

THE INFLUENCE OF *STRONGYLIDAE* INFECTION ON MORPHOLOGICAL CHANGES OF INTESTINAL TISSUES IN HORSES

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Summary. 12 horses infected with *Strongylidae* helminths only were used for this investigation. These horses were infected mainly with *S. edentatus* and *Cyathostomidae* (11.6±1.4 ir 14.1±2.6 helminths/10 cm², respectively (P≥0.05)). Number of *S. vulgaris* helminths was significantly lower compared to *S. edentatus* and *Cyathostomidae* (3.7±0.2 helminths/10 cm²) (P≤0.05). Histological study of horse intestinal tissues, which were affected by *Strongylidae* larvae, showed local changes in the shape of connective tissue capsule around the cysts in the area of implantation of parasites, tissue oedema, vascular congestion and lymphocytic and eosinophilic infiltration in the wall of the small and large intestines.

Keywords: *Strongylidae*, helminths, horse, intestine, morphological changes.

ARKLIŲ ŽARNŲ, PAŽEISTŲ *STRONGYLIDAE* HELMINTŲ, MORFOLOGINIAI POKYČIAI

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Santrauka. Tyrimas atliktas su 12 arklių, užsikrėtusių tik *Strongylidae* helmintais. Tirti arkliai daugiausiai buvo užsikrėtę *S. edentatus* ir *Cyathostomidae* (atitinkamai 11,6±1,4 ir 14,1±2,6 helmintų 10 cm²) (p≥0,05). *S. vulgaris* nustatyta daug mažiau (3,7±0,2 helmintų 10 cm²) (p≤0,05). Arklių žarnų sienelių audinių, kurie buvo pažeisti *Strongylidae* lervų, histologinis tyrimas parodė jungiamojo audinio kapsulės pakitimus aplink cistas parazitų implantacijos vietose, audinių edemą, kraujagyslių išvešėjimą bei limfocitų ir eozinofilinių granulocitų infiltraciją plonųjų ir storųjų žarnų sienelėje.

Raktažodžiai: *Strongylidae*, helmintai, arklys, žarna, morfologiniai pokyčiai.

Introduction. Helminths-related diseases of horses are widespread throughout the world since ancient times (Schultz and Shikhobalova, 1935; Mirzayans et al., 1974; O'Meara and Mulcahy, 2002; Güiris et al., 2010).

Majority of horses worldwide are infected with more than 45 species of *Strongylus vulgaris*, *S. edentatus*, *S. equinus* and *Cyathostomidae*. These helminths called large and small strongyles are the most common and caused pathological changes in horses of different age.

The adult stages are located in the large intestine and the migrating larval stages – various organs of horses and donkeys. All *Strongylidae* type of helminths are geonematodes which have development without an intermediate host. Sexually mature females produce a lot of eggs, which are excreted with the faeces. Under favourable environmental conditions from eggs in 2-8 days develops L₁ stage larvae, which hatch from egg. In 6-14 days L₁ molts twice and become invasive L₃. Horses as a new

host could be infected with feed or water. Larvae penetrate the intestinal wall and then cause various pathological changes (Kuzmina et al., 2005; Šarkūnas, 2005; Stan-campiano et al., 2010).

The histopathological changes around *Cyathostomide* larvae in the horse gut wall were described by Ogbourne (1978), Jasko and Roth (1984), Giles et al. (1985), Church et al. (1986), Collobert-Laugier et al. (2002), Kharchenko and Kuzmina (2010).

The mucosal and submucosal cysts of caecum and ascendant colon are associated with eosinophilic and lymphocytic infiltration. In the submucosa, the cellular infiltration also includes mast cells (Collobert-Laugier et al., 2002; Debra et al., 2006). The role of mast cells, eosinophils and globule leucocytes in immunity against intestinal nematodes has also been investigated in several laboratory species (Miller, 1986; Rothwell, 1989). The response of naturally infected horses intestinal cells was investigated by Kuzmina et al. (2005).

In many ways invasive process is similar to the infectious process and proceeds with specific features. In this connection it is interesting mechanism of formation of the helminthiasis manifestations of in the form of specific syndromes, which determine the diagnosis and differential diagnosis. The basis of these syndromes and their changes in the dynamics of the disease is a successive combination of developing processes, such as dystrophy, local disturbances of blood circulation and microcirculation, inflammation, allergy, immune deficiencies and etc. (Schultz and Shikhobalova, 1935; Raisov et al., 1985; Skosogorenko et al., 1986; Chebyshev et al., 1998). Depending on the course of pathological process in helminthiasis is determined by the specificity of their pathogenetic and symptomatic therapy (Tsvetaeva 1963; Bogogyavlenskij et al., 1992; Chebyshev et al., 1998; Matthee and McGeoch 2004).

The knowing character of morphological changes in tissue structures of damaged organs can be helpful to make the selection of drugs that combine in one hand, pronounced antihelminthic effect, and the other hand, a minimum toxic effect on the host organism (Skosogorenko et al., 1986; Grishina and Suranova 1988; Bogogyavlenskij et al., 1992).

It is very important to detect the horses infected by *Strongylidae* timely because these helminths become resistant to some antihelminthics (Kaplan, 2002; Bonneau et al., 2009; Kharchenko and Kuzmina, 2010; Lyons et al., 2010).

The aim of this work was to investigate the morphological changes in the tissues of the horse intestine during helminthiasis of the intestinal *Strongylidae* larval stage.

Materials and methods. The material for macroscopic studies was taken from 12 infected Bashkirian horses (5-10 years old) during their slaughter immediately after removing of organs. Helminths were counted in 10 cm² of small and large intestine (5 fields). The species of *Strongylidae* were differentiated by helminths' size and structure of their mouth bucal capsule. Results were analysed using the ANOVA program (Statistica Version 5, StatSoft inc.).

The material for microscopic studies (pieces of 1x1x1 cm in size) was cut out from various parts of small and large intestines. They were fixed in the solution of 10% neutral formaldehyde for 10 days. The fixing solution was replaced twice. Pieces were washed under the water flow for 24 hours. Tissues were dehydrated in the ascending concentrations of ethanol (50%, 60%, 70%, 80%, 96% (1), 96% (2) and 100%). Later they were placed in xylene and were kept in the solution of paraffin and xylol saturated at +37°C. Then tissues were put in paraffin and beeswax solution at +56°C. Serial histological sections (5-7 µm of thickness) were stained with hematoxylin and eosin. Microphotographs of tissues were made from histological slides which were made by the microscope OLYMPUS CX 41.

Results. All investigated horses were infected with *Strongylidae* spp. – *S. edentatus*, *S. vulgaris* and *Cyathostomidae*. The intensity of different animals' infection was variable, but it was quite intensive in all selected horses (tab. 1). These horses were infected mainly with *S. edentatus* and *Cyathostomidae* (11.6±1.4 ir 14.1±2.6 helminths/10 cm² respectively (P≥0.05)). *S. vulgaris* helminths were found in significantly lower amounts (3.7±0.2 /10 cm²) (P≤0.05).

Table 1. **The intensity of investigated horses' infestation with *Strongylidae***

N.	Helminths	N per 10 cm ² (X±Sx)
1.	<i>S. edentatus</i>	11.6±1.4 a
2.	<i>S. vulgaris</i>	3.7±0.2 b
3.	<i>Cyathostomidae</i>	14.1±2.6 c

A:b, b:c; P≤0.05; A:c; P≥0.05

In microscopic investigation, the cysts were detected in wall of the small intestine. They were located in the mucosa, usually no deeper than the muscle plate. They were surrounded by thin connective tissue capsule with a small amount of thin-walled vessels, as well as the infiltration of small lymphocytes and eosinophils.

The close microscopic investigation of cysts in the mucosa of the horse's small intestine showed the visualized kind of an emerging "channel" and a partial desquamation of surface epithelium. In some cases, the signs of mucous membranes necrosis were in the zone of the cyst. The signs of the moderate oedema and plethora diffuse lymphocytic and eosinophilic infiltrations were found in the lamina of the mucosa (Fig. 1).

In the submucosa, the signs of oedema and eosinophilic infiltration were defined in varying degrees also (see Fig. 1). The lymphatic nodules showed signs of follicular hyperplasia with markedly enhanced germinal centres, as well as the phenomena of moderate oedema (Fig. 2) with the presence of eosinophils in their cortical substance.

Analysis of the horse's small intestine microslides indicates that a weakly pronounced oedema of the villi, partial desquamation of surface epithelium with the formation of microerosion were marked in areas of mucosa without macroscopic signs of damage by helminthes.

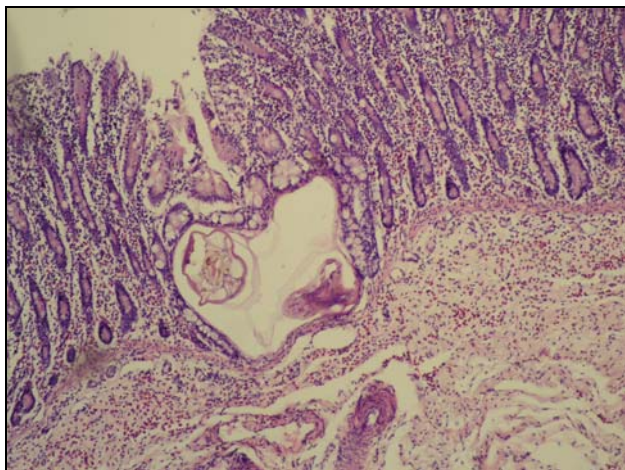


Figure 1. **Section of the horse small intestine (100x)**

Cysts are visible in the small intestine wall. The "channel" is located above the cysts in the mucous membrane. A partial desquamation of surface epithelium is visible in the zone of helminth's implantation. Evidenced lymphocytic and eosinophilic infiltration is observable in the submucosa.

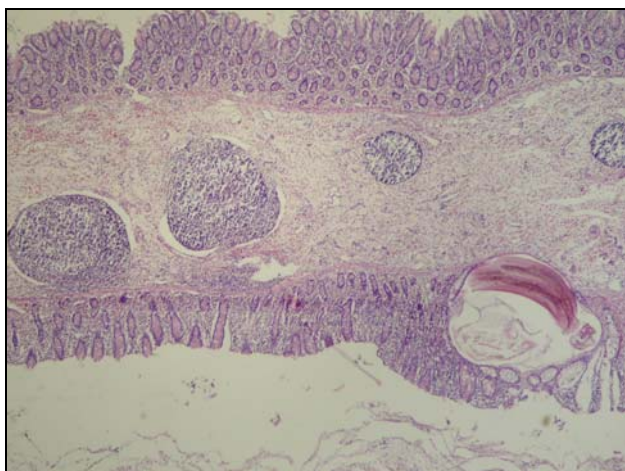


Figure 2. **Section of the horse's small intestine's wall (40x)**

The mucosal cyst is bounded by a thin connective tissue capsule. The oedema, lymphocytic infiltration and hyperplasia of lymphoid follicles are visible in the submucosa.

Villi of intestine had signs of slight oedema, mild lymphocytic infiltration with a small addition of eosinophils. In the deeper parts of the mucosa, the diffuse lymphocytic infiltration was visible that extended a part of the submucosa also.

The signs of a weak oedema were observed in the submucosa. The diffuse lymphocytic and eosinophilic infiltration had varying degrees of intensity (from mild to moderate). Lymphatic nodules which were located in the submucosa of intestine showed the phenomenal follicular hyperplasia with dramatically expanded germinal centres (Fig. 3).

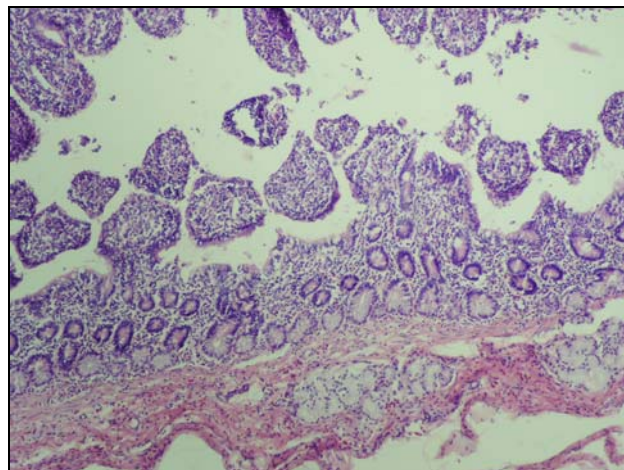


Figure 3. **Section of the wall of the horse's small intestine (100x)**

A weakly villous oedema and partial desquamation of surface epithelium are visible.

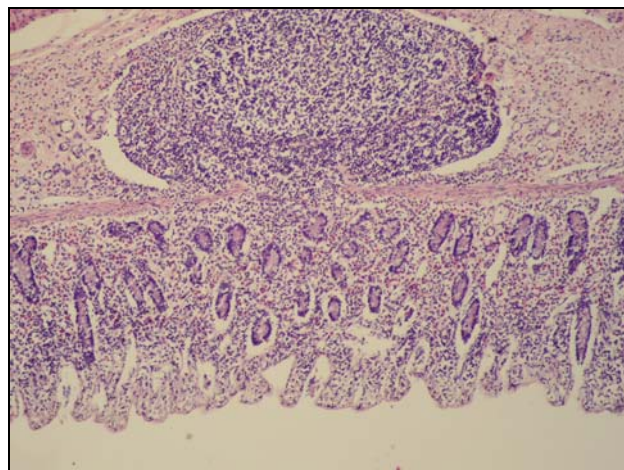


Figure 4. **The section of the horse's large intestine (100x)**

The weak and diffuse oedema, lymphocytic and eosinophilic infiltrations are visible in the mucosa.

The microscopic investigation of the horse's large intestine showed the mucosal oedema outside the location zones of helminthes in surface of crypt. The moderately expressed diffuse lymphocytic and eosinophilic infiltration was visible deeper, which went into submucosa. In submucosa, the signs of a slight oedema were too (Fig. 4). The hyperplasia was visible in the submucosal lymphatic nodules with expansion of germinal centres. The small number of eosinophils recorded in the cortical substance of the regional lymphatic nodules.

Discussion. Detected by us morphological changes in the wall of the intestine of horses with lesions helminthes probably are general biological nature, with no specific features, because the same inflammatory reaction of tissues of the gastrointestinal tract, which accompanied by the presence of eosinophilic infiltrate large numbers of white blood cells, tissue oedema, hyperplasia of the lymphatic nodules and the formation of granulosa structures

also occurred in different species with other helminthes infection (Archer et al., 2006; Kharchenko and Kuzmina, 2010; Lyons et al., 2010). However, according to Chebyshev et al. (1998) granulosa cell composition formations, their structure and their localization depends on the characteristics of a helminthiasis, causing inflammation, although its morphology, they are not always specific, as in the formation of inflammatory granulomas of different origins are involved the same cellular elements in different quantitative ratios: histiocytes, plasma cells, neutrophils and especially eosinophils.

Our findings are consistent with the directions (Bonneau et al., 2009) that the morphological changes in tissues of affected organs are not only in the field introduction of helminthes, but also in areas far away from them, where infection is somewhat less pronounced.

Conclusions

All investigated horses were infected with *S. edentatus* and *Cyathostomidae*, and *S. vulgaris* infection level was significantly lower ($P \leq 0.05$).

There was morphological changes in the wall of the small and large intestine of horses infected with *Strongylidae*.

Local changes were observed directly in the zone of localization of helminthes larvae. The connective tissue capsule was formed around the cysts in the zone of helminthes implantation. The oedema, plethora, lymphocytic and eosinophilic infiltration were detected in the tissues of the small and large intestine. These morphological changes in the wall of horses' small and large intestine showed significant gastrointestinal pathology. Therefore, it must be taken into account in the treatment of horses against gastrointestinal infection of helminthes.

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