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SIGNIFICANCE OF THE RED FOX AS A NATURAL RESERVOIR OF INTESTINAL ZOONOSES IN VOJVODINA, SERBIA

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In the present study, 223 foxes were collected from various localities in the northern part of the Republic of Serbia (Vojvodina province) and examined for intestinal helminths. Among the examined foxes 178 (79.8%) were infected. The most frequently identified parasites were *Mesocestoides* spp. (49.3%) and *Toxascaris leonina* (36.3%). The parasite with the lowest prevalence was *Pterygodermatites affinis* (0.9%), and this is the first confirmed finding in Serbia. The other recovered species were *Alaria alata* (25.6%), *Taenia* spp. (6.3%), *Echinococcus multilocularis* (13%), *Toxocara canis* (16.6%) and *Uncinaria stenocephala* (14.8%). The highest number of foxes infected with *E. multilocularis* were in the Srem area. The results of this study indicate the presence of helminth species in red foxes in Vojvodina which may also infect humans.

Key words: Red fox, helminth, Carnivora, echinococcosis, Serbia, Vojvodina

The red fox (*Vulpes vulpes* L., 1758) belongs to the order Carnivora and is widely distributed worldwide. An increase in fox populations was noted in many European countries (Vervaeke et al., 2003; Goszczyński et al., 2008), explained by the effects of a successful rabies vaccination campaign of wild animals, an increase in anthropogenic food sources, and the impact of environmental factors (Gloor et al., 2001). At the same time, fox habitats extended into urban areas (Schweiger et al., 2007). A growing number of studies point to the colonisation of cities across Europe, the USA and Australia (Christensen, 1985; Adkins and Stott, 1998; Romig, 1999; Robinson and Marks, 2001). Following the trend seen in other European countries, red fox populations have been on the rise in Serbia as well.

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Due to the increased contact between humans and domestic animals on the one hand, and the appearance of populations of so-called urban foxes on the other, the risk of zoonotic transmission of parasitic diseases in urban areas is rising. A study conducted in Switzerland found an increase in the incidence of human alveolar echinococcosis since 2000. This can be explained by the urbanisation of the *Echinococcus multilocularis* Leuckart, 1863 life cycle, due to an increase in the number of infected foxes in urban areas (Deplazes et al., 2004; Schweiger et al., 2007).

Foxes are permanent hosts of many types of helminths, some of which have significant zoonotic potential. One such parasite is the tapeworm *E. multilocularis*, a causative agent of alveolar echinococcosis. This parasite has been recorded in jackals and foxes in Serbia, on the territory of Vojvodina (Lalošević et al., 2016). The rich community and the wealth of parasite species found in foxes is associated with the ability of the host to exploit a wide range of habitats, which affects the availability of prey and the composition of the diet (Eira et al., 2006). The intestinal helminth fauna of red foxes has been well researched throughout Europe (Vervaeke et al., 2005; Eira et al., 2006; Magi et al., 2009; Miterpakova et al., 2009; Vergles-Rataj et al., 2013).

The importance of foxes as natural reservoirs and distributors of zoonotic infections, as well as the deficiency of data on population infections in Vojvodina have indicated the need for more detailed and systematic research. Studies on the helminth fauna conducted in Serbia do not provide a complete epidemiological presentation of the helminth fauna of Vojvodina. The aim of this study was to investigate the prevalence of intestinal helminths of red foxes in all seven districts of this area.

Materials and methods

The Province of Vojvodina occupies the northern part of the Republic of Serbia. It spreads through the Pannonian Plain with an area of 21,506 km², which represents 24.9% of the total territory of Serbia. It borders Hungary to the north, Romania to the east, the River Danube and Sava to the south, Croatia to the west, and Bosnia and Herzegovina to the far southwest. There are three regions within Vojvodina, Srem, Banat and Bačka, which are administratively divided into 7 districts (Fig. 1). Bačka and Banat are mainly flat with arable land, while the landscape of Srem is dominated by the Fruška Gora mountain.

From all 7 districts of Vojvodina, 223 red foxes were collected between January 2015 and February 2018, as part of the routine rabies diagnostic procedure in the Pasteur Institute of Novi Sad, National Reference Laboratory for Rabies. After autopsy, the intestines were washed and parasites collected and determined morphologically. The intestinal scraping technique (SCT) according to Eckert et al. (2001) was also used for the detection of parasite infection.

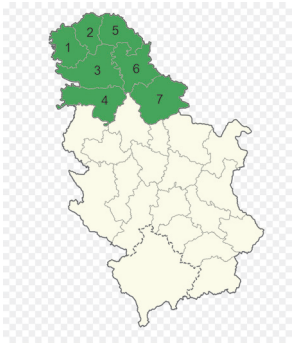

Fig. 1. Location of Vojvodina (green) within Serbia with each of the seven districts demarcated by a border. 1 – West Bačka, 2 – North Bačka, 3 – South Bačka, 4 – Srem, 5 – North Banat, 6 – Central Banat, 7 – South Banat

All quantitative parameters of intestinal helminth infection follow the guidelines of Bush et al. (1997), and were calculated in QPweb (Reiczigel et al., 2019), along with 95% confidence intervals. The dispersion index (d) was also calculated, as a measure of parasite aggregation within hosts (Shaw and Dobson, 1995). Dispersion index values higher than 1 indicate an aggregated distribution of parasites.

Results

Intestinal parasites were detected in 178 red foxes, resulting in a total prevalence of 79.8%. *Mesocestoides* spp. Vaillant, 1863 (49.3%) and *Toxascaris leonina* (Linstow, 1902) (36.3%) were the most prevalent parasite species (Table 1). The parasite with the lowest prevalence was *Pterygodermatites affinis* (Jagerskiold, 1904), which was found in only two foxes (0.9%) collected in 2017. To the best of our knowledge, this is the first confirmed finding of *P. affinis* in Serbia. The genus *Pterygodermatites* Wedl, 1861 is characterised by an apical oral opening, three oesophageal teeth and 29–58 pairs of prevulvar spines (Anderson et al., 2009). The species was identified on the grounds of specific morphological characteristics of two females: body length, egg size and the presence of two rows of cuticular combs or spines throughout the length of the body, which become more sparse and shorter towards the posterior end. The female tail has a spinal terminal spike (Ammar, 2015; Scioscia et al., 2016) (Fig. 2).

With regard to parasite abundance, the most numerous species was *E. multilocularis*, with 1,485 individuals. The second largest group of helminths was *Mesocestoides* spp. (1,282), followed by the nematode *Toxascaris leonina* (483) and the trematode *Alaria alata* (Goeze, 1782) (429). *Echinococcus multilocularis* had the highest values of parameters such as mean abundance (6.7) and mean infection intensity (51.2) (Table 1). It was found in only one fox in Banat (1/58) (1.7%), five foxes in Bačka (5/47) (10.6%) and 23 foxes (23/118) (19%) in Srem.

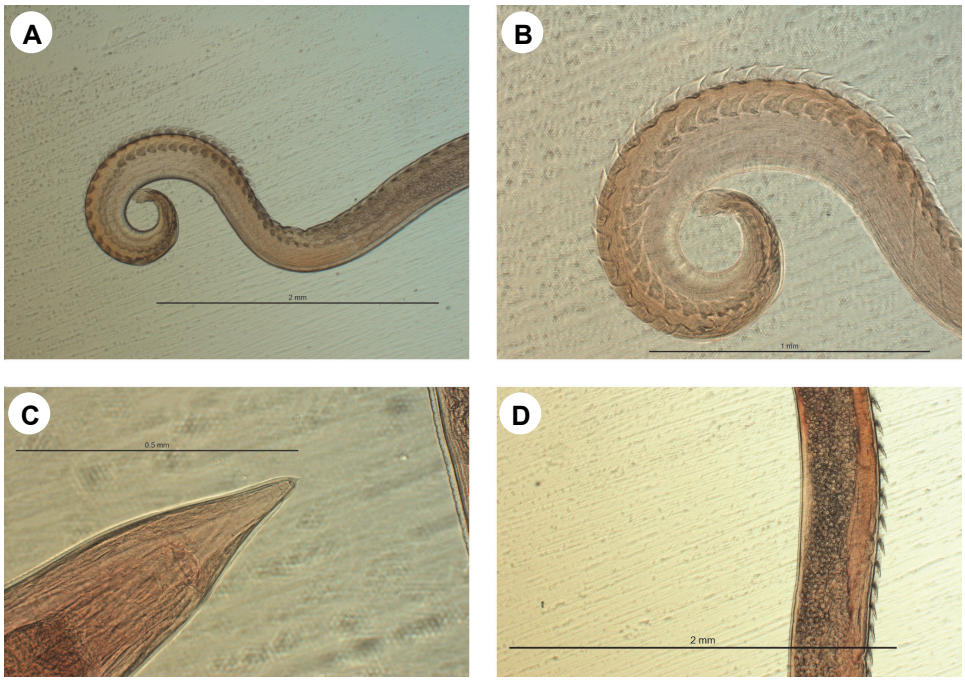

Fig. 2. Female *Pterygodermatites affinis* specimen found in a red fox in Vojvodina. A – anterior end of female, B – detail of anterior end with visible spines, C – posterior end of female with visible terminal spike, D – eggs

Table 1

Quantitative parameters of intestinal helminth infection of the examined red foxes in Vojvodina

Endoparasite species	P (%)	MA	MI	R	d
Alaria alata	25.6 (20.1–31.8)	1.9 (1.1–3.5)	7.5 (4.7–13.9)	1–90	1
Echinococcus multilocularis	13.0 (9.1–18.1)	6.7 (3.1–16.5)	51.2 (27.3–120)	1–540	36.6
Taenia spp.	6.3 (3.7–10.3)	0.1 (0.06–0.2)	1.9 (1.4–2.6)	1–5	26.7
Mesocestoides spp.	49.3 (42.8–56.1)	5.8 (4.4–7.9)	11.7 (9.3–15.6)	1–100	2.4
Toxascaris leonina	36.3 (30.2–42.8)	2.2 (1.5–3)	6 (4.6–8.1)	1–40	249.8
Toxocara canis	16.6 (12.1–22.2)	0.3 (0.2–0.4)	1.7 (1.4–2.4)	1–6	13.3

P – prevalence, MA – mean abundance, MI – mean intensity, R – minimum–maximum range, d – dispersion index; 95% confidence intervals in parentheses, where applicable

In the fox sample, one species of hookworm, identified as *Uncinaria stenocephala* (Railliet, 1884) was found, with a prevalence of 14.8%. Additionally, the

presence of parasitic tapeworms of the genus *Taenia* Linnaeus, 1758 was recorded in a relatively low percentage (6.3%). In the present study, these tapeworms were not identified to the species level.

Multiple infections with intestinal helminths were frequently observed, and were more common (61.4%) than infections with a single species. Intestinal infection with two species of parasites was found in 31.1% (55/177), three species in 22% (39/177), four species in 6.8% (12/177), and five species in 1.7% (3/177) of red foxes.

Discussion

Eight species of intestinal helminths were found in the examined animals, with varying prevalence values. The most prevalent parasites were *Mesocestoides* spp. tapeworms. A wide distribution of *Mesocestoides* spp. with a similar prevalence was observed in other European countries such as the Czech Republic (Jankovska et al., 2016: 40%), Slovakia (Hrčkova et al., 2011: 41.9%), Italy (Magi et al., 2009: 45.5%) and Hungary (Széll et al., 2015: 45.8%). Pavlović et al. (2008) found two *Mesocestoides* species in Serbia: *M. lineatus* with a prevalence of 37.98% and *M. litteratus* with a prevalence of 10.95%. The latter species was recorded in Vojvodina for the first time. There are only two species within the genus *Mesocestoides* (*M. variabilis* and *M. lineatus*) that are recognised as possible causative agents of human infection. Fuentes et al. (2003) summarised 18 cases of human infections caused by *M. lineatus* in Japan, Korea and China.

Considering the pathogenicity of *Mesocestoides* tapeworms (Széll et al., 2015), their presence in the host sample is not to be overlooked. However, morphological differences between species in the genus are subtle, and determination is difficult without the use of molecular methods (Padgett et al., 2005). Since such analyses were not performed in the current study, and since it is unclear whether the foxes were infected via game birds or rodents, these tapeworms were identified only at genus level. Similarly, *Taenia* tapeworms were not identified at species level. They were characterised by low prevalence (6.3%), corresponding to the results of other studies carried out in Serbia which reported 4.65% of foxes infected with these parasites (Ilić et al., 2016).

In a study conducted by Lalošević et al. (2016), *E. multilocularis* was recorded in red foxes in Vojvodina. The current study expands on these results by including other intestinal parasites from a larger host sample. Two years after the published finding of multilocular echinococcosis in Vojvodina, Beck et al. (2018) published the first report of *E. multilocularis* in the red fox in the neighbouring country of Croatia. By observing the distribution map of infected foxes in Croatia and Vojvodina, we can conclude that the distance between the two closest points is about 100 km, and this raises questions about the possible connections between these areas. That the potential for red fox dispersal is not a limiting fac-

tor is demonstrated by the fact that foxes can cover straight-line distances in excess of 108 km in 7 days (Walton et al., 2018). Based on these data, and taking into account the fact that the Danube acts as a barrier for foxes, we may explain why parts of Vojvodina north of the river (Bačka and Banat) have low prevalences of multilocular echinococcosis (only six infected foxes), while the hotspot of echinococcosis is in Srem, a region south of the Danube shared by Serbia and Croatia. Alveolar echinococcosis is one of the most dangerous zoonoses in the world. While there are cases of human infection in some European countries (Horváth et al., 2008; Szilágyiová et al., 2015), there are no documented cases of human alveolar echinococcosis in Serbia at the moment.

In a recent study, *A. alata* was found in the red fox with a prevalence of 25.6%. In a previous study conducted in Serbia (Ilić et al., 2016), the prevalence was higher (49.4% in foxes and 30% in jackals). In their review Möhl et al. (2009) summarise a large number of literature data on this species and state that about 30% of wild canids in Europe are carriers of *A. alata*, with a prevalence range from 0.1% in Germany to 88% in Poland. The authors also state that a particularly high rate of infection can be registered in omnivorous species, such as wild boars living in areas with a high prevalence of *A. alata* in definitive hosts. The first published finding of *Alaria* mesocercariae in wild boar meat in Serbia, in the province of Vojvodina, emphasised the importance of investigating the presence of this trematode in the red fox as the main reservoir of alariosis (Lalošević et al., 2014). The latest research in Serbia on the presence of mesocercariae in pigs (Gavrilović et al., 2019) confirms new cases of *A. alata* in wild boars, but also reports the first registered case of this parasite in domestic pigs in Serbia, again in Vojvodina. According to the authors, this is the only reported case of *A. alata* mesocercariae in domestic pigs over the past several decades in Europe. The life cycle of *A. alata* includes intermediate hosts such as snails of the genus *Planorbis* and amphibians, and is predominantly related to aquatic habitats in which suitable types of hosts are present. It is important to note that Vojvodina has a large quantity of water in the form of ponds, marshes, canals and large plain rivers with slow water flow (Tasić et al., 2007). Although there are no recorded human cases of alariosis, *A. alata* is a parasite that may affect human health.

Toxocarosis is one of the most widespread and economically most important zoonoses, but is nevertheless neglected in international public health in comparison with other helminthic diseases (Macpherson, 2013). *Toxocara canis* (Werner, 1782) was found in 16.1% of foxes in the present survey. Authors from other European countries reported higher prevalence for this species in fox populations: 37.1% and 38.3% in Portugal and Slovenia, respectively (Eira et al., 2006; Vergles-Rataj et al., 2013). Still higher values were recorded in the United Kingdom (55.9%, Richards et al., 1995), Denmark (59.4%, Saeed et al., 2006) and the Netherlands (73.7%, Borgsteede, 1984). Outside Europe, the species has

been found in Tunisia (Lahmar et al., 2014) and Kyrgyzstan (Ziadinov et al., 2010). According to Dybing et al. (2013), *T. canis* is also present in Australia. In Serbia, the prevalence of this nematode in red foxes was 49.4% (Ilić et al., 2016). High prevalence of *T. canis* in urban foxes could provide a source of infection for man and his pets (Richards et al., 1993). The main path of infection of humans is by ingestion of embryonated eggs from the soil, especially in urban areas and playgrounds, which is the reason why children are most exposed to infection (Lalošević et al., 1993).

Pterygodermatites affinis (syn. *Rictularia affinis*) is a rare parasitic nematode in Europe, registered in only a few countries. This species was found in two foxes (0.9%) in our survey and, to the best of our knowledge, this constitutes the first confirmed finding of *P. affinis* in Serbia. Similarly low prevalences in foxes were reported in Slovenia (4.2%; Vergles Rataj et al., 2013), France (4%; Deblock et al., 1988), and Italy (5.3%; Fiocchi et al., 2016). Higher prevalence was noted in Spain (54%) (Martinez-Carrasco et al., 2007). *Pterygodermatites affinis* is a typical nematode of carnivores with an indirect life cycle, and the infection of definitive hosts depends on the frequency of intermediate hosts such as reptiles in their diet (Papadopoulos et al., 1997; Martinez-Carrasco et al., 2007). This nematode, although not often registered in publications, has been recorded in different types of carnivores outside Europe and shows cosmopolitan distribution. It was reported from jackals and foxes in Tunisia (Lahmar et al., 2014), coyotes in Canada (Liccioli et al., 2012), cougars in Oregon, USA (Rausch et al., 1983), and domestic cats in Dubai, United Arab Emirates (Schuster et al., 2009). Scioscia et al. (2016) published the first report of this nematode in Argentina, and it was the first case of *P. affinis* infection in the pampas fox (*Lycalopex gymnocercus*), a new species added to the list of definitive hosts of the parasite.

In the present study, *Toxascaris leonina* had the second highest prevalence value of all intestinal helminth species. Compared to our findings, the prevalence of *T. leonina* in foxes was significantly lower in other countries, ranging from 0.6% in Denmark, 1.5% in United Kingdom and 2.5% in Slovenia to 4.7% in Australia and 5.4% in Italy (Richards et al., 1995; Saeed et al., 2006; Magi et al., 2009; Vergles-Rataj et al., 2013; Dybing et al., 2013). Higher percentage of infected foxes was found in Belgium (47.9%; Vervaeke et al., 2005) and Turkey (65%; Gicik et al., 2009). In a survey of the helminth fauna of foxes in Serbia, Lozanić (1966) cites a prevalence of 20.5%. The presence of this nematode in a high number of examined foxes can be explained by favourable climatic conditions for this species in Vojvodina, as well as its simpler life cycle compared to other ascaridids.

One of the most prevalent intestinal nematodes of red foxes in Europe is *Uncinaria stenocephala* (Reperant et al., 2007; Fiocchi et al., 2016). This species has a worldwide distribution in dogs and wild canids (Otranto and Deplazes, 2019). In a review article, Seguel and Gottdenker (2017) quote that higher preva-

lence of *U. stenocephala* is found in canids which inhabit temperate or circumboreal areas. The same authors also consider the influence of host spatial density on nematode prevalence. With higher spatial density, the number of infective larvae in soil increases. Cutaneous larva migrans (CLM) is a zoonotic disease caused by nematodes of the hookworm family, such as *Ancylostoma caninum* and, rarely, *U. stenocephala*. Humans can also be infected, but the life cycle of this parasite cannot be completed in a human host. In Europe, CLM is usually registered in travellers who return from tropical and subtropical countries. However, autochthonous human cases have been noted in Serbia and other European countries (Tomović et al., 2008; Perić et al., 2017; Gutiérrez García-Rodrigo et al., 2017). Tomović et al. (2008) published the first report of such infections in Serbia, stating that abiotic factors such as unusually hot and sunny weather with heavy rain in the summer of 2005 and 2006 created favourable conditions for parasite development in the soil.

In conclusion, the present study has shown that red foxes living in the Vojvodina area are hosts of numerous parasite species, some of which have veterinary and human medical importance. The results indicate that among other helminths *E. multilocularis* is present in the area. While data on the presence of intestinal helminth species parasitising the red fox is relevant in its own right, this study can also be seen as a preliminary report pointing to the need of future research in the field. This would particularly have to deal with the identification of *Mesocestoides* and *Taenia* species occurring in foxes in Vojvodina, as well as studying the extent of *E. multilocularis* presence and the patterns of its potential expansion into the northern part of Serbia.

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