Biodiversity of Lumbricidae Intermedial Hosts of Metastrongylides of Pigs in the Belgrade Area

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Abstract
Metastrongylidosis or pulmonary strongylidosis of pigs is a disease caused by several species of nematodes from the genus Metastrongylus. Metastrongylides belong to biohelminths whose causative agents use transitional hosts for their development and maintenance of the biological cycle, in this case numerous species of lumbricides (earthworms). The larvae acquire infectivity only when they eat by earthworms - intermedial host. The seasonal variation of microclimate conditions in the soil has a large part in the life of earthworms, and the inhibitory factors in their seasonal dynamics are directly related to the spread of metastrongylidosis. Overview of research conducted in the five-year period in the area of Belgrade it was established that the dominant species of earthworms which are intermediate hosts to lungworm: *Eisenia foetida, Eisenia rosea, Dandreobena rubida, Allopbophora caliginosa, Allopbophora jassyensis, Lubricus terrestris and Lubricus rubbelus.* Also, species occur as transitional hosts of metastrongylidosis was *Eisenia veneta, Eisenella tetraedra, Allopbophora longa, Octolasium complanatuum, Octolasium lacteum, Octolasium rebeli, Dendrobaena octaedra, Dendrobaena subrubicunda, Dendrobaena mariupoliensis, Bimastus tenius* and less often species from the genus *Heledrillus spp*.

Keywords: earthworms, metsrongylides, pigs.

1. Introduction

Metastrongylidosis or pulmonary strongylidosis of domestic and wild pigs is a disease caused by several species of nematodes from the genus Metastrongylus. A total of 6 species of nematodes were found in this genus: *Metastrongylus elongatus* (syn. *M. paradoxus, M. apri*), *M. pudendotectus* (syn. *M. brevivaginatus Choerostrongylus pudendotectus*), *M. salmi, M. confusus, M. madagascariensis* and *M tschiauricus* [1,2,3,4,5]. Most of them have a cosmopolitan distribution, and *Metastrongylus elongatu* and *M. pudendotectus* are found in our area [6,7,8,9,10]. The occurrence of infection is dominant in environments where extensive animal husbandry (herding, discharge housing) and housing hygiene are practiced [11,12,13]. The most susceptible to infection are young pigs aged 2-8 weeks [14,15,16,17].

Metastrongylides belong to biohelminths, the causative agents of which use transitional hosts for their development and maintenance of the biological cycle, in this case numerous types of lumbricides - earthworms [15,18,19]. The biological cycle of metastrongylids was resolved as early as 1929, when the migratory flow of the larvae was established after the eggs of the parasite reached the external environment through faeces [1,2,5,17,20]. Eggs are very resistant in the external environment and can remain viable for up to 2 years in a moist environment [3,21]. At a

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relative humidity of 95%, individual eggs remain viable for up to 20 months with undiminished vitality, while at low temperatures of -5°C to -8°C, vitality is maintained for up to three weeks. Depending on the external conditions, larvae are released from the eggs, which can survive in the external environment for up to three months, but are not infectious for the real host. The larvae acquire infectivity only when the larvae eat earthworms. Depending on the geographical environment, numerous representatives of lumbricides persist as intermedial hosts. [22,23].

2. Materials and methods

During the study of the biodiversity of pulmonary strongylids of pigs in extensive farming in the territory of Serbia, carried out in the period 2008-2015, earthworms, potential transitional hosts, were also examined. Study of the biodiversity of pulmonary strongylids and its intermediate hosts in the Belgrade area was carried out as a continuation of these studies and performed in the period 2015-2018. Earthworms were determined based on morphological characteristics [24,25,26,27].

3. Results and discussion

During examination in various parts of Serbia, including Belgrade area, these are the dominant species of earthworms: *Eisenia foetida, E. rosea, Dandreobena rubida, Allopbophora caliginosa, All.jassyensis, Lubricus terrestris and L. rubbelus* [19,20], *Eisenia veneta, Eisenella tetraedra, Allopbophora longa, Octolasium complanatum, O. lacteum, O. rebeli, Dendrobaena octaedra, D. subrubicunda, D. mariupoliensis, Bimastus tenius* and less commonly species from the genus *Heledrillus* spp. [19,20].

All mentioned types of earthworms belong to the class *Oligochaeta*. This class includes mostly earthworms, many species that live in fresh water and a small number of marine species. External and internal segmentation are clearly expressed, but without the pronounced external regional specializations that we find in *Polycha*eta [28]. Depending on the species, their size ranges from 0.5 mm to two meters. Unlike *Polychaet*a, their head is reduced and there are no eyes, tentacles or palpus on it [24,26,28]. The prostomium is a small rounded lobe, clearly separated from the peristomium, although in some species they are so fused that they form an inseparable unit forming a multi-segmented head region. They also do not have parapodia, although the chaetae are preserved, which are of different shapes, most often in the shape of the letter s with a thickening in the middle that can be shorter or longer. Short setae are characteristic of terrestrial species and are usually arranged in four groups on each segment ventrally ventralaterally and dorsolaterally. In species from the family *Lumbricidae*, the genera *Lumbricus, Eisenia, Dandreobena* and *Allopbophora* in each segment have eight chaetae arranged two each in four groups, so it is taken as one of the determining parameters [24,29].

The histological structure of coelom is similar to that of *Polychaeta*, namely on the surface there is a single-layered epithelium that secretes a cuticle containing fine pores through which the secretion of single-celled glands scattered in the epithelium is secreted. Beneath the epidermis is connective tissue and radially and transversely distributed muscle layers, next to which are the peritoneum. The septa that divide the coelom have openings through which the nerve chain and blood vessels pass, and the intersegmental fluid flow is regulated by muscular sphincters located between the two layers of the septi-deseptiment. In larger species, the head segment's depressions are elongated backwards in the form of muscle pockets, the contraction of which increases the pressure of the coelomic fluid, which enables easier penetration into the ground. The coelomic cavities are open to the external environment through the dorsal pores through which the coelomic fluid that moistens the body surface comes out and whose flow is regulated by muscular sphincters [1,2,22].

Most burrowing Oligochaeta feed on detritus and in doing so come into contact with metastrongylid eggs and larvae. In worms, the larvae are localized in the walls of the blood vessels of the oesophagus and the foregut, and from there, after maturation, the larvae migrate into the blood vessels. This is made possible by the morphological characteristics of earthworms from the Lumbricidae family. In them, as in other Oligochaeta, the intestinal tract is a real tube with pronounced regional differentiation. It starts with the oral opening in the segment behind the prostomium and continues into the oral cavity and

that into the muscular oesophagus, which in Lumbricidae has a protease enzyme [12,36]. There are also calcium glands in the oesophagus, the surface of which forms pockets that are connected to the lumen of the oesophagus by channels. The walls of the calcium glandular surfaces are highly vascularized and represent the entry point for metastrongylid larvae [1,2,27].

The blood system is a closed type and is built from a dorsal vessel that is separated from the intestinal sinus and extends the entire length of the body, then from a lateral sub-neural blood vessel and from two blood vessels laterally from the nerve chain. Dorsal and ventral blood vessels are connected by lateral blood vessels in each segment, whereby the dorsal blood vessel and lateral vessels play the role of a pulsating organ (heart) [28]. Pulsating blood vessels have endothelium valves that regulate the flow of blood so that it flows from the back to the front of the body in the dorsal vessel and vice versa in the ventral vessel. From the main blood vessels, the blood is drained by smaller vessels into the body wall and other organs, where it spills into the capillary network. This type of vascularization allows excellent even distribution of metastrongylid larvae throughout the worm's body.

The degree of infection of earthworms is directly dependent on the species and their biological characteristics - the place of life above all. Namely, some earthworms such as *Eisenia foetida* lives in the surface layers of the soil, so their contact with parasite eggs and larvae is much more frequent than species that have vertical migration. According to research by Tričković [23] the species *E.foetida, E.rosea* and *Lubricus rubbelus* are most often found localized around manure pits and stables, while other species are found in orchards, yards and pastures, and they are usually infected with a smaller number of larvae in contaminated areas [1,30,31]. Earthworms are most abundant in moist and loose soil that is rich in humus and animal waste, where hundreds of earthworms per m2 can be found. Seasonal variation of microclimatic conditions in the soil has a large role in the life of earthworms and inhibitory factors in their seasonal dynamics are directly related to the spread of metastrongylidosis [19,20,32]. In worms, the larvae are localized in the walls of the blood vessels of the oesophagus and the foregut. Here, they change their clothes twice and in 10-25 days they develop into an infectious form. The speed of development is directly dependent on the external temperature, so they have the fastest maturation at a temperature of 22-23^{o}C (up to 15 days), at 15-16oC it takes place in 21 days and at 10-11^{o}C in 219 days [1,2,19,27]. Bioclimatic conditions affecting earthworms are directly related to the development of metastrongylid larvae. After maturation, the larvae migrate into the blood vessels of the earthworm and remain infective there for up to 7 years. The larvae never spontaneously leave the earthworms. Only in case of damage to earthworms (cutting during tillage, etc.) do they come out of the earthworm and from there reach the surface layers of the soil, where depending on humidity they can live up to 2 weeks. The average number of larvae in infected worms ranges from 33.7 in *E.foetida* to 4.1 in *All.caliginosa* [20,27].

Pigs become infected orally when they eat infected earthworms [33,34]. From the pig's digestive tract, the larvae reach the mesenteric lymph nodes, where they moult and reach the bloodstream and lungs via the right heart via the lymphatic system. In the bronchi and bronchioles, the larvae reach the adult stage after 24 days [34,35]. Affected animals show signs of dyspnoea, frequent vesicular breathing, a weak and later hoarse cough that takes the form of an attack when the animals are stressed [36,37]. Mucous membranes are pale, appetite is reduced and eosinophilia is present in the blood. Body temperature is elevated only when secondary infections are present [11,38,39,40]. The predilection site of the parasite is the rear parts of the diaphragmatic lobe - margo acutus and margo obtusus, and in case of severe infections, other parts of the lungs. Bronchiolitis, bronchitis, diffuse pneumonia, alveolar emphysema, and connective tissue growth and cellular infiltration of macrophages, eosinophils, lymphocytes, olimorphonuclear leukocytes, and giant cells are noted. Certain groups of lobules are voluminous, grey-white, lobular emphysema is present, and the changes are wedge-shaped, at the base of which is a bronchus filled with parasites [11,36]. In bronchioles and bronchi, we find parasites in various stages of development either free in the mucous exudate or surrounded by cellular infiltrate [11,21].

4. Conclusions

The study of i hosts of certain parasitic infections provides significant data on the potential possibility of their spread. The study of helminths of transitional hosts of swine pulmonary strongyloidosis is an under-researched field that should focus more attention and continue this research.

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