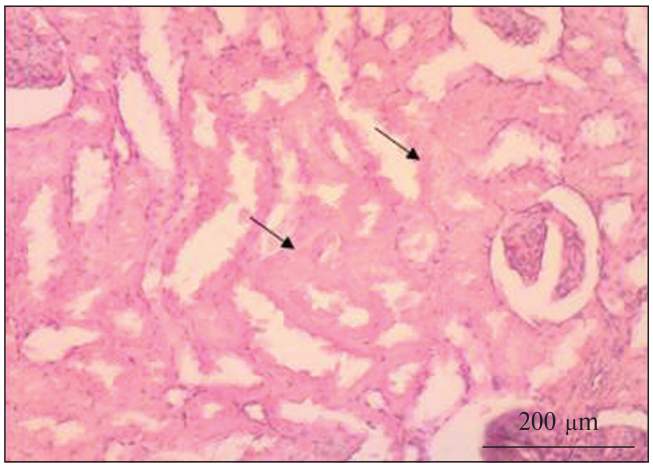

Fig. 5. Necrosis features in the cortical matter of rat kidneys. Karyopyknosis and karyorhexis were noted in the cells (magnification ×400)

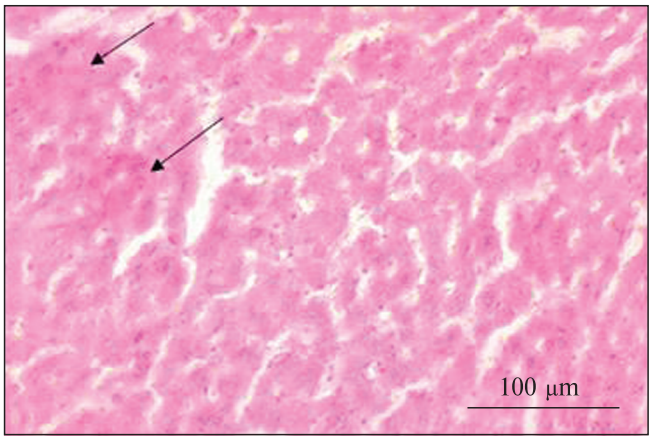

Fig. 6. The accumulation of leukocytes in perisinusoid spaces of the rat liver (magnification ×400)

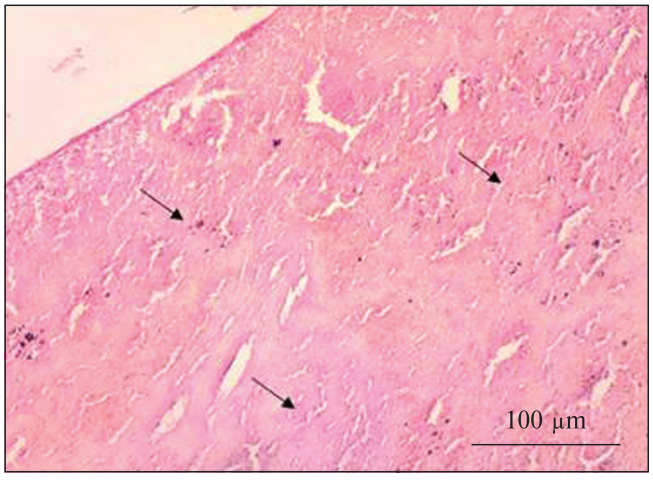

Fig. 7. Necrotic changes in lymphoid follicles of white pulp of the rat liver. The features of «septic spleen» (magnification ×400)

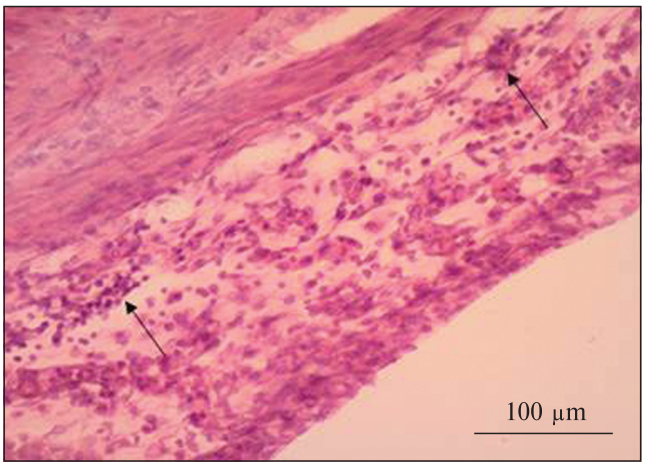

Fig. 8. Purulent inflammation of the serous coat of small intestine of the rat. Significant leukocytic infiltration of tissues (magnification ×400)

structure. Some veins of soft tissues had uneven blood flow, sometimes they contained accumulations of leukocytes. Most arteries of soft tissues did not contain any blood, the walls of single arteries were thickened and had fibrosis. In the places with adjacent muscle tissue (elements of the abdominal wall), the muscle fibrils were sharply swollen, with homogenized or granular bright red cytoplasm, and had necrotic changes. The stroma between them was disintegrated, some stroma veins had uneven blood flow, and most arteries of the stroma did not contain blood.

Thus, under experimental eustrongylidosis, the laboratory rats had acute exudative-proliferative inflammation of the mesentery of the small and large intestine and the formation of non-specific granulomas therein, the formation of non-specific granulomas in the liver and acute serous-purulent enterocolitis.

Thus, under the chronic course of eustrongylidosis, notable is the syndrome of systemic inflammatory reaction, which is the response of the organism of rats to the long-term impact of powerful, diverse factors (allergic, mechanic, inoculatory, and toxic impact of parasites on the organism).

DISCUSSION

The nematodes of genus *Eustrongylides* pose a threat to the health of people who consume fish and fish products that have not been subjected to sufficient cooking (Guerin, 1982; Gunby, 1982; Eberhard et al, 1989; Wittner et al, 1989; Narr et al, 1996). These invasions were characterized by gastritis, perforation of the intestinal wall, peritonitis, and the formation of non-specific subcutaneous granulomas (Panesar et al, 1979; Deardorff et al, 1991; Eberhard et al, 2014).

After penetrating the organism of the final host, the agent of eustrongylidosis does considerable mechanical damage to the tissues, disrupting the integrity of hollow and parenchymal organs, causing hemorrhages, inflammatory processes in the peritoneum, and general septic phenomena. These pathologies may often result in the death of the host (Karmanova, 1968).

While estimating the capability of the eustrongylidosis agent to infect rabbits *per os*, Shirazian D et al (1984) and Barros LA et al (2004) registered some pathological changes: the inflammation in the peritoneum, the formation of granulomas on the surface of the liver, and abscesses in the abdominal cavity, the presence of parasites in the thoracic cavity. There were also histological changes in the gastric wall: intense inflammatory reaction in the gastric wall with the prevalence of eosinophils in the tissues, damage to the mucous and submucous layers, hemorrhages; there was necrosis and inflammatory infiltrate in the muscle layer. There were various inflammatory reactions with the prevalence of mononuclears, necrosis, and abscesses in the depth of the abdominal wall. According to different data, experimentally infected rabbits had clinical symptoms of eustrongylidosis (some animals even died) (Barros et al, 2004), but as per other researchers (Shirazian et al, 1984), no visible clinical signs of eustrongylidosis and cases of death were registered.

Under experimental infection of laboratory rats with *E. excisus* nematode larvae (which were extracted from perch – *P. fluviatialis*), the changes in the clinical state of laboratory rats were registered: poorer appetite and mobility, inhibition of the general state, tachypnea, painful abdominal wall (Honcharov, 2019). The post-mortem examination demonstrated serofibrinous and purulent-fibrinous peritonitis, perforation of the wall of the gastric-intestinal channel, and inflammatory phenomena of the wall of the stomach and intestines. There were also microabscesses under the hepatic capsule, secondary pathologies of kidneys and thoracic organs. Alive larvae and larvae without any signs of life were found in the lumen of the intestines and directly in the abdominal cavity (Honcharov, 2020a). The impact of *E. excisus* larvae, extracted from roach (*Rutitlis rutilis*), on the organism of laboratory rats was somewhat less significant as compared to those, extracted from predatory fish. The abbreviated course of the disease was registered: spontaneous recovery with improved clinical state. The post-mortem examination registered mainly serofibrinous peritonitis and single cases of purulent-fibrinous peritonitis. Some animals had sple-

nomegaly. Dead nematode larvae and their elements were found in the abdominal cavity and the lumen of the stomach and intestines. It should be noted that nematode larvae, which were developing in the body of roach – non-specific intermediate host of *E. excisus*, caused less manifested pathological changes in the organism of laboratory rats as compared to *E. excisus* larvae, extracted from predatory fish (Honcharov, 2020b).

In nature, the clinical and post-mortem changes are most frequently registered among fish-eating birds. The post-mortem examination of sick birds registered considerable mechanical damage to the gastrointestinal organs up to the loss of integrity of the latter, which often led to the development of peritonitis and severe pathological states that often resulted in death. There were also reports on the complete obturation of the intestines of the birds by helminths (Locke, 1961; Franson et al, 1994). The post-mortem examination of gannets that had eustrongylidosis demonstrated the degenerated Eustrongylidosis larvae in the depth of the gastric wall of the birds. In the places, where helminths penetrated, there were registered hemorrhages and ulcers, the formation of granulomas at different stages of the inflammatory process, which consisted of fibrous connective tissue components (El-Dakhly et al, 2012). A similar case of registering eustrongylidosis in fish-eating birds was reported based on post-mortem examination: the perforation of the gastrointestinal tract, peritonitis, and the formation of fibrous granulomas with necrotic content and nematodes inside (Cole, 1999).

Our experimental work demonstrated that the main mechanism launching the pathological process in the organism of an animal that had eustrongylidosis, was perforation of the wall of the gastrointestinal tract and the penetration of the intestine content and parasites themselves into the abdominal cavity, mechanic damage to the tissues of parenchymatous organs and the development of peritonitis.

For instance, it is known that peritonitis does inflammatory damage to the peritoneum, which is accompanied by intestinal paresis, endogenous intoxication, and homeostasis impairment, against the background of which there are impairments in systemic and regional blood circulation, pulmonary gas exchange, functional state of the liver and kidneys with the following prominent syndromes: systemic inflammatory reaction, endothelial dysfunction, enteral insufficiency, and intraabdominal hypertension (Clements et al, 2021).

The described processes lead to acute pathological states: purulent-fibrinous inflammation of the peritoneum with expressed necrotic component. A severe toxic state is a consequence of peritonitis, which occurs in the organism of the rats, develops in the course of the disease, and has a general impact on the tissues and organs of animals. In particular, this is confirmed with pathological changes in the cells of the liver, heart, kidneys, etc. (necrotic changes in the cytoplasm, loss of cellular membrane, fragmentation of cells, re-orientation of nuclei).

While comparing the results of histological testing of the pathological material selected from rats under the acute course of eustrongylidosis (Honcharov et al, 2022), it should be noted that under the chronic course, the proliferative processes with considerable connective tissue and fibrous components prevailed. Noteworthy is the presence of a high number of eosinophils in the leukocytic infiltration, which was not registered by us under the acute course. Under the chronic course of eustrongylidosis, there was a noted formation of non-specific granulomas with the elements of necrotic detritus and some features of autolytic processes; also there were signs of hypertrophy and sclerosis of the tissues of parenchymous organs.

We described the formation of non-specific granulomas, localized in the tissues of the abdominal cavity, under eustrongylidosis in laboratory rats. The findings of the study demonstrated the elements of parasite larvae in a state of considerable destruction inside such granulomas. Some researchers believe that a parasite has its life strategy, which lies in a specific impact on the host organism, thus activating different immunologic factors (Rohlenová et al, 2011). The formation of capsules and granulomas in the host organism is a result of mutual adaptation between the immune system of the host and the parasite and the element of strategic compromise between both organisms for the survival of the latter (Bruschi et al,2011).

The formation of granulomas under eustrongylidosis is widely common among other intermediate hosts for this type of parasite – fish. The post-mortem of fish, infected with *E. excisus* larvae, registered the signs of inflammation around the encapsulated larvae: hyperemia, induration of adjacent tissues, and swelling. Especially vivid inflammatory phenomena were found in the tissues of mesentery and muscles (Goncharov et al, 2018). The histological testing of the muscle tissue of perch (*Perca fluviatialis*) demonstrated that nematode larvae were surrounded by a capsule. The foci of necrosis were noted in the places where the parasite penetrated the tissues. The muscle tissue was in a state

of degeneration. There was a considerable aggregation of macrophages and the formation of multifocal fibroepithelial granulomas in the thickness of the capsule at the ends of the spindle-shaped capsules (Dezfuli et al, 2015). Our studies demonstrated the prevalence of proliferation processes and the formation of connective tissue granulomas in the organism of rats, which had eustrongylidosis. These granulomas seem to be the reaction of the animal's organism to the penetration of the xenogenic object to the tissues of the internal environment. There were dystrophic and morphological changes in the tissues as a result of the secondary impact of parasites on the host organism. The formation of granulomas and capsules in the animal's organism is a mechanism of defense and localization of the internal environment of the host from the parasite. These inflammatory processes are accompanied by the aggregation of the cells of the leukocytic and macrophagic series.

It should be noted that under the chronic course, there was a prevalence of proliferative processes with a considerable connective tissue and fibrous component. Noteworthy is the presence of a high number of eosinophils in the leukocytic infiltration, which was not registered by us under the acute course. Under the chronic course of eustrongylidosis, there was a noted formation of non-specific granulomas with the elements of necrotic detritus and some features of autolytic processes, the signs of hypertrophy and sclerosis of the tissues of parenchymous organs.

CONCLUSION

The findings of the histological (microscopic) studies of the biological material, selected by us from the infected laboratory rats, demonstrated severe pathological changes in organs and tissues with features of the syndrome of general inflammatory response. This fact is conditioned by the specificity of pathogenesis under the chronic course of eustrongylidosis. In particular, we registered pathological changes in the organism of rats, which occurred directly due to the mechanical damage to the tissue by the helminth at the time of their migration, and also the secondary changes due to endogenous intoxication and impaired homeostasis. The mechanically injured tissues had inflammatory phenomena, mainly of a purulent nature, and there was considerable cellular infiltration with the prevalence of neutrophils, eosinophils, lymphocytes, plasma cells, and macrophages. We determined the signs of purulent pleurisy, acute purulent interstitial pneumonia, acute exudative-proliferative inflammation of the liver and its capsule, the signs of autolysis in the pancreas, acute exudative-proliferative inflammation of mesostenium and mesocolon, serous-purulent enterocolitis. At the same time, there were registered hypoxemic-ischemic changes, dystrophies, and necroses in the tissues of the brain, heart, kidneys, spleen, and liver as a consequence of endogenous intoxication, occurring against the background of purulent peritonitis development. The phenomena of hypertrophy and sclerosis of heart tissues were also noted under the chronic course of eustrongylidosis.

The formation of non-specific granulomas was registered in the extraenteric space. The elements of helminths were found inside these granulomas. The granulomas were characterized by exudative-proliferative inflammation and manifested cellular infiltration with a considerable necrotic component.

A more detailed study of the agent biology and pathological changes in the host organism are urgent objects of further scientific research.

Conflict of interests. The authors deny any conflict of interest.

Compliance with standards of working with animals. All the studies were conducted in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, dated March 18, 1986, and other regulatory documents governing the protection of animals from cruelty.

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Гістопатологічні зміни в організмі щурів за експериментального інвазування личинками *Eustrongylides excisus* (nematoda: Dioctophymatidae) за хронічного перебігу

С. Л. Гончаров [1], Н. М. Сорока [1],
М. В. Галат [1], І. Ю. Пашкевич[1],
Р. О. Слободян [1], А. І. Дубовий [2], О. П. Литвиненко [3]

[1] Національний університет біоресурсів і природокористування України,
Міністерства освіти і науки України,
Вул. Полковника Потєхіна, 16, м. Київ, Україна, 03041
[2] Відділення молекулярної медицини та патології,
Університет Окленда, 85 Park Road, Auckland,
Grafton 1023, Нова Зеландія
[3] Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи,
Державна служба України з питань безпечності харчових продуктів та захисту споживачів

Вул. Донецька, 30, Київ, Україна, 03151
e-mail: sergii.honcharov@gmail.com*,
5278823@ukr.net, maryna.galat@gmail.com,
pashk_ira@ukr.net, sunraissa@gmail.com,
dr.dubovoy.andrey@gmail.com, litvinenkoop@gmail.com
orcid: https://orcid.org/0000-0001-7464-6689,
https://orcid.org/0000-0003-4559-6666,
https://orcid.org/0000-0001-8881-0865,
https://orcid.org/0000-0003-0673-6249,
https://orcid.org/0000-0001-8446-3851,
https://orcid.org/0000-0003-1979-9163,
https://orcid.org/0009-0003-0682-8917

Мета. З метою оцінки патогенного ефекту личинок нематоди *Eustrongylides excisus* (L3–L4) на організм лабораторних щурів, останніх заражали *per os* зазначеними паразитами. Личинок *Eustrongylides excisus* отримували від окуня *(Perca fluviatialis,* Linnaeus, 1758) виловленого в акваторії Дніпро-Бузького лиману виловленого в акваторії Дніпро-Бузького лиману – 46°37’48.45» N, 31° 51’43.72» E, поблизу села Дніпровське, Миколаївська область (Україна) **Методи.** Експериментальні дослідження, що тривали 30 діб, проводили на нелінійних 15 лабораторних щурах (самці) із яких і було сформовано дослідну групу за принципом аналогів; окремо три тварини були використані в якості контролю. Оцінку глибини патологічного процесу за хронічного перебігу еустронгілідозу проводили методом гістологічних досліджень уражених органів дослідних тварин, яких по закінченню термінів очікування піддавали евтаназії. **Результати**. За результатами гістологічних досліджень було встановлено патологічні зміни у тканинах головного мозку, легень, селезінки та органах шлунково-кишкового каналу. Відмічали утворення неспецифічних гранульом (тобто таких, що не були виявлені у контрольній групі тварин), що складалися із жирової та сполучної тканин брижі тонких та товстих кишок і шлунку. Зазначені гранульоми були з вираженим ексудативно-проліферативним запаленням та містили елементи личинок паразита (залишки тіла личинок: фрагменти головного та хвостового кінців, кутикули), що були піддані значним деструктивним змінам. Стінки таких гранульом складалися із фібрину та некротичного детриту. Встановлено, що у тварин групи (термін очікування 30 діб) реєстрували переважно гнійно-фібринозний тип запальної реакції з переважанням ексудативно-проліферативних процесів та утворенням неспецифічних гранульом. **Висновок.** За результатами проведення наукових досліджень виявлено некротичні та гіпоксично-ішемічні зміни в тканинах головного мозку; ознаки серцево-судинної недостатності, дистрофічні та гіпертрофічні зміни тканин міокарду; гнійне запалення плеври та інтерстеційної тканини легень; встановлено зміни тканин нирок у вигляді балонної та зернистої дистрофії епітелію канальців; гостре ексудативно-проліферативне запалення капсули та паренхіми печінки; відмічали проліферацію білої пульпи з некротичними змінами лімфоїдних фолікулів; товща стінок органів шлунково-кишкового каналу значно інфільтрована еозинофілами, плазмоцитами та макрофагами. Також було встановлено явища гіпертрофії та склерозу тканин, як патоморфологічна реакція організму на хронічний перебіг захворювання.

Ключові слова: *Eustrongylides excisus,* щури, експериментальне зараження, гістологічне дослідження, хронічний перебіг.

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