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OCCURRENCE OF *DIROFILARIA IMMITIS* (NEMATODA, ONCHOCERCIDAE) IN RED FOXES (*VULPES VULPES*) FROM THE SUBURBS OF KHARKIV (UKRAINE)

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Occurrence of *Dirofilaria immitis* (Nematoda, Onchocercidae) in Red Foxes (*Vulpes vulpes*) from the Suburbs of Kharkiv (Ukraine). Liulin, P. V., Prykhodko, Yu. O., Mazannyi, O. V., Fedorova, H. V., Nikiforova, O. V., Kryvoruchenko, D. O. — Dirofilariasis of animals and humans is a disease caused by nematodes of the genus *Dirofilaria* (Railliet et Henry, 1911). The role of wild predators, particularly the red fox (*Vulpes vulpes*) (Linnaeus, 1758) in the distribution of pathogen of the genus *Dirofilaria* in the world remains unclear. We studied the occurrence of *Dirofilaria immitis* (Leidy, 1856) in a population of the red fox. Twenty-seven foxes were examined during the winter hunting season of 2019–2020 in Dergachi district, Kharkiv region of Ukraine. All 27 examined foxes were older than 1.5–2 years old, 5 of them (18.5 %) were 4–5 years old. *Dirofilaria immitis* was found in 22.2 % of foxes. The intensity of infection ranged from 2 to 4 specimens of nematodes per host. In four foxes (66.7 % of infected hosts), the same number of male and female nematodes was detected, while two foxes (33.3 %) had each 1 male and 2 females of *D. immitis*. The length of *D. immitis* males (7 specimens) was 13.96 ± 0.29 cm and the length of females (9 specimens) was 19.58 ± 0.55 cm. Prevalence of infection of foxes with *D. immitis* presumably depends on their habitat and the presence of mosquitos (vectors of the nematode) in these areas.

Key words: dirofilariasis; *Dirofilaria immitis*; fox; Kharkiv, Ukraine.

Introduction

From twenty six species of the genus *Dirofilaria* two species are recorded in Ukraine. The most pathogenic species is *Dirofilaria immitis* (Leidy, 1856), whose mature stage lives in the heart, pulmonary artery, and other blood vessels in domestic and wild carnivorous animals as well as humans (Arkipov et al., 2004).

Definitive hosts are infected with participation of obligate biological vectors, numerous species of blood-sucking mosquitoes (Ionică et al., 2017).

In Ukraine, dirofilariasis of wild canids, such as foxes and wolves, caused by *D. immitis* and *D. repens* was first recorded in Crimea (Kadenatcii, 1957). The parasites were considered to be imported from tropical and subtropical areas. Up to date, there are reports of 264 cases of dirofilariasis in human (Maiboroda, 2004; Bodnya, 2006). These days it is a steady tendency in Ukraine, and in some regions, the situation becomes drastic as the number of infected dogs (Kryvoruchenko et al., 2019; Nikiforova & Reshetylo, 2019), cats (Vinokurova, 2011) and humans increased significantly (Filipsova et al., 2016). Dirofilariasis caused by *D. repens* has become a serious medical problem in Ukraine. The number

of people affected by dirofilariasis exceeded 1500 cases by 2014 (Salamatin et al., 2013; Pavlikovska et al., 2014). During the same time period (1998–2010), domestic dogs and cats were examined in the Kyiv region. Both species of dirofilaria were recorded. *D. repens* was found in cats and dogs, and *D. immitis* in dogs only (Varodi et al., 2017).

The role and impact of wild predatory animals, particularly red foxes (*Vulpes vulpes*) in the transmission and the spread of dirofilariasis in the world remain unstudied (Ionică et al., 2017).

The purpose of the work was to investigate the occurrence of *D. immitis* in the population of foxes in the vicinity of Kharkiv, Ukraine.

Material and methods

The study was conducted in the Department of Normal and Pathological Morphology and in the Scientific Laboratory of the Parasitology Department of Kharkiv State Zooveterinary Academy. The material for the research was 27 dead foxes (*Vulpes vulpes*), which were shot during the winter hunting season in 2019–2020. The foxes were collected in six settlements located in suburbs of Kharkiv (fig. 1).

Anamnestic data, sex and age of animals, and sampling areas were taken into account.

Prior to dissection, the dead foxes were examined by veterinarians of the State Food and Consumer Service of suburbs of Kharkiv city, and negative results of virologic examination for rabies were obtained. Subcutaneous tissue, body cavities, heart, pulmonary arteries, and other blood vessels were thoroughly examined. The site, number, and sex of helminths were recorded. The infection rates were established including the prevalence (P, %), and the intensity of the infection calculated as the number of helminths per host. Helminths were identified following Skryabin et al., 1949

Results

Six animals, of which 2 males and 4 females, were infected with *D. immitis*. Hence, the prevalence of infection was 22.2 %. All collected foxes were older than 1.5–2 years old, and 5 of them (18.5 %) were 4–5 years old. Eight foxes were obtained from the meadows and wetlands of Lopan river floodplain near the settlements Mala Danylivka, Luzhok, and Caravan of Malodanylivska united territorial community (UTC) (table 1). There, prevalence (P) was 50 % (fig. 2). Four other foxes were collected on wetlands and meadows near Solonytsivka and Vilshany villages. Only one of those was infected with *Dirofilaria* (P = 25 %).

Only one of 15 foxes obtained on dry land in fields, forest belts at a distance of 3–5 km from Dergachi to Zolochiv settlement, was infected with *D. immitis* (P = 6.7 %). Presumably, intensity of infection in foxes could depend on the presence of fox and mosquito (intermediate hosts of dirofilaria) habitats. In the natural ecosystem with meadows and wetlands the intensity of infection of foxes reached 50 %. In infected hosts, 16 specimens of

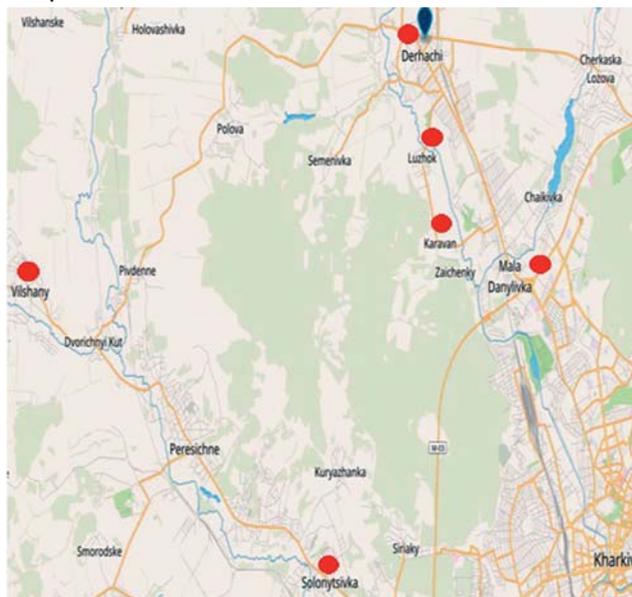


Fig 1. The sites of fox collection in the suburbs of Kharkiv City.

D. immitis were found. They localized in the right ventricle (*ventriculus dexter*) of the heart, pulmonary trunk (*truncus pulmonaris*) and pulmonary arteries.

Species and sex of nematodes were identified according to morphological and morphometric parameters.

The nematodes are white to light yellow. The head (fig. 3, A) and tail ends (fig. 3, B, C) of body are thinner than the mid-region in females and males. The tail end of male is curved as a corkscrew (fig. 3, D). Seven of 16 collected nematode specimens were males (43.8 %). The length of body in males was 13.96 ± 0.29 cm (from 13.2 to 15.3 cm), the width of body was 0.59 ± 0.02 mm. Males had two spicules of different size (fig. 3, E), the right spicule was

Table 1. The results of detection nematodes in hearts of foxes (n = 27, M ± m)

N	Fox gender, (♂, ♀)	Fox age, years old	<i>D. immitis</i> , number		<i>D. immitis</i> ♂		<i>D. immitis</i> ♀			
			♂	♀	length, cm	width, mm	length, cm	width, mm		
Dergachi										
1	♂	2	1	1	15.3	0.6	18.6	0.9		
Malodanylivska UTC Mala Danylivka settlement										
2	♀	1.5–2	1	1	13.3	0.55	20.5	0.85		
3	♀	2	1	1	13.3	0.55	17.4	0.85		
Luzhok village										
4	♂	3	2	2	13.3	0.5	21.6	1.0		
					14.0	0.6	18.0	0.9		
Karavan village										
5	♀	3	1	2	14.5	0.7	18.7	0.9		
							21.3	1.0		
Other localities Solonytsivka, Vilshany settlements										
6	♀	2	1	2	14.1	0.6	18.6	0.9		
								21.5	1.0	
Total			♂ — 11	♀ — 16	7	9	13.96 ± 0.29	0.59 ± 0.02	19.58 ± 0.55	0.92 ± 0.02

1.5 times longer than the left one. The number and location of the papillae varied; there were 4–5 preloacal ventrolateral papillae on the right side and 3–4 on the left side, and from 3 to 6 postloacal pairs (fig. 3, C).

The length of body in nine females (56.3 % of all specimens) was 19.58 ± 0.55 cm (from 17.4 to 21.6 cm), the width of body was 0.92 ± 0.02 mm. The anus opening is subterminal, the tail end is rounded. The vulva opens at the anterior end of body.

The intensity of infection (II) ranged from 2 to 4 nematodes in one animal, with an equal number of males and females in four hosts (66.7 %). Other two foxes (33.3 %) had 2 females per 1 male of *D. immitis*.

Discussion

According to the literature, the *Dirofilaria* spp. are common nematode parasites of domestic dogs in the Kharkiv City and Kharkiv Region. That is confirmed by the results of *in vivo* laboratory diagnostics and dissections (Maiboroda, 2004; Kryvoruchenko et al., 2019; Nikiforova & Reshetylo, 2019).

Four dogs with severe clinical symptoms of dirofilariasis have been studied after death in Romania

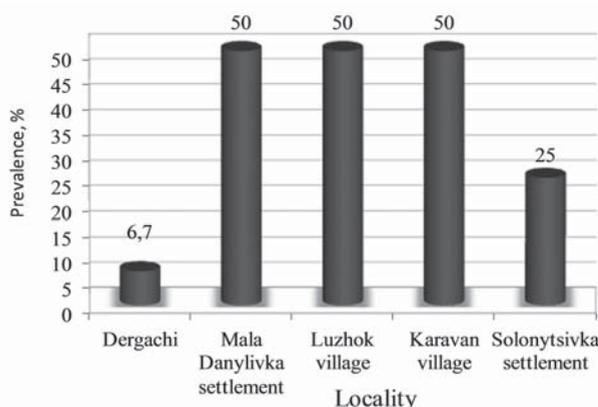


Fig 2. The prevalence of *Dirofilaria immitis* infection in foxes from separate localities.

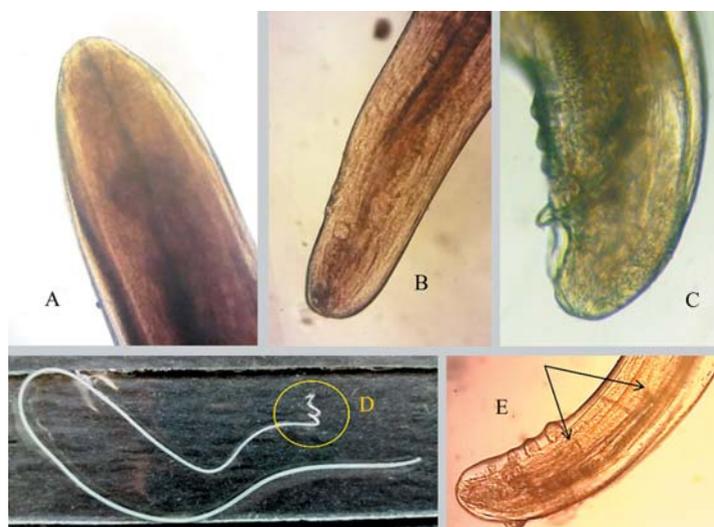


Fig 3. Morphological features of *Dirofilaria immitis*: A — head end of female; B — tail end of female; C — tail end of male; D — general view of male showing typical shape of posterior part of body; E — spicules (arrows) and papillae on the tail end of male. In A, B, C — magnification $\times 100$.

in 2010–2011. Only one of these dogs had severe infection by 25 specimens of *Dirofilaria immitis* localized in the right ventricle and pulmonary arteries (Pasca et al., 2012). Infestation with *Dirofilaria* spp. in Romania is restricted to domestic dogs. Despite the large population of wild predators in the country, their role in the epizooty and the distribution of filarial parasites among domestic dogs is unknown (Ionică et al., 2017).

Vinokurova (2011) examined 60 dogs from Krasnodarsky area (Russia), and found mature *D. immitis* in 33 animals with typical localization for this species. Prevalence in males was lower (52.9 %) than in females (57.7 %). Prevalence depended on the type of local dog habitats: it was 58.6 % in urban areas, and 47.3 % in rural areas. Intensity of dog infection ranged from 10 to 35 specimens per dog, with fewer number of female nematodes than males (Vinokurova, 2011).

Omelianenko et al. (2016) reported changes in all parenchymal organs of six dogs with cardiac dirofilariasis, but the most dangerous changes were observed in the locations of dirofilaria. The helminths caused interstitial pneumonia, venous hyperaemia, haemorrhage in the lungs, and granular and fatty dystrophy, necrosis and destruction of cardiomyocytes in the myocardium (Omelianenko et al., 2016). Thus, mature nematodes not only cause disorders in bloodstream, but also lead to profound changes directly in their localization.

Dirofilaria immitis was found in red foxes, golden jackals and domestic dogs in the study of 115 red foxes, golden jackals, wolves, domestic dogs, martens, and cats in Bulgaria in 2012–2013 (Panayotova-Pencheva et al., 2016). Mature filariae were found in the heart of 55.24 % of animals, and in the heart and pulmonary arteries of 27.97 % hosts. Prevalence was 25.22 % in red foxes, and 33.33 % in domestic dogs. Intensity of infection ranged from 1 to 15 specimens (on average 4.79 specimens) in foxes, and thrice more, from 1 to 34 specimens (on average 14.43 specimens) in dogs. The obturation changes in the inner surface of the pulmonary arteries and the damage of the atrioventricular valves were observed in areas parasitized by dirofilaria (Panayotova-Pencheva et al., 2016).

In northern Greece, four *D. immitis* females were found in the heart and pulmonary arteries of 4-year-old brown bear which died accidentally, in 2014 (Papadopoulos et al., 2017). The nematodes were 10.2 ± 0.5 cm long. The known range of reservoir hosts of the nematode has expanded.

Dirofilaria was found in 23 of 132 collected foxes ($P = 17.4$ %) in Tuscany, Central Italy, in 2005–2006 (Magi et al., 2008). In 2013–2014, *D. immitis* was recorded in two of 28 red foxes ($P = 7.1$ %) in the Italian province of Modena (Fiocchi et al., 2016).

Several species of wild predators were used in a parasitological study in Serbia in 2009–2013 (Penezić et al., 2014). The prevalence of mature *D. immitis* in 738 examined hearts and lungs was as follows: 1.55% in red foxes, 1.43 % in wolves, 7.32 % in jackals and 7.69 % in wild cats. In foxes, the infection was more common in males (1.75 %) than in females (1.26 %). In Vojvodina, northern Serbia, 83 red foxes were examined in 2017–2018, and *D. immitis* was found in hosts collected near alluvial rivers (P = 4.8 %). Two male nematodes were found in two host animals, two female parasites in one host, and four female dirofilaria in another host (Gavrilović et al., 2019).

In Hungary, red foxes and jackals were studied in 2007. *D. immitis* was detected in 20 of 534 red foxes (P = 3.7 %), and in golden jackals, the prevalence of infection was 7.4 %. However, the authors claim that the 1.5 % prevalence of dirofilaria infection of wild canids is lower in Hungary than in the Mediterranean Europe, where the prevalence is 0.4–12.7 % and intensity is 2.9–4.4 specimens/host (Tolnai et al., 2014).

In the central region of the Russian Federation, 40 dogs and 427 red foxes were dissected in 2003–2016. In one fox (P = 0.23 %), 10 specimens of *D. immitis* were found in the right ventricle of heart, and in one dog (P = 2.5 %), seven dirofilaria were recorded (Andreyanov et al., 2016). Thus, dogs are more susceptible to dirofilaria than red foxes, which are also confirmed by the results in other studies (Vinokurova, 2011; Pasca et al., 2012; Panayotova-Pencheva et al., 2016).

Dirofilaria immitis has been found in foxes on other continents as well, including North America and Australia (Wixsom et al., 1991; Henderson, 2009).

The results of world-wide research are partially consistent and confirm that in domestic dogs the prevalence and intensity of infection are significantly higher than in wild animals of the family Canidae.

In our study, the number of dirofilaria in six infected foxes in Kharkiv Region did not exceed 4 specimens. Our data concerning localization of *D. immitis* in the body of foxes coincide with the data of scientists from different countries, and the localization in dogs does not differ either (Vinokurova, 2011; Omelianenko et al., 2016).

Scientists from different countries agree that the role of wild carnivores in the epidemiology process and in the spread of dirofilariasis has not been fully identified. However, it is confirmed that foxes contribute to the transmission of *D. immitis* in the natural ecosystems, and they probably act as a reservoir and a source of infection and distribution of these nematodes to susceptible animals.

Conclusion

Dirofilaria immitis is a common parasite (P = 22.2 %) in the fox population in Dergachi Dstrict, Kharkiv Region of Ukraine. Prevalence of infection of foxes with *D. immitis* may depend on their habitat and the presence of vectors in these areas.

This study was conducted in close collaboration between Kharkiv State Zooveterinary Academy and hunting farm of Kharkiv Regional Department of Forestry and Hunting of the State Agency of Forest Resources of Ukraine.

Conflict of Interest

Authors state no conflict of interest.

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