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EARTHWORMS (LUMBRICIDAE) AS INTERMEDIATE HOSTS OF LUNG NEMATODES (METASTRONGYLIDAE) OF SWINE IN KYIV AND ZHYTOMYR REGIONS OF UKRAINE

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Earthworms (Lumbricidae) as Intermidiate Hosts of Lung Nematodes (Metastrongylidae) of Swine in Kyiv and Zhytomyr Regions of Ukraine. Antipov, A. A., Bakhur, T. I., Feshchenko, D. V., Romanishina, T. A., Avramenko, N. V., Goncharenko, V. P., Zghozinska, O. A., Solovyova, L. M., Koziy, N. V., Pidborska, R. V., Shahanenko, V. S., Dzhmil, V. I., Tyshkivska, N. V. — The article presents the data on prevalence and intensity of earthworms' infection with metastrongylid larvae in pork production enterprises of Kyiv and Zhytomyr Regions of Ukraine. In the investigated areas, six species of lumbricids were collected and identified: *Eisenia fetida* (Savigny, 1826), *Aporrectodea rosea* (Savigny, 1826), *A. caliginosa* (Savigny, 1826), *Dendrodrilus rubidus* (Savigny, 1826), *Lumbricus rubellus* (Linnaeus, 1758), *L. terrestris* (Linnaeus, 1758). The studies were carried out from April to September, 2016, both in pig farms and on pastures. We observed no pronounced changes in prevalence and intensity of infection during the warm period of the year. The highest infection rates were detected in earthworms living under the wooden floor of pigpens and in its cracks. In the studied localities, metastrongylid larvae were found in the most common worms of the Lumbricidae family: *E. foetida, D. rubidus, A. caliginosa*, and *L. rubellus*.
Key words: Lumbricidae, *Metastrongylus*, intermediate host, prevalence, intensity, *Eisenia foetida, Aporrectodea rosea, A. caliginosa, Dendrodrilus rubidus, Lumbricus rubellus, L. terrestris.*

Introduction

Anthropogenic environmental transformation destabilizes natural ecosystems resulting in severe epizootics of sinanthropic helminthiases (Dovgiy, 2012). The implementation of dehelminthization only cannot prevent the reinfection of susceptible animals if the infective stages (helminth eggs and larvae) are able to survive in the environment (Koryachkov et al., 2009).

On the territory of Kyiv and Zhytomyr Regions, the family Lumbricidae Claus, 1876, is represented by 11 species of earthworms belonging to the genera *Aporrectodea* Orley, 1885; *Eisenia* Michaelsen, 1900; *Eiseniela* Savigny, 1826 and *Lumbricus* Linnaeus, 1758 (Goldin, 2009). Lumbricids are potentially able to form permanent foci of helminthiasis of pigs due to their participation as intermediate and paratenic hosts in life cycles of metastrogylid nematodes.

According to the results of our previous studies (Feshchenko & Zghozinskaya, 2014; Antipov et al., 2016), the greatest danger of metastrongylid infection is observed in the pig farms practicing keeping of animals on summer paddock or in livestock houses equipped with wooden flooring, and with manual removal of manure.

Metastrongylus spp. (Nematoda, Metastrongyloidea) are rather common lung parasites of pigs in Ukraine (Antipov, 2017). The development of these nematodes needs the participation of various species of earthworms as intermediate hosts (Anderson, 2000; Dunn, 1955, Poinar, 1978). The problem of metastrongylosis in pigs is relevant for veterinarians, farmers, scientists and even medical doctors in the Americas (Calvopina et al., 2016), China (Li et al., 2016), India (Rama Devi et al., 2011) and Europe (Nosal et al., 2010, Neghina et al., 2011, García-González et al., 2013).

In Ukraine, on the territory of Polissya, Steppe and Forest-steppe regions, infection of pigs with lung nematodes occurs mainly on the territory of farms, with participation of earthworms *Eisenia foetida*, *Aporrectodea caliginosa*, *Lumbricus rubellus*, *L. terrestris* in the life cycle of metastrongylid nematodes. This is due to the presence of earthworms in pens, poorly-equipped barnyards near pig farms and even in livestock buildings (Antipov, 2016).

The regional peculiarities of lumbricid biology as a part of the epizootiology of swine helminthiasis in Zhytomyr and Kyiv Regions are still not studied. In view of this, we investigated the infection of the various species of earthworms with eggs and larvae of lung nematode *Metastrongylus elongatus* in connections with the seasonal weather and climatic conditions, and with the particular site of sample collection.

Material and methods

The studies were carried out in eight pork production farms in Kyiv and Zhytomyr Regions. At all those farms, metastrogylid infection of pigs has been previously detected.

To study the seasonal dynamics of environment contamination with lung threadworm larvae, the earthworms were collected during warm season (April–September, 2016) in places accessible to pigs such as soil, manure and organic remains of pigsties, on pig farms, in pens and on pastures, using the method described by I. I. Malevich (1950). Altogether, 3,300 earthworms were collected on various sites, approximately 550 specimens per month.

The dependence of earthworm infection by lung threadworm larvae on the place of sample collection was studied in June, 2017. For this purpose, 2,970 specimens of earthworms were collected in pigsties and pens, on pig farms and on pastures. Before fixation, the earthworm intestines were cleared by placing the earthworms into Petri dishes with saline solution and damp cotton wool for 12 hours. For short-term storage (up to 24 hours), the earthworms were fixed with 10 % formalin solution or 40 % ethanol. The earthworms were identified to the genus and species levels using the light microscope Biomed XSM-20. To detect, identify and count the nematode larvae, the compressors and trichinoscopes MBU-6 were used.

The digital data were processed biometrically using the Microsoft® Excel 2016 program using methods of variation statistics and the Student–Fisher tables. The arithmetic mean of the data (M) and its standard error (m) — M ± m, were calculated. The intensity of infection per one earthworm and the prevalence of infection (in %) were calculated.

Results and discussion

Of 3,300 earthworm specimens collected, 923 (28 %) belonged to the species *Eisenia foetida* (Savigny, 1826), 267 (8 %) — to *Aporrectodea rosea* (Savigny, 1826), 598 (18 %) — to *Aporrectodea caliginosa* (Savigny, 1826), 115 (3 %) — to *Dendrodrilus rubidus* (Savigny, 1826), 760 (23 %) — to *Lumbricus rubellus* (Linnaeus, 1758) and 637 (20 %) — to *Lumbricus terrestris* (Linnaeus, 1758) (fig. 1).

The seasonal dynamics of levels of infection in earthworms are shown in figures (figs 2 and 3). The infection did not show any pronounced seasonality in the warm period of the year. The differences in levels of earthworms' infection with nematode larvae were not statistically significant both on average (prevalence was from 35.1 to 43.5 %; intensity was from 8.8 ± 1.6 to 11.5 ± 1.9 specimens), as well as for data collected in different months ($p > 0.05$).

The slight increase in prevalence and intensity of earthworm infection detected in September was associated with a certain accumulation of the nematode larvae during the warm period of the year. Thus, the pattern of prevalence and intensity of earthworm infection by lung nematode larvae from April to September, probably, showed the lack of expression in the dynamics of the level of these indicators during the whole warm period of the year.

The earthworm species observed in the present study showed different ecological preferences. *Aporrectodea caliginosa* and *L. rubellus* are better adapted to pastures, pens and pig farm premises, whereas *E. foeida* and *D. rubidus* prefer to accumulate in organic

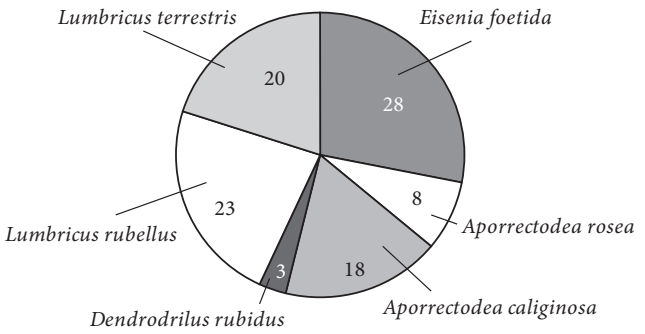

Fig. 1. The ratio (in %) of earthworm species found on the territory of the pig farms of Kyiv and Zhytomyr Regions of Ukraine in April–September, 2016 (n = 3,300).

matter (on the floor and under feeders, in cracks of livestock buildings). Therefore, in June 2017, the lung threadworm infection of earthworms was studied taking into account the place of their collection at farms.

Five earthworm species (*A. caliginosa*, *A. rosea*, *D. rubidus*, *E. foetida*, and *L. rubellus*) were identified in soil, manure, and organic matter of pigsties, territories of pig farms, pens and pastures at eight pig farms in Zhytomyr and Kyiv Regions of Ukraine. Nematode larvae were found in 1,244 of 2,970 collected earthworms (prevalence = 41.9 %, intensity = 20.9 ± 5.8). Earthworms of four species were found to be infected: *A. caliginosa* (prevalence = 26.2 %, intensity 26.3 ± 9.2), *D. rubidus* (prevalence = 42.2 %, intensity 13.7 ± 2.0), *E. foetida* (prevalence = 56.15%, intensity 25.3 ± 4.8), and *L. rubellus* (prevalence = 23.45%, intensity 14.9 ± 5.6).

The studied specimens of *A. rosea* and *L. terrestris* were not infected with the lung threadworm larvae; only *A. caliginosa, L. rubellus*, *E. foeida* and *D. rubidus* were infected (figs 4 and 5).

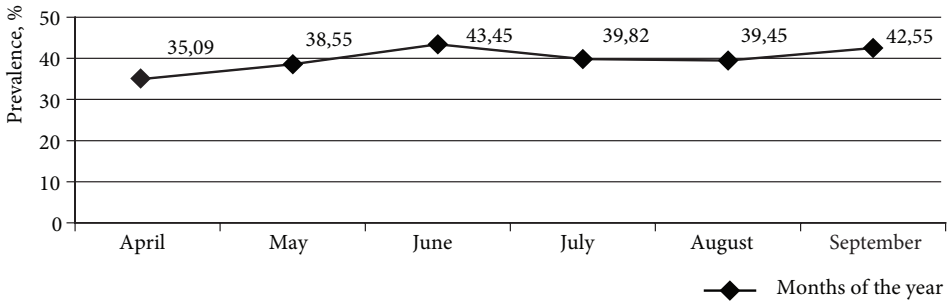

Fig. 2. Seasonal dynamics in prevalence of the metastrongylid larvae in earthworms from Kyiv and Zhytomyr Regions of Ukraine in 2016 (n = 3,300).

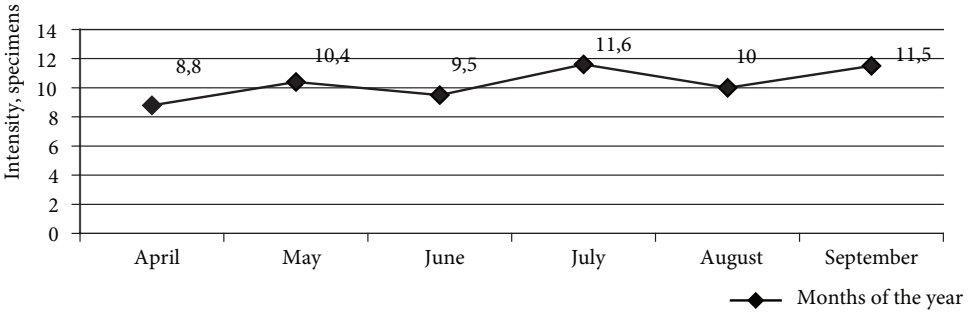

Fig. 3. Seasonal dynamics of the mean intensity of the earthworm infection with metastrongylids in Kyiv and Zhytomyr Regions of Ukraine in 2016 (n = 3,300).

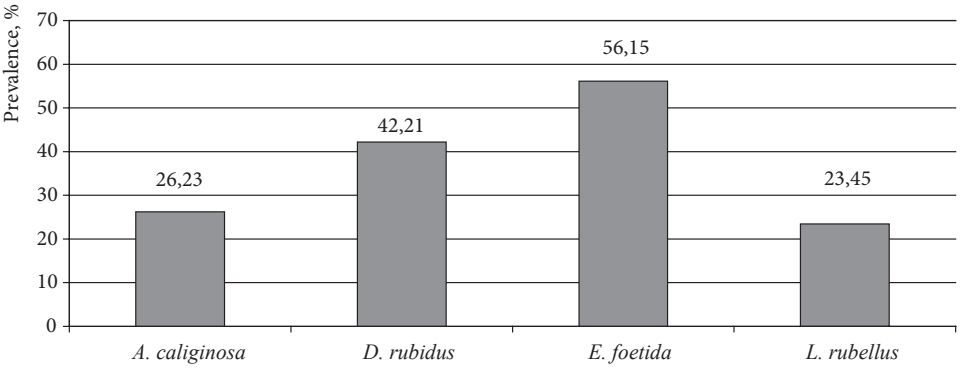

Fig. 4. Prevalence of metastrongylid infection in separate earthworm species at pig farms of Kyiv and Zhytomyr Regions of Ukraine (n = 2,970).

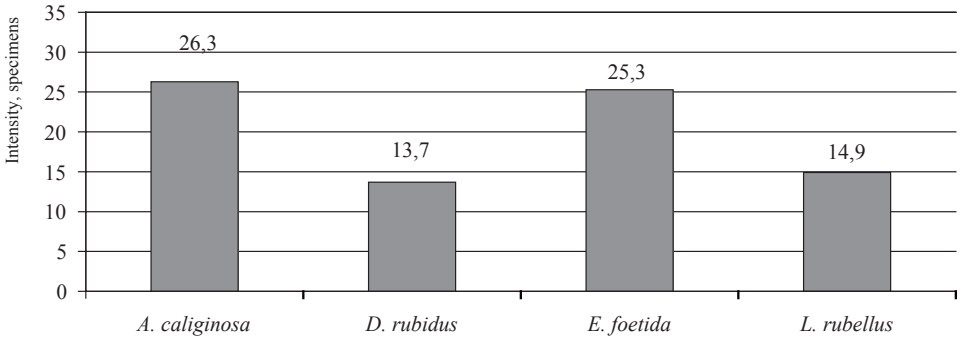

Fig. 5. Intensity of metastrongylid infection in separate earthworm species at pig farms of Kyiv and Zhytomyr Regions of Ukraine (n = 2,970).

The rates of metastrongylid infection and the species composition of earthworms collected in different types of environment (pigsties, premises of the swine enterprises, pens and swine pastures) are summarized in table 1.

Two species of earthworms: *E. foetida* and *D. rubidus*, were collected in the pigsties (1,160 specimens). These species were infected by lung nematode larvae more than earthworms from other places: infection prevalence in *E. foetida* was 70.3 %, with intensity of 28.3 ± 2.8 specimens; prevalence in *D. rubidus* was 54.8 % and intensity 14.6 ± 1.3 specimens.

Four species of earthworms: *E. foetida*, *D. rubidus*, *A. caliginosa*, and *L. rubellus* were found in soil, manure and organic remains of the premises of the swine enterprises.

Earthworms are an important component of soil fauna on the territory of Eastern Europe and especially Ukraine, which owns a third world reserve of fertile land. The last fact is largely due to the spread and vital activity of earthworms. Areas with black and forest soils, as well as peat-lands (including the territory of Kyiv and Zhytomyr Regions) are ecologically optimal zones for the evolutionary development of variable populations of earthworms. However, a complete inventory of species of worms in Ukraine taking into account the zonal features (Polissya, Forest-steppe, Steppe, etc.) has not yet been carried out. Only fragmentary data regarding the biology of earthworms on the territory of Ukraine are published (Ivanciv, 2007; Cron et al., 2010; Buslenko & Schepna, 2011). At the same time, influence of the anthropogenic factors and global climate change, the taxonomic features of the earthworm species can be different (Novo et al., 2011; Blakemore, 2016).

In our studies, we used museum collections of earthworms to identify the species found and based on their morphological features. All specimens of 6 species found in our study (*E. foetida, A. rosea, A. caliginosa, D. rubidus, L. rubellus* and *L. terrestris*) fully corresponded to the classical identification keys (Malevich, 1950).

Table 1. The rates of infection of the earthworms by lung threadworm larvae in different types of environment

Place of earthworm collection	Earthworm species	Number of earthworms	Prevalence, %	Intensity, specimens
Pigsties	*Eisenia foetida*	580	70.3	28.3 ± 2.8
	Dendrodrilus rubidus	580	54.8	14.5 ± 1.3
	TOTAL	1160	62.6	22.3 ± 2.4
Soil of the swine enterprises premises	*Eisenia foetida*	336	44.1	17.7 ± 4.6
	Dendrodrilus rubidus	210	21.4	11.1 ± 3.3
	Aporrectodea caliginosa	255	16.9	9.7 ± 2.9
	Lumbricus rubellus	174	14.9	5.4 ± 1.5
	TOTAL	975	26.9	14.0 ± 2.2
Pens	*Eisenia foetida*	160	39.4	24.1 ± 6.6
	Dendrodrilus rubidus	160	23.8	8.5 ± 2.5
	Aporrectodea caliginosa	225	26. 7	24.9 ± 8.4
	Lumbricus rubellus	130	31.5	15.7 ± 5.3
	TOTAL	675	29.9	19.7 ± 2.9
Pastures	*Eisenia foetida*	30	6.7	2.0 ± 1.0
	Dendrodrilus rubidus	30	–	–
	Aporrectodea caliginosa	50	72.0	48.4 ± 5.9
	Lumbricus rubellus	50	32.0	28.4 ± 5.9
	TOTAL	160	33.8	40.7 ± 13.4

It is known that earthworms are included in the biological cycles of several helminths including *Metastrongylus* spp., *Filiponema* spp. (Nematoda: Drilonematoidea), *Ascaris suum* (Nematoda, Ascaroidea), *Heterakis gallinarum* (Nematoda, Heterakidae), etc. In Ukraine, where pig production is traditionally well developed and the private agricultural sector is very large, precise knowledge on the specific and seasonal features of the earthworm infection by the *Metastrongylus* larvae, which cause parasitic bronchitis in pigs, is needed.

According to the present findings, in two regions of Ukraine, four earthworm species, *A. caliginosa, D. rubidus, E. foetida*, and *L. rubellus* are the intermediate hosts of lung nematodes. The leading role in the transmission of metastrongylids belongs to *E. foetida* in pig pens, sties and farms, and to *A. caliginosa* at pastures.

Conclusions

1. Six earthworm species were found on the territory of Zhytomyr and Kyiv Regions of Ukraine: *Eisenia foetida* (28 %), *Aporrectodea rosea* (8 %), *Aporrectodea caliginosa* (18 %), *Dendrodrilus rubidus* (3 %), *Lumbricus rubellus* (23 %) and *Lumbricus terrestris* (20 %). Four of these species, *A. caliginosa, D. rubidus, E. foetida* and *L. rubellus* were found to be the intermediate hosts of lung threadworms.

2. Prevalence and intensity of metastrongylid infection in earthworms was stable during the whole warm season, from April to September (prevalence 35–43 % and intensity 8.8–11.6 specimens, respectively).

3. The role of various earthworms in transmission of lung nematodes was found to be different; in general, *E. foetida* was the species most affected by the metastrongylid larvae. At pig farms, the highest rate of infection with the larvae was observed in *E. foetida* (prevalence 39.4 % to 70.3 %, intensity 17.7 ± 4.6 to 28.3 ± 2.8); on pastures *A. caliginosa* was found to be the most infected (prevalence 72 %, intensity 48.4 ± 5.9). Larvae of lung nematodes were not detected in *A. rosea* and *L. terrestris*.

4. The prevalence of infection of earthworms at pig farms was on average 41.89 %, and intensity was 20.9 ± 5.8 specimens. The worms most infected with the larvae were found under the wooden floor of pigsties and in cracks of buildings (prevalence 62.6 %, intensity 22.3 ± 2.4). The average rates of metastrongylid infection in earthworms were the following: on pastures — prevalence = 33.8 %, intensity = 40.7 ± 13.4, in pens — prevalence = 29.9 %, intensity = 19.7 ± 2.9, on territories of the swine enterprises — prevalence = 26.9 %, intensity = 14.0 ± 2.2 specimens.

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