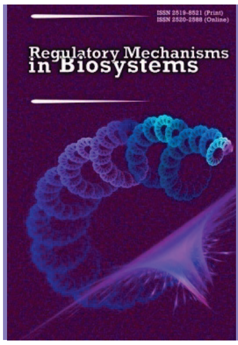

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Pathomorphological changes in the large intestine subject to chronic trichuriasis

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Trichuris spp. are endoparasites identified in many neotropical rodents. The study was conducted on nutrias (*Myocastor coypus* Molina, 1782) – semi-aquatic rodents of the family Echimyidae Gray, 1825, held at individual private farms in Poltava Oblast. This study gives us understanding of the species *Trichuris myocastoris* (Enigk, 1933), diagnosed in the gastrointestinal canal of nutrias. Thus, the objective of the study was to describe pathomorphological changes occurring in the large intestine of nutrias suffering trichuriasis. During the dissection, by macroscopically examining the cavity of large intestine, we detected small white spindle-shaped helminths. *Trichuris myocastoris* was found to have specific morphological features. Nematodes had a long thread-like front part and thickened, shorter, posterior part. The cuticle was finely striped transversally. In the lumen of large intestine of the nutrias, the average numbers *T. myocastoris* (n = 15) were found to be 36.7 ± 2.1 in males and 47.7 ± 2.5 in females in summer; and 11.1 ± 1.3 and 16.5 ± 1.2 in winter, respectively. For the histological studies, we chose fragments of the wall of large intestine. Microscopically, in the intestinal lumen, there were recorded transversal or diagonal sections of bodies of the pathogens. The nematodes' cuticle was stained basophically, and their internal organs eosinophilically. The superficial epithelium of the intestine was ruined in places, and some epitheloicytes were in the intestinal lumen. The intestinal glands were enlarged, filled with mucous content, mostly stained blue-violet, especially in the superficial sections of the glands, i.e. basophilous color. The lamina propria of the mucous membrane, especially between the intestinal glands, was notably infiltrated by lymphoid cells, and individual lymphoid cells were also found in the lumen of intestinal glands and even in the organ's lumen. Single lymphoid cells also were present in the layer of the mucous membrane. Under large increases, we saw signs of mucous hypersecretion in the goblet cells of the intestinal glands in the form of concentrations of poorly stained mucus granules in cytoplasm. Some goblet cells were ruined, desquamated, and their remains were in the lumens of glands together with mucus. The provided data suggest the relevance of *Trichuris* invasion as an etiological factor in the emergence of chronic lymphohistiocytic colitis.

Keywords: *Trichuris myocastoris*; *Myocastor coypus*; parasites; histological changes; colitis; macroscopic changes.

Introduction

Trichuriasis is a disease of mammals caused by nematodes of the *Trichuris* genus (Nematoda, Trichuridae). Around 80 species of those pathogens have been identified so far. Most of them have specific mammalian hosts (Ghai et al., 2014; Zhou et al., 2022). Reports have confirmed the spread of pathogens of the *Trichuris* genus in primates, carnivores, swine, and wild and domesticated ruminants (Hawash et al., 2015; Gul & Tak, 2016; Xie et al., 2018; Yevstafieva et al., 2018, 2019; Boyko & Brygadyrenko, 2020; Win et al., 2020; Jones, 2021; Saichenko et al., 2021). Some species parasitize people (Hawash et al., 2016). Some epidemiological studies indicate quite high level of the helminthiasis morbidity in the population, with the most affected being people of developing countries (Niyas et al., 2019). Nematodes of the *Trichuris* genus and other intestinal parasites have been given much research over recent years all around the globe due to exponential increase in the level of invasion of people and animals (Riveroet al., 2022; Bathobakae et al., 2024). *Trichuris trichiura*, which localize in the intestines, infect about 465 million people around the world. In Latin America and the Caribbean Basin, trichuriasis in people is considered the commonest helmianthiasis (12.3%). The main source of infection of people in the region is soil (95.0%) (Cruz et al., 2021; Thomas et al., 2023).

Rodents (Rodentia Bowdich, 1821) are considered the most diverse order of mammals, along with the Gires clade (Glires Linnaeus, 1758). Rodents include around 40.0% of all mammals, the largest group being the Muridae family (Muridae Illiger, 1811) (Musser & Carleton, 2005). Not only are they common around the globe, but also have a long evolutionary history. At the same time, we should note that rodents are also the reservoirs/specific hosts for a variety of pathogens of infectious diseases. Therefore, researchers have been concentrating their efforts on identifying rodents' parasites (Petružela et al., 2021). For example, molecular studies confirmed circulation of new causative agents of *Trichuris* spp. in rodents. Using the molecular method with internal transcribed spacer (ITS1-5. 8S-ITS2), a number of researchers revealed differences among *T. arrizabalagai* n. sp., *T. cossoni* n. sp., and *T. muris* in the rodents of the Muridae family on the continent and the island part of the Southeast Asia. The new species from Thailand was named *T. cossoni*, and the species from Borneo – *T. arrizabalagai*. The authors highlight the necessity of further studies of phylogeographically different species of *Trichuris* spp., which were earlier considered *T. muris*, despite one specific host (Ribas et al., 2020). *Mastomys natalensis* (Smith, 1834) is one of the commonest and dominating rodents in Africa south of the Sahara, including Tanzania. For the Morogoro region, the overall morbidity of *M. natalensis* with the *Trichuris* nematode accounted on average for 36.0%, and in the Iringa region, central part of Tanzania, the morbidity was higher, measuring 65.0%. It has to be noted that no significant difference was found between infected males and females in those two regions. At the same time, the authors indicated seasonality of the disease. Trichuriasis was much higher during the rainy season in the Iringa region (80%) than in Monogoro (42%). In the conditions of dry season, *Trichuris* invasion reached 50% and 30%, respectively for Iringa and Morogoro (Thomas et al., 2023).

The review by Jones, 2019 generalized the data on parasite fauna of neotropical animals. They inhabit a biogeographic region spanning the entire South America, part of Central America, islands of the Caribbean Basin and the southern part of Florida. In the rodents such as red-rumped agouti (*Dasyprocta leporina* Linnaeus, 1758), lowland paca (*Cuniculus paca* Linnaeus, 1766), and capybara (*Hydrochoerus hydrochaeris* Linnaeus, 1766); opossum (*Didelphis marsupialis insularis* Linnaeus, 1758), and collared peccary (*Tayassu tajacu / Peccari tajacu* Linnaeus, 1758), and red brocket (*Mazama americana* Erxleben, 1777), the commonest parasites were *Paraspidodera uncinata*, *Strongyloides* spp., *Eimeria* spp., *Giardia* spp., *Moniezia benedeni*, *Trichuris* spp., and *Physocephalus* spp. Zoonosis-pathogenic endoparasites include *Giardia* spp., *Echinoccocus* spp., *Cryptosporidium* spp., and *Trichuris* spp. (Jones et al., 2019; Jones, 2021).

Continuing the study of trichuriases in neotropical rodents, researchers found the following results. Guinea pig (*Cavia porcellus* Linnaeus, 1758), capybara (*Hydrochoerus hydrochaeris*), lowland paca (*Cuniculus paca*), and red-rumped agouti (*Dasyprocta leporina*), grown for meat, were diagnosed with *Trichuris* spp. In particular, EI in agouti ranged 4.6% to 53.9%, half of the capybara population was found to be infected, and the invasion of lowland paca and Guinea pigs measured 4.2–10.0% and 1.0–31.0%, respectively. In most cases, *Trichuris* spp. was diagnosed in combination with other endoparasites (Jones, 2021). The new species *Trichuris cutillasae* was described in capybaras living in the Corrientes province, Argentina (Eberhardt et al., 2019). Copro-ovoscopic studies of feces of wild nutrias, conducted in South America in 2009–2010, found circulation of the nematodes *Strongyloides myopotami* (26.7) and *Trichuris myocastoris* (13.8). According to the studies, those endoparasites were identified to the commonest helminthiases (Martino et al., 2012).

Most researchers came to the conclusion that the said animals were reservoirs of parasites that could infect domesticated cattle (Jones et al., 2019; Jones & Garcia, 2019; Jones et al., 2021).

However, there is a lack of data on the *Trichuris* spp. species that parasitize nutria in Ukraine. The available on-line resources provide fragmented information for Ukraine, reported only by Osadcha & Zon (2016). The authors identified a spectrum of parasitic fauna of nutrias in amateur-held farms of Sumy Oblast. At the same time, no data regarding the effects the pathogens have on the rodent were found in the available literature sources.

Researchers confirmed the antagonistic relationships emerging between the host and helminths. Some parasites circulate in a definitive host for a long time, settling in various organs and tissues. Parasitism entails deteriorated absorption of nutrients in the intestine, and lack of microelements and vitamins is diagnosed. Once a parasite infiltrates an organism, the gut microflora starts changing, and conditionally pathogenic microflora becomes prevailing. Causative agents produce toxins, which affect the immune response and facilitate their adaptation (Oliveira et al., 2022). A host organism tries to oppose metabolic and immunologic impairments. Reactivity mechanisms of the immune system and blood biochemical parameters that become involved can affect the progression of other occurring diseases or others that may be acquired in the future (Osadcha & Zon, 2019; Souza et al., 2021).

The objective of the study was to analyze pathomorphological changes in the large intestine during *Trichuris* spp. infection, and identifying specifics of morphofunctional changes in the tissues in case of the chronic disease. To achieve our goal of filling the knowledge gap on the effects of *Trichuris* spp. on nutrias, we set ourselves the following tasks: carry out pathoanatomic necropsy of nutrias infected with *Trichuris* spp., study macroscopic and microscopic changes in the intestine subject to this disease.

Materials and methods

The studies were conducted in 2023–2024, at the research laboratory of the Department of Parasitology and Veterinary-Sanitary Expertise (Poltava State Agrarian University). All the procedures with the experimental animals were performed adhering to the ethical norms of international and Ukrainian law. Protocol of the current study was approved by the Ethics Committee of the Poltava State Agrarian University (number of adoption 5, 2023/09). The nutrias were kept at private farms in Poltava Oblast, Ukraine. The experiments were carried out on slaughtered 8–12 months-old semi-aquatic rodents (30 individuals). *Trichuris* spp. were collected using the method of complete helminthological dissection of some organs according to Skrjabin (1928). Nematodes were collected from the large and small intestines and kept in 70% ethanol. For histological studies, we gathered samples of the large intestine (Zon et al., 2005; Horalskyi et al., 2019). The nematodes were identified according to the guide (Ryzhikov et al., 1970) and reports (Barus et al., 1975). The specimens were examined and identified using a light microscope Zeiss Axio Imager M1 at the I. I. Schmalhausen Institute of Zoology (Kyiv, Ukraine).

Samples of pathological material were fixated in 10% buffer neutral solution of formalin according to Lillie (1969) and then engulfed in paraffin. We prepared 5 µm paraffin samples and stained them with ready-to-use solutions of hematoxilin and eosin, manufactured by Leica. The obtained preparations were examined and photographed under a MC 100 LED light microscope, equipped with a Canon EOS 550D. The general structure, appearance of the tissues, and specific morphological changes were studied at x 100 and x 400 increases.

Statistical analyses were performed using MedCalc for Windows, version 20.2 (MedCalc Software, Ostend, Belgium, 2022). The number of males and females was expressed as mean arithmetic value ± SE (standard error). The Shapiro-Wilk test was used to check the distribution of indicators for normality. A posteriori comparisons between the groups were performed using the Mann-Whitney criterion. Differences between parameters in the groups were considered significant at $P < 0.05$.

Results

As a result of the conducted copro-ovoscopic studies, we diagnosed trichuriasis in nutrias in the conditions of farms in Poltava Oblast (Ukaine) according to the specific structure of eggs of the *Trichuris* worms (elongated, lemon-like shape, with caps on both poles, well visible membrane) (Fig. 1). Analysis of morphological features of nematodes confirmed presence of only one species of the genus – *T. myocastoris*.

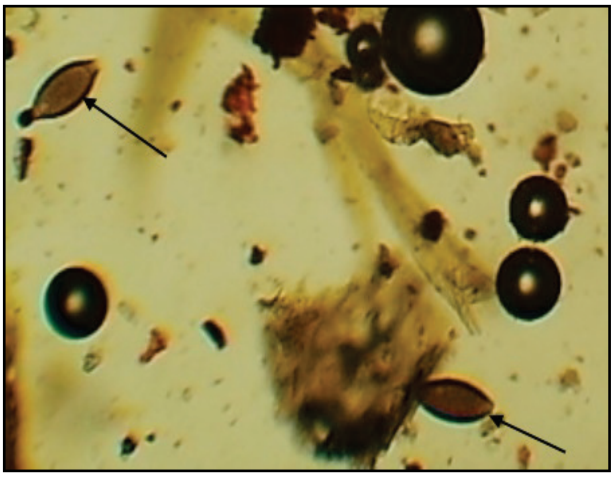

Fig. 1. General appearance of eggs of *T. myocastoris*

In the cavity of large intestine, we found small, spindle-like, white helminths (Fig. 2a). The nematodes had an elongated thread-like frontal part and a thickened, shorter, posterior part (Fig. 2b). The cuticle was finely striped transversally (Fig. 3). The esophagus was thin and elongated (Fig. 4a). This parasite was characterized by pronounced sexual dimorphism. Therefore, the sexual apparatus of females was represented by vulva, vulvar tube, and uterus. The vulva has a slit-like opening without protrusions, leading to an insignificantly bent vagina that gradually turns into the vulvar tube and then into the uterus, filled with eggs (Fig. 5). On the surface of the frontal part of female, there are bubbly cuticular elevations (Fig. 5a). Males of this species have a specific structure of the spicule (Fig. 4b).

The number of collected helminths, according to the results of post-mortem diagnostics of trichuriasis in the nutrias in Poltava Oblast varied depending on season of the year. Therefore, the intensity of invasion in summer ranged 51 to 111 nematode specimens against 10–39 in winter. Taking into account differentiated species features of male and female *T. myocastoris*, we counted them depending on sex. We found that the ratio of male and females *T. myocastoris* depended on season of the year. In the lumen of large intestine of the nutrias (n = 15), there were recorded on average 36.7 ± 2.1 male and 47.7 ± 2.5 female (P < 0.01) nematodes in summer and 11.1 ± 1.3 and 16.5 ± 1.2 (P < 0.01) in winter. Ratio of detected male to female *T. myocastoris* in winter equaled 1:1.5, whereas in summer, it was on average 1:1.3 (Fig. 6).

Histological studies revealed that the epithelial layer of the large intestine was ruined, and the fragments of epithelium were in the intestinal lumen (Fig. 7a). The intestinal glands were enlarged, overfilled with mucous content, which was stained pink, and sometimes basophilically in the glands' deep sections (Fig. 7b).

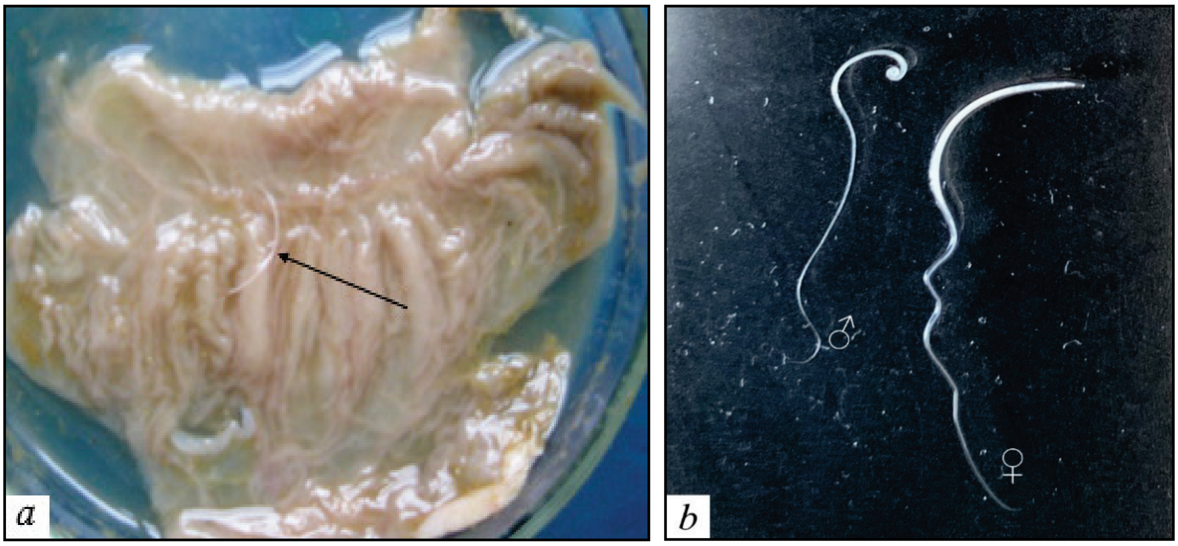

Fig. 2. General appearance of *T. myocastoris* nematodes

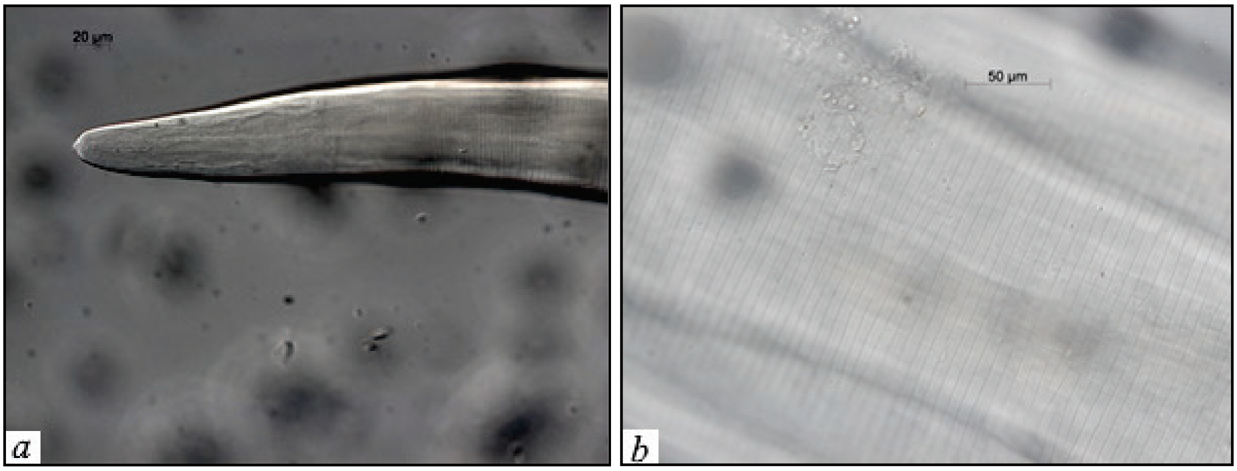

Fig. 3. Morphological structure of female ♀ *T. myocastoris*: *a* – front end; *b* – transversal striping on the cuticle

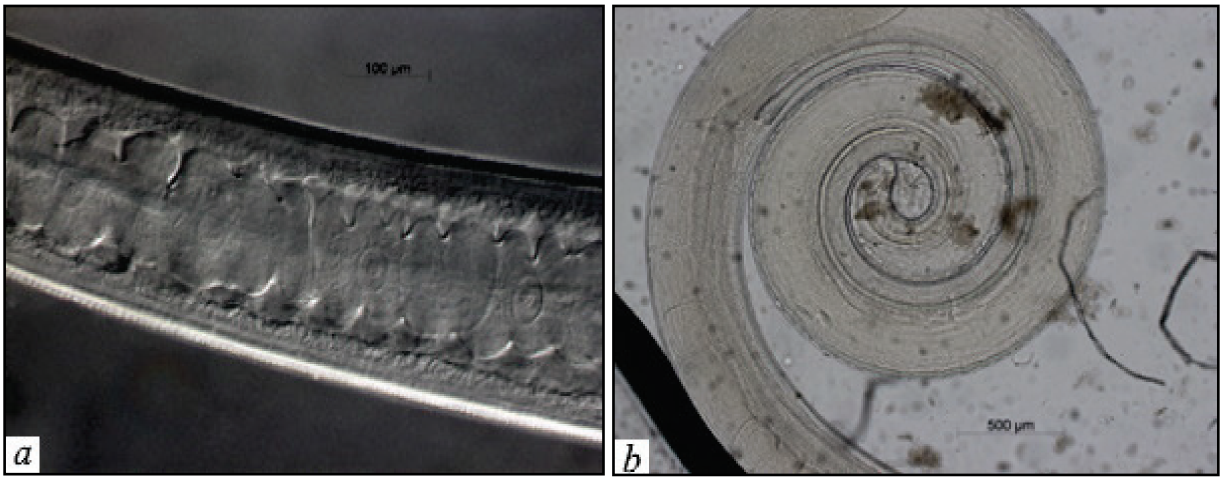

Fig. 4. *T. myocastoris*: *a* – region of esophagus; *b* – tail end of male with spicule

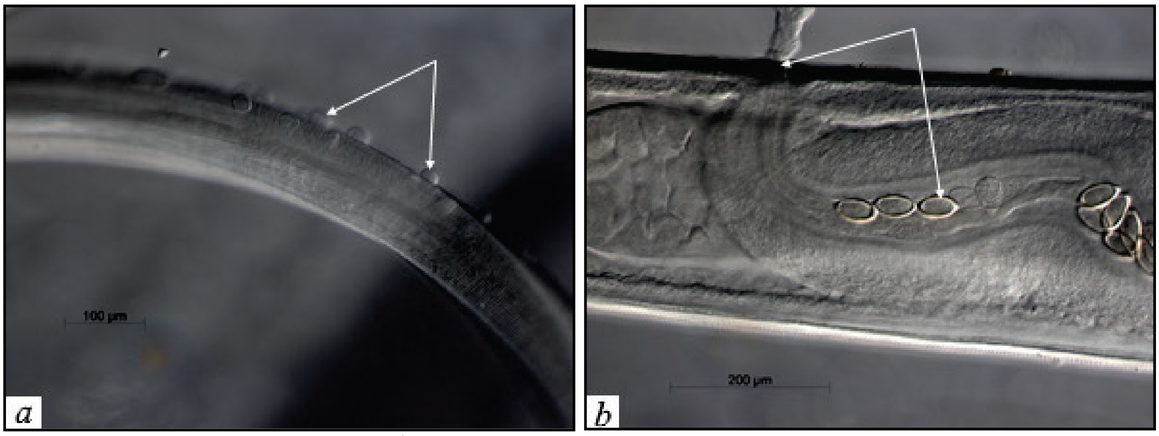

Fig. 5. Morphological structure of female ♀*T. myocastoris*: *a* – cuticular processes on the front end; *b* – region of vulva and eggs

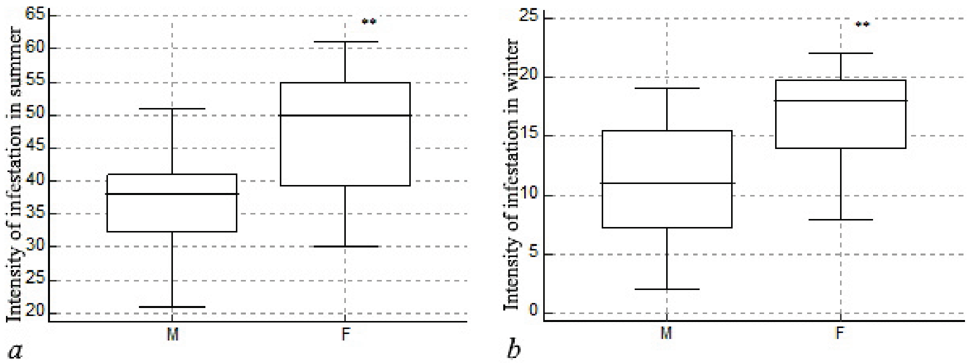

Fig. 6. Ratio of detected male (M) to female (F) *T. myocastoris* (according to the results of post-mortem diagnostics): *a* – in summer (n =15); *b* – in winter (n = 15)

The lamina propria of the mucous membrane, especially between the intestinal glands, was heavily infiltrated by lymphoid cells, and individual lymphoid cells were also found in the lumens of intestinal glands and even in the lumen of the organ. Single lymphoid cells also occurred in the layer of mucous membrane. Under large increases, we saw features of hypersecretion of mucus in goblet cells of the intestinal glands in the form of accumulations of poorly stained mucus granules in cytoplasm. Some goblet cells were ruined, desquamated, and their remains were in the glands' lumens together with mucus (Fig. 7). In the intestinal lumen, there were seen transversal or diagonal sections of bodies of *T. myocastoris* helminths (Fig. 7d, 8). The parasites' cuticle was stained basophilically, and their internal organs eosinophilically. Their internal cavity (coelom) was filled with structureless pink substance (Fig. 8).

Discussion

Nutria (*Myocastor coypus*) is a mammal, semi-aquatic rodent, of the Echimyidae family (Echimyidae subfamily), which lives alongside stretches of freshwater bodies, and swims well. Its body reaches up to 85 cm in length, with a tail of up to 45 cm. The natural range is southern part of South America. Nutrias have acclimated in many countries of the world, including Ukraine. This animal is also considered a neotropical rodent, whose meat is in demand among population. Therefore, timely prediction and diagnosis of nutria parasites are crucial (Saadoun & Cabrera, 2019; Adhikari et al., 2022).

Nutrias grown at farms in the Czech Republic were recorded to have various extent of helmianthiases, measuring 57.0% for *Trichuris* spp., 11.5% for *Strongyloides* spp., and 4.0% for *Trichostrongylus* spp. (Nechybová et al., 2018). Researchers proved that by morphological features, *T. myocastoris* is much closer to trichuriasis of rodents according to metric parameters than to species localized in ruminants. A number of authors recommend considering morphology a necessary tool for differentiation of male *Trichuris* (Garcia et al., 2011; Yevstafieva et al., 2018; García-Sánchez et al., 2019). At the same time, *T. myocastoris* was found to be different from any other species of *Trichuris* spp. of rodents (Rylková et al., 2015). Our results are coherent with the data of many other authors. Specific differentiation differences found in the morphological structure of the parasite body confirmed the circulation of *T. myocastoris* in the Poltava region. At the same time, according to results of estimates of ratio of male to female *Trichuris* spp. depending on season of the year, we found that the number of female nematodes prevailed in winter, forming the degree of environmental contamination with invasive elements.

Experiments on various models of mice (Lawson et al., 2021) gave us the understanding of how helminths interact with a host. The study of *Trichuris muris* as a mouse model of *Trichuris trichiura* in people confirmed the negative effect on the definitive host: there were recorded changes in microbiota, increase in permeability of the intestinal barrier, and inflammatory process was diagnosed (Yousefi et al., 2021). Schachter et al. (2020) found that parasitism of *T. muris* (82.5 specimens) in mice was accompanied by decrease in their mass. Average mass was 36.5 g for control mice and 33.2 g for infected ones. At the same time, there were diagnosed anemia, dysbalance of microbiota, and hypertrophy of the cecum. Histological study of the large intestine of *Trichuris*-infected mice revealed pathological changes in all three intestinal membranes. Therefore, morphometric studies confirmed a significant enlargement of the mucous membrane (466.0 µm), submucous layer (327.0 µm), and the mucous membrane (169.0 µm) in the infected mice. The data obtained by Schachter et al. (2020) correlate with the study by Klementowicz et al. (2012), who also confirmed hyperplasia of goblet cells in case of parasitism by *T. muris* (Klementowicz et al., 2012; Hurst & Else, 2013). Similar pathomorphological changes were diagnosed in the cecum of the rodent *Thrichomys apereoides* parasitized by *T. thrichomysi*. Moreover, the authors indicate that the pathogen was localized exclusively on the surface of the mucous membrane, rather than in deeper layers, forming a tunnel (parasitic niche) (Lopes Torres et al., 2011). Similar data were reported by Tilney et al. (2005), indicating that *T. muris* also infiltrated the epithelium of mucous membrane, ruining lateral membranes, leaving the surface of apical and basal cells undamaged. At the same time, we assumed that pathogenic bacteria may infiltrate the cecum and individual leukocytes through damaged mucous membrane.

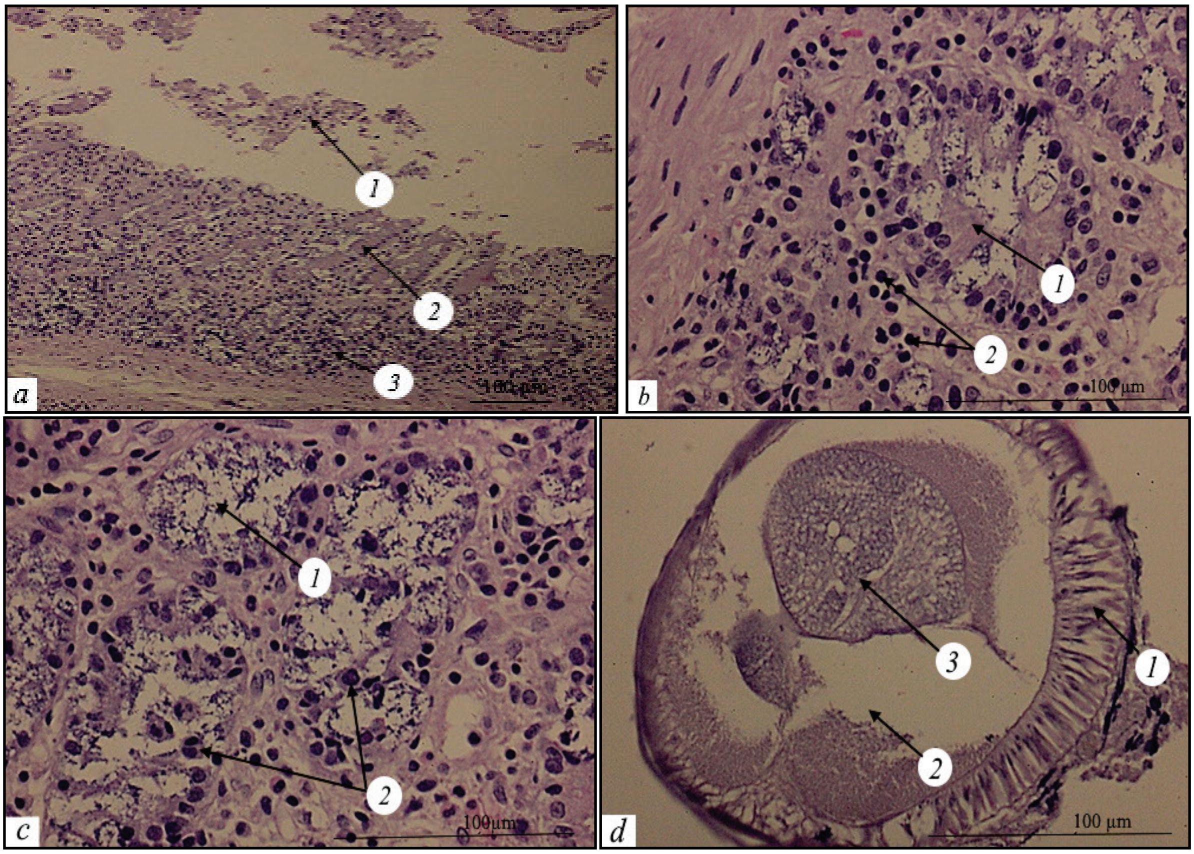

Fig. 7. Large intestine of nutrias: *a* – fragments of ruined epithelium in the intestinal lumen (*1*), overfilling of the lumens with mucus (*2*), lymphocytic infiltration of lamina propria (*3*); *b* – overfilling of intestinal gland with mucus (*1*); lymphocytes in lamina propria (*2*); *c* – basophilically stained mucus (*1*) and desquamated epitheliocytes (*2*) in the lumens of glands; *d* – transversal section of a *T. myocastoris* helminth in the organ's lumen, cuticle (*1*), coelom (*2*), internal organs of helminth (*3*); staining with Carazzi hematoxylin and eosin

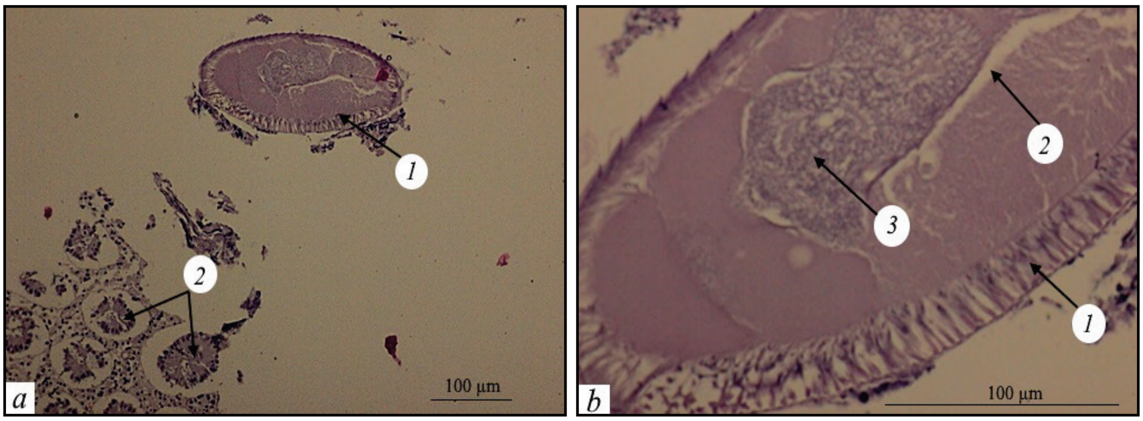

Fig. 8. Large intestine of nutrias: *a* – transversal section of a *T. myocastoris* helminths in the organ's lumen (*1*); intestinal glands (*2*); *b* – transversal section of the helminths *T. myocastoris* in the organ's lumen, cuticle (*1*), coelom (*2*), internal organs of helminths (*3*); staining with Carazzi hematoxylin and eosin

There has been given a considerable amount of evidence that Trichuris that live in the intestines of agricultural animals survive by influencing the organism of definitive host. Researchers found the following pathological histological changes in the cecum of sheep: infiltration of lymphoid cells, eosinophiles, and fibroblasts around the body of the parasite *T. ovis* (Iliev et al., 2017). Morphological changes against the background of trichuriasis have been best researched in swine. Pathanatomical changes in cases of chronic course of swine trichuriasis and high-intensity nematode invasion of the large intestine indicate purulent-diphteria typhlitis, colitis, and proctitis. The disease was accompanied by hyperplasia of lymph nodes, dystrophy of the liver and kidneys, and hyperemia (Zon, 2005). Less expressed changes were observed by Jenkins (1970). The author noted slight cellular ruination in cases of *Trichuris* infection in natural conditions. Negative impact of *T. suis* was pointed out in studies by other researchers, who observed presence of inflammatory process in the cecum and colon of swine on the 21st day of the experiment after inoculation of *Trichuris* eggs. Products of vital activity of helminths, and also proteins and cells of the host that had been altered during pathogenesis, become a powerful immune stimulus and activate the mechanisms of the general and local immunity, both humoral and cellular (Myhill et al., 2018; Dawson et al., 2020). Histological study of the thin and large intestines of swine infested with whipworm revealed a notable eosinophile infiltration in the lamina propria and signs of hyperfunction of goblet cells in the intestinal glands. Cranial parts of nematodes that infiltrated the mucous membrane were found by a number of authors during histopathological study of the intestine (Krag et al., 2006). In general, all existing results of histological studies confirmed the clinical diagnosis of swine trichuriasis (Bünger et al., 2022).

There are very few literature sources where authors report effects of *Trichuris* on nutrias, and likewise, they are now obsolete, including studies by foreign scientists (Enigk, 1933; Jacob, 1947). Changes against the background of nutria trichuriasis, according to the results of our research, are not as heavy as those described by Zon (2005) in his study on swine. Obviously, those cases of helminth-induced damages were exacerbated by inoculation of conditionally pathogenic gut microflora, because purulent and fibrinous inflammations are typical of lesions of bacterial etiology. It has to be noted that the course of disease and severity of *Trichuris*-caused damages depend on the intensity of invasion and how deep into the intestinal wall nematodes had infiltrated with the cranial part, and its possible perforation.

For the first time in Ukraine, we illustrated a transversal section of the helminth *T. myocastoris* in the large intestine and obtained first data on morphology of this helminth according to the results of histological study. Also, we described the influence of the pathogen on the large intestine of nutria, which were kept at farms in Poltava Oblast, Ukraine. We found confirmation of lesions of the mucous membrane of the nutrias' large intestine, damage to the integrity of the blood vessels, and ruination of the epithelium of the mucous membrane. We described the formation of passages, around which small extravasated fluids emerged, and also the lymphoid-histiocytic reaction.

Conclusions

In nutrias suffering chronical course of trichuriasis in the large intestine, a distinct complex of pathomorphologial changes occurs. The examined animals were observed to have signs of chronic catarrhal colitis, which was accompanied by lymphoid-histiocytic infiltration of the intestinal wall. The described changes in the large intestine occur as a result of mechanical and immunological actions of Trichuris towards the mucous membrane, and also as a result of its irritation by the products released from nematodes. Detection and identification of the pattern of pathomorphological changes against the background of nutria trichuriasis allow effective treatment to be developed that would have not only a etiotropic but also pathogenetic component, making the treatment more successful and fostering recovery.

The prospects of using methods of post-mortem diagnostics will help control the degree of invasion at nutria-breeding farms and timely introduction of measures against this disease.

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The authors declare no conflicts of interest.

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