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Intestinal helminth infections in the golden jackal (*Canis aureus* L.) from Vojvodina: Hotspot area of multilocular echinococcosis in Serbia

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RESEARCH ARTICLE

MILAN MILJEVIĆ[1*], DUŠAN LALOŠEVIĆ[2], VERICA SIMIN[2], JELENA BLAGOJEVIĆ[1], BORISLAV ČABRILO[3] and OLIVERA BJELIĆ ČABRILO[3]

[1] Department of Genetic Research, Institute for Biological Research 'Siniša Stanković', National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia

[2] Department for Microbiological and Other Diagnostics, Pasteur Institute Novi Sad, Novi Sad, Serbia

[3] Department of Biology and Ecology, Faculty of Science, University of Novi Sad, Novi Sad, Serbia

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ABSTRACT

In the present study, 64 golden jackals were examined for intestinal helminths in three regions of Vojvodina, Serbia. Among the examined jackals 57.8% were infected with at least one parasite species. Using the intestinal scraping technique (SCT), eight species of intestinal helminths were found: *Alaria alata* (7.8%), *Toxascaris leonina* (9.4%), *Toxocara canis* (4.7%), *Uncinaria stenocephala* (20.3%), *Echinococcus multilocularis* (14.1%), *Mesocestoides* sp. (42.2%), *Taenia pisiformis,* and *Taenia hydatigena* (the overall prevalence of *Taenia* infection was 6.3%). To the best of our knowledge, this is the first report of *T. leonina* in jackals from Serbia. In comparison with the SCT results, coprological tests were less sensitive and specific for parasite identification, as only two nematode species (*T. leonina* and *T. canis*) as well as ancylostomatid and taeniid eggs were identified. The total prevalence of intestinal helminths was higher in males (71.9% males, 45% females), but the difference was not statistically significant ($\chi^2 = 3.76$; $P = 0.052$). Co-infection with two species of intestinal helminths was found in 35% of the examined golden jackal individuals, three-species co-infection was demonstrated in 21.6%, whereas four-species co-infection was detected in 2.7% of the golden jackals examined. *Echinococcus multilocularis* has previously been recorded in jackals and foxes in Serbia, but only in Vojvodina. Our results corroborate the findings of previous studies, and indicate that the Vojvodina Province, more specifically the Srem region, is probably a high-risk area for *E. multilocularis* transmission to humans.

KEYWORDS

intestinal helminths, parasite, zoonoses, *Canis*, *Echinococcus multilocularis*, Serbia

INTRODUCTION

The golden jackal (*Canis aureus* L., 1758) is a widely distributed carnivore species that inhabits Europe, the Middle East, and Central and Southeast Asia (Moehlman and Hayssen, 2018). Recent research has shown that populations from North and East Africa are lineages genetically distinct and independent from the Eurasian golden jackals. The former lineage likely represents a separate species, the African golden wolf (*Canis anthus* Cuvier, 1820), which is morphologically similar to the golden jackal, but more closely related to the wolf (Koepfli et al., 2015). While golden jackal populations suffered a decline in the first half of the 20th century, an opposite trend of population growth and range expansion has been noted from the Balkan Peninsula into Central and Western Europe since the 1980s (Arnold et al., 2012; Spassov and Acosta-Pankov, 2019). The species is adapted to life in various agro-

*Corresponding author. Tel.: +381 (11) 207 8332.
E-mail: miljevic.milan@uns.ac.rs

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ecosystems, rural and semi-urban settlements (Clutton-Brock et al., 1976; Šálek et al., 2014). It is considered an opportunistic omnivore, with its diet affected by season, geographic area, and regional climate (Lanszki and Heltai, 2002). As a generalist species, the golden jackal feeds on mouse-like rodents, ungulates, invertebrates, plant matter and human-derived foods (Penezić and Ćirović, 2015). Such a diet inevitably results in exposure to a wide range of parasite species (Ćirović et al., 2015a; Sindičić et al., 2018; Széll et al., 2013; Takács et al., 2014), some of which have significant zoonotic potential. One of them is *Echinococcus multilocularis* Leuckart, 1863 that may cause one of the most dangerous parasitic zoonoses in the world. In Serbia, *E. multilocularis* has previously been recorded from jackals and foxes only on the territory of Vojvodina Province (Lalošević et al., 2016; Miljević et al., 2019). It is estimated that 43% of human zoonotic infections with this tapeworm originate from wild carnivores (Cleaveland et al., 2001), which are generally known to play an important role in the development and distribution patterns of numerous parasites. Following an increase in jackal population density, range expansion and recolonisation of its natural habitats (Arnold et al., 2012; Ćirović et al., 2014), the risk of parasitic infections spreading to other carnivores and humans also becomes greater. That is why studies of wild carnivores have gained relevance in the field of public health, with a strong emphasis on their role in zoonotic disease transmission.

In contrast to red foxes (*Vulpes vulpes* L., 1758), which have been surveyed on a European and global scale (Wolfe et al., 2001; Rajković-Janje et al., 2002; Vervaeke et al., 2003; Barbosa et al., 2005; Letková et al., 2006; Reperant et al., 2007; Hanosset et al., 2008; Kharchenko et al., 2008; Barabási et al., 2010; Learmount et al., 2012; Franssen et al., 2014; Fiocchi et al., 2016), data on the intestinal parasites of golden jackals are relatively scarce (Kirkova et al., 2011; Széll et al., 2013; Takács et al., 2014; Lahmar et al., 2014; Ilić et al., 2016; Gherman and Mihalca, 2017; Sindičić et al., 2018). The studies on jackals in Europe and Serbia tend to focus on population dynamics and trophic ecology (Ćirović et al., 2014; Šálek et al., 2014). Research on the intestinal parasites of *C. aureus* in Serbia has mostly been conducted outside of Vojvodina Province (Ćirović et al., 2015a; Ilić et al., 2016). In Vojvodina, respiratory and cardiopulmonary nematodes have been reported from golden jackals (Bjelić Čabrilo et al., 2018). To the best of our knowledge, this study represents the first detailed report on the intestinal helminth fauna of jackals in the Pannonian region of Serbia.

MATERIALS AND METHODS

Study area

The province of Vojvodina is situated in the south-eastern part of the Pannonian Basin, a large basin in Central Europe. It occupies the northern part of the Republic of Serbia, and spreads through the Pannonian Plain with 21,506 km^2, which represents 24.9% of the whole territory of Serbia. It shares borders with Bosnia and Herzegovina, Croatia, Hungary and Romania. There are three regions within Vojvodina: Banat, Bačka and Srem. Bačka and Banat are mainly flat regions with arable land, while the Fruška Gora mountain dominates in the landscape of Srem. The river Danube separates Srem from Bačka and Banat to the north and northeast, while the river Sava surrounds Srem on the south side. Only the western side of Srem is without a river border and is connected with Croatia.

Sampling and parasitological procedures

The host sample consisted of 64 golden jackals captured from various sites in the Vojvodina Province of Serbia between January 2015 and February 2018 (Fig. 1). The animals were caught as part of routine rabies diagnostic procedures of the Pasteur Institute of Novi Sad, which serves as the National Reference Laboratory for rabies. Following autopsy, the intestines were cleaned so that parasites could be collected and examined morphologically. Parasites were detected using the intestinal scraping technique (SCT) procedures described by Eckert et al. (2001). Sex was determined in 52 jackals (32 males and 20 females). Coprological analysis was performed according to the Lőrintz method on 46 faecal samples (Simin, 2014). The animals were delivered by hunters and some of the individuals were in the autolysis phase, which made it impossible to sample faeces.

Data analysis

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. Differences in the prevalence of infection between males and females were analysed using Chi-squared [χ^2] and Fisher's exact test. The significance level was defined as $P < 0.05$.

RESULTS

Quantitative structure

A total of 8 species of intestinal helminths were identified: one trematode [*Alaria alata* (Goeze, 1782)], three nematodes [*Toxascaris leonina* (von Linstow, 1902), *Toxocara canis* (Werner, 1782) and *Uncinaria stenocephala* (Railliet, 1884)] and four cestodes (*E. multilocularis, Taenia pisiformis* Block, 1780, *Taenia hydatigena* Pallas, 1776 and *Mesocestoides* sp. Vaillant, 1863) (Table 1). Due to the frequent morphological damage of individuals, all species of the genus *Taenia* are classified as *Taenia* spp. in further analysis. From 37 of the 64 examined jackals infected with at least one intestinal helminth species (57.8%) a total of 657 parasites were collected. As regards the quantitative infection parameters, *E. multilocularis* was the dominant species, with the largest number of individuals (284), the greatest infrapopulation size range (1–120) and the highest mean

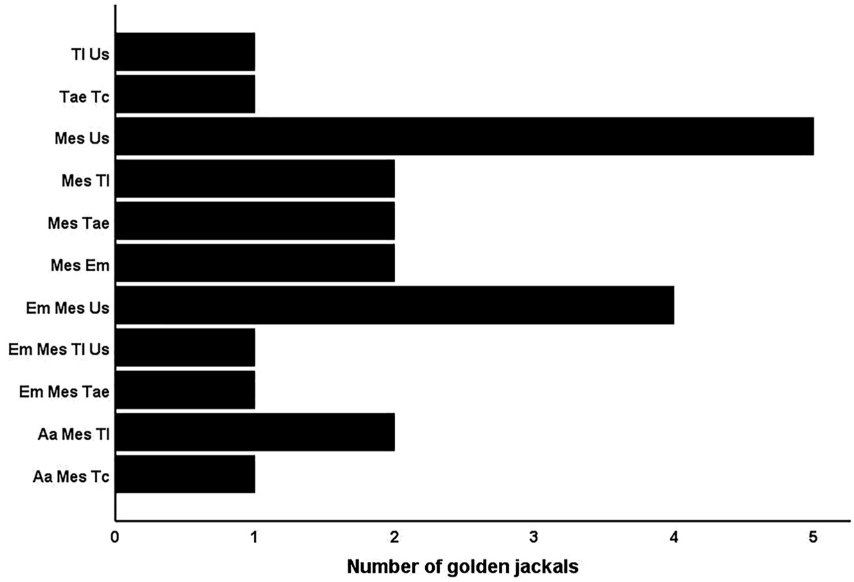

Fig. 1. Number of golden jackals infected with particular combinations of intestinal helminth species in the total sample. Aa – *Alaria alata,* Em – *Echinococcus multilocularis,* Tae – *Taenia* spp., Meso – *Mesocestoides* sp., Tl – *Toxascaris leonina,* Tc – *Toxocara canis,* Us – *Uncinaria stenocephala*

Table 1. Quantitative infection parameters of the intestinal helminth fauna of the examined golden jackal (*Canis aureus*) specimens in Vojvodina, Serbia

Helminth species	P%	MA	MI	R	n
Alaria alata	7.8 (3.1–17)	0.19 (0.05–0.5)	2.4 (1.2–3.8)	1–5	12
Toxascaris leonina	9.4 (4.2–19.3)	0.17 (0.05–0.3)	1.8 (1.2–2.3)	1–3	11
Toxocara canis	4.7 (1.3–13.1)	0.2 (0.02–0.9)	4.3 (1–7.7)	1–11	13
Uncinaria stenocephala	20.3 (13.1–33.5)	1.1 (0.5–3.1)	5.1 (2.6–13.1)	1–21	71
Echinococcus multilocularis	14.1 (7.3–24.9)	4.4 (1.3–12.6)	31.6 (21.9–45.2)	1–120	284
Taenia spp.	6.3 (2.2–15.4)	0.06 (0.02–0.13)	1 (NA)	1–2	4
Mesocestoides sp.	42.2 (30.3–54.7)	4.3 (2.7–7.1)	10.1 (6.7–15.7)	1–50	262

P% – infection prevalence in percentage; MA – mean abundance; MI – mean infection intensity; R – infrapopulation size range; n – total number of collected parasite individuals; 95% confidence intervals in parentheses, where applicable.

infection intensity (31.6) and mean abundance (4.4) values. However, the prevalence of *Mesocestoides* infection (42.2%) was the highest. The lowest infection prevalence was detected for *T. canis* (4.7%) (Table 1). *Echinococcus multilocularis* was recorded in the Srem region on a regional level, whereas the species was absent in the remaining regions of Vojvodina (Bačka and Banat). Similarly, *Taenia* tapeworms were registered only in jackals from Srem, while *T. canis* was not registered in jackals from the Bačka.

Community structure

Infracommunity species richness of intestinal helminths varied from one to four species, with most jackals (59%) carrying mixed infections. Infection with two species of intestinal helminths was found in 35%, three species in 21.6% and four species in 2.7% of jackals (a single host). Eleven different combinations of parasites were noted: six combinations of two species, four combinations of three species and one combination of four species. The most common combination was *Mesocestoides* sp. and *U. stenocephala*, followed by the three species combination of *E. multilocularis*, *Mesocestoides* sp. and *U. stenocephala* (Fig. 1).

Effect of host sex on intestinal helminth prevalence

Of the 52 examined jackals, 32 were males and the remaining 20 were females. The overall prevalence of intestinal helminths was higher in males (71.9% in males and 45% in females), but the difference was not significant statistically ($\chi^2 = 3.76$; $P = 0.052$). No significant effect of host sex was found on the infection prevalence of specific parasites, although prevalence was generally higher in male jackals. Two helminth species (*A. alata* and *T. canis*) were not found in female hosts (Table 2).

Coprological analysis

Coprological analysis of 46 jackals found evidence of parasitic infection in 22 individuals, resulting in a prevalence of 48%. Two nematode species were detected in coprological

Table 2. Comparison of intestinal helminth prevalence between the examined male and female golden jackals sampled in three areas of Vojvodina, Serbia

Helminth species	P%		x^2	*P*
	Male	Female		
Alaria alata	12.5	0	2.71	0.099
Toxascaris leonina	15.6	5	1.36	0.243
Toxocara canis	6.3	0	1.30	0.254
Uncinaria stenocephala	28.1	20	0.43	0.510
Echinococcus multilocularis	12.5	20	0.53	0.465
Taenia spp.	3.1	10	1.07	0.301
Mesocestoides sp.	50	40	0.50	0.481
Total	71.9	45	3.76	0.052

P% – infection prevalence in percentage; *P* – statistical significance level.

material, *T. leonina* (6.5%) and *T. canis* (8.7%). Additionally, ancylostomatid and taeniid eggs were also found. While ancylostomatid eggs were commonly observed (37%), the faeces of only a single jackal contained taeniid eggs (2.2%).

DISCUSSION

The present results demonstrate that golden jackals from Vojvodina are hosts of eight intestinal helminth species. A previous study found a similar parasite fauna in red foxes from the same sampling area in Vojvodina examined between 2015 and 2018 (Miljević et al., 2019); however, in the present study, *Pterygodermatites affinis* was not recorded in jackals. These findings indicate that identical ecological conditions and physical environment may have a greater impact on the presence of certain parasite species, regardless of their definitive host. In comparison to the SCT results, the coprological test was less sensitive and specific for parasite identification; only two nematode species (*T. leonina* and *T. canis*) and ancylostomatid and taeniid eggs were identified. A technical limitation of coprological tests may explain this difference. The eggs from cestodes and trematodes float at a specific gravity (SG) of 1.35 and higher, while the nematode eggs float at a SG > 1.15. When the SG of the solution is higher than that of the worm eggs, eggs float to the surface (Speer, 2015). This may explain the detection of taeniid eggs only in a single jackal, and the absence of trematode eggs, with the predominant detection of nematode eggs. Thus, the method of coprological diagnostics can give only an apparent review of helminth fauna, without the possibility of detecting all parasite species and determining the other quantitative and qualitative parasitological parameters. In carnivorous species, authors described a lower sensitivity of coprological methods (Magi et al., 2009, 2016; Byrne et al., 2018).

While studies of red foxes in Serbia date back more than 50 years (Lozanić, 1966), the first study of the intestinal helminth fauna of jackals was published in 2015 (Ćirović et al., 2015a). A total of thirteen species are known to infect golden jackals in Serbia: two trematodes (*A. alata* and *Pseudamphistomum truncatum*), eight cestodes (*Dipylidium caninum*, *T. pisiformis*, *T. hydatigena*, *Multiceps serialis*, *Multiceps multiceps*, *Mesocestoides litteratus, Mesocestoides lineatus* and *E. multilocularis*) and three nematodes (*Ancylostoma caninum*, *Gongylonema* sp. and *T. canis*) (Ćirović et al., 2015a; Ilić et al., 2016; Lalošević et al., 2016). *T. leonina* has not been reported until now, and it increases the number of intestinal helminth species known to infect golden jackals in Serbia. Since half of the registered helminth species require rodents as intermediate hosts, the proportion of rodent prey taken by jackals is assumed to be an important factor in determining infection levels. Studies of jackal diet in Hungary, Croatia, Bulgaria and Serbia indicate that small mammals have an important role, either as the dominant or the subdominant prey category with regard to frequency and consumed biomass (Lanszki et al., 2006; Kirkova et al., 2011; Bošković et al., 2013; Ćirović et al., 2014).

In the present study, the overall infection prevalence of intestinal helminths in jackals was 57.8%. A slightly higher prevalence (66.7%) was reported by Meshgi et al. (2009) in Iran. In Serbia, Ćirović et al. (2015a) detected a lower prevalence of 10.3% from a sample of 447 jackals. In our study, the most prevalent taxon was *Mesocestoides* sp. that infected 42.2% of hosts, whereas the highest mean infection intensity (31.6) and mean abundance (4.4) were noted in *E. multilocularis*. *Echinococcus multilocularis* can be viewed as a driver of the increased interest in jackal helminth fauna, since the few studies in the topic focus specifically on this species (Széll et al., 2013; Sindičić et al., 2018, Balog et al., 2021). The parasite species was first reported in golden jackals in Vojvodina, Serbia (Lalošević et al., 2016). This is the only province in Serbia where the presence of *E. multilocularis* has been reported (Lalošević et al., 2016; Miljević et al., 2019), with the exception of one isolated case from Mačva district (a region separated from the southern part of Vojvodina by the river Sava), where the parasite was found in its paratenic host, the European beaver (*Castor fiber* L., 1758) (Ćirović et al., 2013). Other studies of the definitive host out of Vojvodina have not detected affected animals (Ćirović et al., 2015a, 2015b; Ilić et al., 2016). Thus, our results indicate that Vojvodina Province, especially the Srem region, is a suitable area for *E. multilocularis* transmission (Fig. 2). More recently, *E. multilocularis* has been found in jackals in neighbouring Croatia, where four individual tapeworms were found in one host near the border with Bosnia and Herzegovina (Sindičić et al., 2018). The distance between this locality and our study area is approximately 250 km. *Echinococcus multilocularis* was reported from jackals in another neighbouring country: in Hungary, one female carried 412 individual tapeworms. This finding was the first report of *E. multilocularis* in jackals on the European continent (Széll et al., 2013). In the current study, the prevalence of *E. multilocularis* was 14.1%, with all infected jackals originating from the Srem region, which reflects the infection seen in red foxes as reported by Miljević et al. (2019). To explain the presence of the parasite in this part of Vojvodina, the authors hypothesise that infection can freely

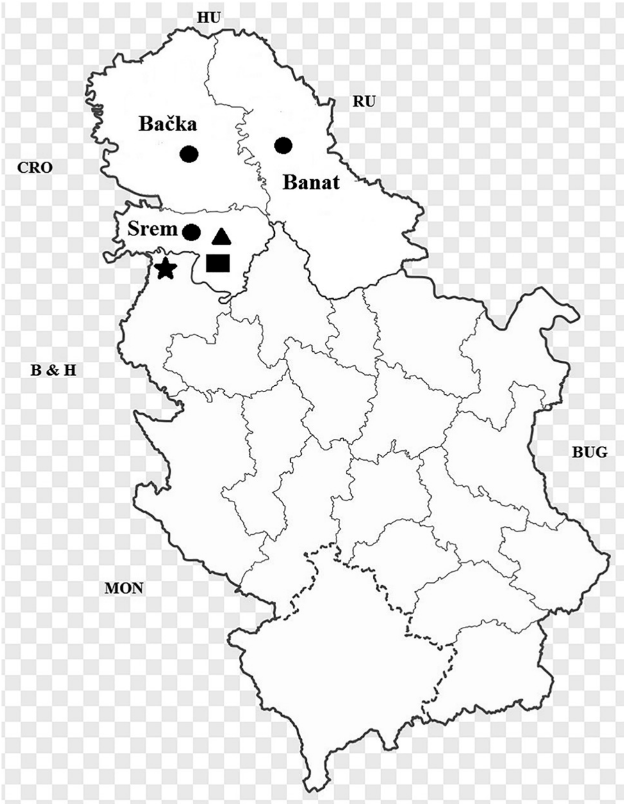

Fig. 2. A map of Serbia highlighting Vojvodina province as a hotspot area of multilocular echinococcosis. Black circles indicate *E. multilocularis* infected foxes in all three regions of Vojvodina, while black triangles indicate *E. multilocularis* infected jackals in Srem in previous studies in Serbia (Lalošević et al., 2016; Miljević et al., 2019). Black star represents an infected European beaver in Mačva district (Ćirović et al., 2013). Black square indicates *E. multilocularis* infected jackals in our study in Srem region

spread via mobile wildlife between Croatia and Srem, which are geographically continuous; the river Danube would act as a barrier preventing the disease from spreading further north into Bačka and Banat. The results also suggest that similar environmental conditions influence the quantitative representation of parasite species, regardless of their definitive hosts. Additionally, the samples of *E. multilocularis* from two jackals and two red foxes, both from Vojvodina, participated in the study conducted by Umhang et al. (2021), the aim of which was to complete the understanding of parasite expansion from the historical focus using EmsB microsatellite as a marker. The three EmsB profiles obtained from samples from Serbia had been previously identified in Europe, and these data may support the assumption that *E. multilocularis* has expanded from the historical focus and spread to Croatia and Serbia, most likely via Hungary. However, firm conclusions require further surveys in Croatia and Serbia (Umhang et al., 2021). The risk of alveolar echinococcosis in humans is highly dependent on the presence of infective eggs in the environment and their availability to humans (Eckert et al., 2011). The degree of environmental contamination, in turn, depends on the infection loads of the hosts, with heavily infected animals contributing the highest number of infective stages. Because of this, in an epidemiological sense, infection intensity and parasite abundance carry far more information than infection prevalence: a few heavily infected animals may have a greater impact than a large number of hosts carrying small numbers of parasites (Otero-Abad et al., 2017). If there is evidence of spatial heterogeneity in infection, anthelmintic baits may be placed in areas frequented by hosts with high infection intensity, to reduce environmental contamination and prevent infections spreading into human populations (Hegglin et al., 2003). High values of mean infection intensity and mean abundance of *E. multilocularis*, coupled with the appearance of heavily infected hosts in the Srem area, serve as evidence of a micro-hotspot of multilocular echinococcosis in the region, which show an urgent need for the optimisation of control strategies.

In the current study, *Mesocestoides* tapeworms were identified only at a genus level. Due to their high morphological plasticity, identification of *Mesocestoides* species based on morphometric data is not reliable without the use of molecular markers (Crosbie et al., 2000; Padgett et al., 2005). Gherman and Mihalca (2017) reported that tapeworms of the genus *Mesocestoides* are among the most frequently registered parasites of jackals, found all over Europe, Asia, and Africa. *Mesocestoides lineatus* was the most prevalent (70%) parasite of jackals in Iran (Dalimi et al., 2006). In Serbia, Ćirović et al. (2015a) list two *Mesocestoides* species from jackals: *M. lineatus* and *M. litteratus*. These cestodes were found to be the most prevalent parasites, albeit with lower prevalence values (5.8 and 4.7%, respectively) and higher mean infection intensity (69.7 and 64.3, respectively) than in the current study. In contrast, Ilić et al. (2016) did not report *Mesocestoides* sp. in jackals from eight sites in Serbia, noting ancylostomatid nematodes as the most prevalent parasites (40.9%). In an earlier study in Vojvodina, *Mesocestoides* tapeworms infected a higher percentage of red foxes than other parasites present in the sample (Miljević et al., 2019).

The two *Taenia* species (*T. pisiformis* and *T. hydatigena*) reported in the current study were also reported by Ćirović et al. (2015a) in golden jackals in Serbia. Due to the frequent damage of tapeworms in the course of sample collection, only two individuals could be identified at the species level, thus other specimens were classified as *Taenia* spp. The overall prevalence of *Taenia* spp. infection was relatively low (6.3%), in correlation with the results of other research studies carried out in foxes and jackals in Serbia, revealing prevalences ranging from 0.9% to 6.3% (Ćirović et al., 2015a; Ilić et al., 2016; Miljević et al., 2019).

Alaria alata is the only digenean species found in the present study, with a prevalence of 7.8%. Ćirović et al. (2015a) also report it, along with *P. truncatum*, in jackals in Serbia, with a prevalence of 0.9%. While 27 species of digeneans are known to infect jackals, *A. alata* is the most frequently reported one, and it is found over the widest geographic range (Gherman and Mihalca, 2017). The species was also found in foxes in Serbia, where it was the only digenean identified, with a prevalence of 25.6% (Miljević et al., 2019). The presence of *A. alata* larvae has been confirmed in domestic pigs and wild boars in Vojvodina (Lalošević et al., 2014; Maleševič et al., 2016; Gavrilović et al., 2019), and these animals are presumably infected by parasites released by jackals and foxes. Humans can be infected with mesocercariae of *A. alata* via pork, which is commonly consumed in Vojvodina. Human alariosis manifests through various clinical symptoms, including mild respiratory issues, muscle pain, blindness, and anaphylactic shock, with cases caused by *A. americana* reported in North America between 1973 and 1993 (Möhl et al., 2009). While *A. alata* may pose a threat to public health, there is currently no legislation in Serbia dealing with its control and eradication.

Toxocara canis is a parasite with high zoonotic potential and the causative agent of toxocariosis. This species was found in 4.7% of jackals in our survey. Similarly, low prevalence in jackals was previously reported in Serbia (1.6%; Ćirović et al., 2015a); in contrast, a higher prevalence of 23.33% was noted by Ilić et al. (2016). This nematode is often overlooked, and its monitoring is lax: in Serbia, toxocariosis is not a notifiable disease by law. The prevention of toxocariosis is even more complicated due the complexity of its infection and transmission patterns (Ma et al., 2018). Humans are primarily infected by ingesting embryonated eggs found in the soil. The two reported cases of toxocariosis in Serbia, in children under three years of age, have been attributed to geophagy and poor hygiene (Kuzmanović and Lalošević, 2009; Lalošević, 2019). The number of currently reported cases of human toxocariosis in Serbia is relatively high (Kuzmanović and Lalošević, 2009; Považan et al., 2011; Čolović-Čalovski et al., 2014; Gabrielli et al., 2017; Lalošević, 2019), and the investigation of jackals as reservoirs of this zoonosis is an important step towards disease prevention, as they may play an active role in the dissemination of the parasite into urban and rural areas.

Toxascaris leonina has not been reported in jackals in Serbia previously. In the present study, it was found in 9.4% of hosts. Various authors studying small jackal samples have reported different prevalence values: 36% in Bulgaria (Trifonov et al., 1970), 30% in Iran (Dalimi et al., 2006) and 15% in Hungary (Takács et al., 2014). According to Miljević et al. (2019), *T. leonina* had the second highest prevalence (36.3%) of all intestinal helminth species of red foxes in Vojvodina.

The present study shows that 20.3% of jackals were infected with *U. stenocephala*. This is the only nematode in the sample that is characterised exclusively by a direct life cycle. The jackals in Serbia are known carriers of ancylostomatids, with a prevalence of 33.3% (Ilić et al., 2016). Jackals may act as reservoirs that contribute to the spread of this nematode, which is known to cause cutaneous and visceral larva migrans. Cutaneous larva migrans (CLM) is usually detected in travellers who return from tropical and subtropical countries. The first autochthonous human cases in Serbia were described by Tomović et al. (2008). A decade later, Perić et al. (2017) also described two new autochthonous cases of CLM in the country.

Our random sample of jackals from the territory of Vojvodina, Serbia, was found to harbour species of intestinal helminths that are known to harm human health, most significantly *E. multilocularis* that causes one of the most serious zoonotic infections known in humans. These results are consistent with the findings of previous research on red foxes and golden jackals (Lalošević et al., 2016; Miljević et al., 2019) and confirm that Vojvodina (especially the Srem region) is a hotspot area of multilocular echinococcosis in Serbia.

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