Journal of Helminthology (2015) **89**, 28–33
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doi:10.1017/S0022149X13000552

Levels of infection of intestinal helminth species in the golden jackal *Canis aureus* from Serbia

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(Received 22 November 2012; Accepted 9 July 2013; First Published Online 13 August 2013)

Abstract

During the past decade, golden jackal populations have substantially increased, yet little is known of their potential for transmitting parasites within animal and human hosts. In the present study, between 2005 and 2010, 447 jackals from six localities in Serbia were examined for intestinal parasites. Two species of trematodes (*Alaria alata*, *Pseudamphistomum truncatum*), three nematodes (*Toxocara canis*, *Ancylostoma caninum*, *Gongylonema* sp.), and seven cestodes (*Taenia pisiformis*, *Taenia hydatigena*, *Multiceps multiceps*, *Multiceps serialis*, *Mesocestoides lineatus*, *Mesocestoides litteratus*, *Dipylidium caninum*) were identified. *Pseudamphistomum truncatum* and *M. serialis* species were recorded for the first time. The overall prevalence of parasitic infection was 10.3%. No significant differences were found in the prevalence of infection between males and females ($P > 0.817$), between localities ($P > 0.502$), or with regard to annual cycles ($P > 0.502$). In the infected jackal population, 65% harboured multiple infections and one individual was a host to five different types of parasite species, the highest number of parasites we recorded in a single host. These findings indicate that although the prevalence of gastrointestinal helminths in the jackal population in Serbia is significantly lower than expected from earlier studies, further monitoring is required given the jackal's rapid population increase.

Introduction

The golden jackal (*Canis aureus* Linnaeus 1758) is a canid of medium size with one of the widest distribution ranges in the world, a range that is still expanding (Demeter & Spassov, 1993; Kryštufek *et al.*, 1997; Mitchell-Jones *et al.*, 1999). In the past few decades, it has undergone several changes, especially in Europe, where the stretch of the Danube through ex-Yugoslavia and Romania has traditionally been described as the northern border of residential populations (Kryštufek *et al.*, 1997). However, the present distribution of the jackal additionally comprises parts of central Europe (Mitchell-Jones *et al.*, 1999; Arnold *et al.*, 2012). Apart from Greece, where the jackal population has decreased (Giannatos, 2004; Giannatos *et al.*, 2005), European populations are both rapidly increasing and widening their ranges (Kryštufek *et al.*, 1997; Arnold *et al.*, 2012). In Serbia, jackals neared extinction due to extensive poisonings organized after World War II, initially aimed at controlling the size of the wolf population and

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lessening the damage they caused to domestic animals. Only two relic populations survived in Srem and in eastern Serbia (Milenković, 1983, 1987). At the beginning of the 1980s, the species started to spread quickly and to increase in number (Savić *et al.*, 1995), which resulted in the fusion of the two relic populations and the widening of their range. This range now covers more than half of the territory of modern Serbia (Ćirović *et al.*, 2008; Zachos *et al.*, 2009).

The increasing jackal range in Europe was not automatically followed by detailed parasitological research. Only two studies in Europe, namely in Bulgaria and Greece, have been undertaken on the intestinal helminths of the golden jackal, and these are based on small numbers of hosts (Bulgaria $n = 13$, Greece $n = 5$) examined (Trifonov *et al.*, 1970; Papadopoulos *et al.*, 1997). More work has been done in Asia, where comprehensive studies, particularly in Iran, showed high prevalences in jackals, ranging from 66.7 to 100% (Sadighian, 1969; Dalimi & Mobedi, 1992; Dalimi *et al.*, 2006; Meshgi *et al.*, 2009). In central Asia, 13 species of intestinal helminths were identified in Tajikistan and 8 species in Uzbekistan, but, again, data were limited (Heptner & Naumov, 1967). In Israel, two helminth species, *Ancylostoma caninum* and *Dipylidium caninum*, were identified using faecal flotation techniques (Shamir *et al.*, 2001). High prevalences of *Toxocara leonina*, *A. caninum* and *Mesocestoides lineatus* were found in Iran (Sadighian, 1969; Dalimi & Mobedi, 1992; Dalimi *et al.*, 2006; Meshgi *et al.*, 2009) and also *Uncinaria stenocephala* and *Taenia hydatigena* have been found in Europe (Trifonov *et al.*, 1970; Papadopoulos *et al.*, 1997).

However, these studies were limited by the small number of jackals examined. The aim of the present investigation, therefore, was to identify species of gastrointestinal helminths in a large sample of hosts and to evaluate the potential role of the golden jackal in enhancing transmission of these helminths in Serbia and Europe.

Material and methods

Collection and examination of jackals

In cooperation with local hunting associations, the carcasses of all available, legally hunted jackals were collected in six locations in Serbia between 2005 and 2010. This collection was conducted throughout the main hunting season, the winter period from December until February, when jackals are sexually mature and therefore presumed to be adults. In total, 447 animals (239 males and 208 females) were collected: 40 from Negotin (MGRS 34T FP29), 162 from Veliko Gradište (MGRS 34T EQ45), 49 from Velika Plana (MGRS 34T EQ00), 119 from Smederevo (MGRS 34T DQ75), 39 from Svilajnac (MGRS 34T EP19) and 38 from Surčin (MGRS 34T DQ46) (fig. 1). The date of collection, location and sex of each jackal were noted. After morphometric analysis of each individual, the stomach and intestine were removed in the field and immediately frozen at $-20°C$. For safety reasons, the material was additionally frozen at $-80°C$ for 3 days and then thawed at room temperature prior to parasitological investigation in the laboratory of the Scientific Institute of Veterinary Medicine of Serbia.

The analysis of the gastrointestinal helminths was part of wider research on the jackal's diet (Ćirović *et al.*, 2009). For this reason, the stomach was first scrutinized for food contents. Parasites found in the stomach were removed and stored in 70% ethanol until eventual determination. The intestines were opened with a longitudinal cut and the entire intestinal contents were collected and washed with a water jet over a 200 μm sieve. All visible parasites were transferred to Petri dishes. Furthermore, to detect the presence of small parasites embedded in the mucosa, intestinal walls were scraped with a wooden spatula or a glass microscope slide. All helminths found were first washed in warm water and then fixed. Nematodes were fixed in 70% ethanol, while cestodes and trematodes were fixed in a mixture composed of 5% formalin, 85% ethanol and 10% glacial acetic acid. Nematodes were studied in depression slides using lactophenol wet mounts. Cestodes and trematodes were stained with acetic carmine, and after dehydration were mounted with Canada balsam. Scolices of taeniids were severed and mounted in lactophenol; sufficient pressure was applied to the coverglass to cause the rostellar hooks to lie flat. Identification was based on the number, size, shape and arrangement of rostellar hooks.

All helminths were identified using the relevant keys (Kozlov, 1977; Soulsby, 1982), counted and deposited in the collections of the Scientific Institute of Veterinary Medicine of Serbia and the Faculty of Biology of the University of Belgrade.

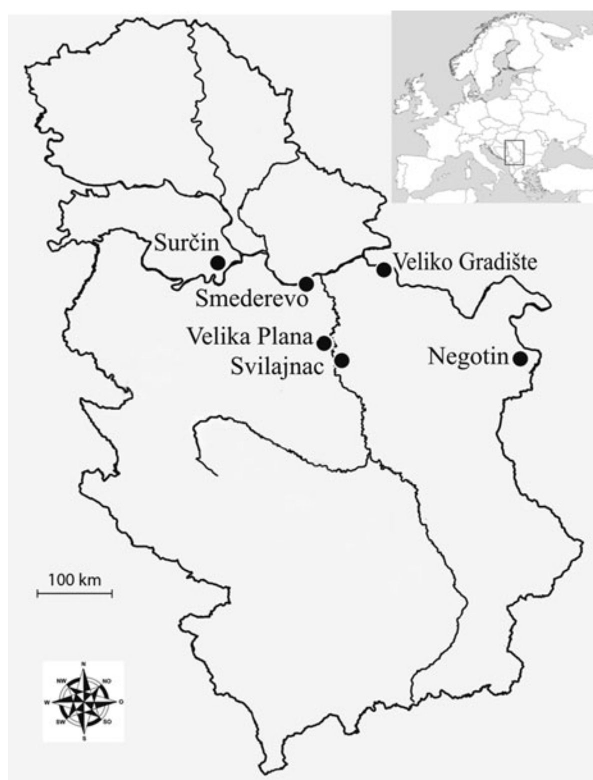

Fig. 1. The collection sites of golden jackals examined from Serbia between 2005 and 2010.

Table 1. The prevalence (P%) and mean intensity (MI ± SE) of gastrointestinal helminths in the golden jackal *Canis aureus* from six collection sites in Serbia; *N* = number of hosts examined.

Helminth species	Negotin $N = 40$		Veliko Gradište $N = 162$		Velika Plana $N = 49$		Smederevo $N = 119$		Svilajnac $N = 39$		Surčin $N = 38$		Total $N = 447$	
	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI
Trematoda														
Alaria alata	2.5	16	0.6	12	–	–	0.8	19	2.6	29	–	–	0.9	19 ± 3.63
Pseudamphistomum truncatum	–	–	0.6	2	–	–	–	–	–	–	–	–	0.2	2
Nematoda														
Toxocara canis	–	–	1.8	8 ± 5.57	4.1	8 ± 1	1.7	7.5 ± 1.5	–	–	–	–	1.6	7.86 ± 2.14
Gongylonema sp.	–	–	–	–	2	1	–	–	–	–	2.6	1	0.4	1
Ancylostoma caninum	–	–	–	–	–	–	0.8	2	–	–	–	–	0.2	2
Cestoda														
Taenia pisiformis	5	8 ± 1	0.6	17	–	–	3.4	11.5 ± 3.33	2.6	2	–	–	1.8	10.12 ± 2.17
Taenia hydatigena	–	–	1.8	4.7 ± 2.19	–	–	0.8	1	–	–	–	–	0.9	3.75 ± 1.80
Multiceps multiceps	2.5	3	1.8	3.7 ± 1.2	–	–	2.5	2.3 ± 0.33	–	–	–	–	1.6	3 ± 0.53
Multiceps serialis	–	–	1.8	3	–	–	0.8	2	–	–	–	–	1.1	2.75 ± .25
Mesocestoides lineatus	10	133.5 ± 23.04	3.7	75 ± 19.57	2	87	10.1	54.7 ± 10.30	2.6	31	5.3	27 ± 2	5.8	69.73 ± 9.38
Mesocestoides litteratus	7.5	41 ± 12.05	4.9	102.5 ± 36.24	4.1	43 ± 20	5	39.5 ± 6.44	2.6	64	2.6	21	4.7	64.33 ± 15.14
Dipylidium caninum			1.2	5 ± 1			3.4	4.7 ± 1.11			2.6	5	1.6	4.86 ± 0.63

Data analysis

Prevalence (P) and mean intensity (MI) were calculated according to Bush *et al.* (1997). For each intestinal helminth, prevalences were noted for each locality and for the entire sample of jackals. In addition, prevalence was calculated according to annual cycles (2005–2010) and host sex. The G-test was used for statistical evaluation of differences between the six localities (Sokal & Rohlf, 1995) in addition to the number of infected/uninfected hosts for each year. The chi-square test was used to compare the prevalence of intestinal helminths in male and female hosts. The mean intensity was calculated for the entire host sample and at each locality. Data were analysed using Statistica 5.1 (Statsoft, Tulsa, Oklahoma, USA) with the level of significance being $P < 0.05$.

Results

Up to 46 of 447 jackals (10.3%) were infected with at least one helminth species and a total of 12 species were identified, namely two trematodes, *Alaria alata* and *Pseudamphistomum truncatum*, three nematodes, *Toxocara canis*, *Ancylostoma caninum* and *Gongylonema* sp. and seven cestodes, *Taenia pisiformis*, *Taenia hydatigena*, *Multiceps multiceps*, *Multiceps serialis*, *Mesocestoides lineatus*, *Mesocestoides litteratus* and *Dipylidium caninum* (table 1).

The highest prevalence value was recorded at Smederevo (15.1%), followed by Veliko Gradište (10%), Negotin (10%), Velika Plana (6.1%), Surčin (7.9%) and Svilajnac (5.1%). These differences, which varied from 5.3% in 2007 to 15% in 2008, were not significant ($P > 0.9999$), relative to locality. Similarly, no significant differences in prevalence were found between years ($P > 0.502$) nor relative to host sex ($P > 0.817$). The smallest number of infected jackals (4 from 75 examined) was recorded in 2007, while the highest number (11 from 102 examined) was recorded in the last year – 2010. The most frequent parasites found in this study were the cestodes *M. lineatus* (found in 26 individuals) and *M. litteratus* (found in 21 individuals), with the respective prevalences of 5.8 and 4.7% and mean intensities 69.73 ± 9.38 and 64.33 ± 15.14. These two species also had the widest distribution range, having been recorded at all six locations.

Generally, all cestodes had a relatively higher prevalence in comparison to nematodes and trematodes. *Toxocara canis* was the nematode with the highest prevalence (1.6%) and a mean intensity of 7.86 ± 2.14, while among trematodes *A. alata* had the highest prevalence (0.9%) with a mean intensity of 19 ± 3. *Pseudamphistomum truncatum* and *A. caninum* were the only two parasites recorded in one animal only. With the exception of *Gongylonema* sp., which were found in the stomach, all parasites were found in the intestine.

Regarding the diversity of parasites, the highest number of species (10) was recorded at Veliko Gradište and Smederevo, while the smallest number of parasite species (4) was recorded at Velika Plana, Svilajnac and Surčin. While 34.8% (16) of the infections were monospecific, the majority (65.2%, 30) of individuals had multiple infections. Within the group with multiple infections: 37% (17) of jackals had two species of helminths, 21% (10) had three, while 4% (2) hosted four species. Only one animal had five species of parasites in its intestine, the highest number of recorded parasites per jackal in Europe. Most of the analysed animals were hosts to cestodes only (76%, 35), while 10% (5) were hosts to nematodes only. Four animals were hosts to both cestodes and trematodes, two animals were hosts to both cestodes and nematodes, and one animal was infected with both nematodes and trematodes. No single animal was infected with trematodes only. The findings of *P. truncatum* and *M. serialis* represent the first record of these parasites in jackals.

Discussion

The present analysis has shown that jackals in Serbia are hosts to 12 species of intestinal helminths. According to the available literature, 32 species of intestinal helminths have been recorded across the entire distribution range (table 2). Of the 12 species found in this research, *P. truncatum* and *M. serialis* have never been found infecting jackals before, and *M. lineatus* and *D. caninum* are recorded for the first time in jackal populations in Europe. Acanthocephalan species were not found in this study nor in other studies in Europe (Trifonov *et al.*, 1970; Papadopoulos *et al.*, 1997) (table 2), although they have been found in Iran and Tajikistan (Heptner & Naumov, 1967; Sadighan, 1969; Dalimi *et al.*, 2006; Meshgi *et al.*, 2009).

The total prevalence of gastrointestinal parasites in Serbia was significantly low (table 1) at only 10.3%. All previous studies have indicated a much higher helminth infection of jackals (Sadighian, 1969; Trifonov *et al.*, 1970; Dalimi & Mobedi, 1992; Papadopoulos *et al.*, 1997; Dalimi *et al.*, 2006; Meshgi *et al.*, 2009). Similarly, the prevalence of each helminth species was considerably lower than in previous studies.

These differences could be partly explained by seasonal variations in prevalence, which is well known in canids (see Rajković-Janje *et al.*, 2002; Saeed *et al.*, 2006). The low

Table 2. The occurrence (+) of intestinal helminths in the golden jackal from parts of Europe including Bulgaria (Trifonov *et al.*, 1970), Iran (Sadighian, 1969; Dalimi & Mobedi, 1992; Dalimi *et al.*, 2006; Meshgi *et al.*, 2009), Greece (Papadopoulos *et al.*, 1997), Tajikistan (Heptner & Naumov, 1967), Uzbekistan (Heptner & Naumov, 1967) and Serbia. N = number of hosts examined.

	Bulgaria $N = 13$	Iran $N = 10–100$	Greece $N = 5$	Tajikistan $N = ?$	Uzbekistan $N = ?$	Serbia $N = 447$
Trematoda						
Alaria alata	+		+			+
Alaria canis		+				
Pseudamphistomum truncatum						+
Ascocotyle sinoecum (*Phagicola sinoecum*)	+					
Echinochasmus schwartzi		+				
Nematoda						
Toxocara canis		+	+	+	+	+
Toxocara leonina	+	+		+		
Uncinaria stenocephala	+	+	+	+	+	
Ancylostoma caninum		+	+	+	+	+
Rictularia cahirensis		+			+	
Rictularia affinis					+	
Strongyloides stercoralis		+				
Trichocephalus vulpis (*Trichuris vulpis*)		+				
Oxynema crassispiculum		+				
Cestoda						
Taenia pisiformis	+	+	+	+		+
Taenia hydatigena	+	+		+		+
Taenia ovis		+		+		
Hydatigena taeniaeformis		+		+	+	
Taenia endothoracicus		+				
Multiceps multiceps	+	+				+
Multiceps serialis						+
Mesocestoides lineatus		+		+	+	+
Mesocestoides litteratus	+					+
Dipylidium caninum		+		+	+	+
Diplopylidium nolleri		+				
Joyeuxiella pasqualei		+				
Sparganum mansoni				+		
Diphyllobothrium mansonoides				+		
Echinoccocus granulosus	+	+				
Acanthocephala						
Macracanthorhynchus hirudinaceus		+				
Macracanthorhynchus catulinus				+		
Oncicola canis		+				

infection rate we found in Serbia could be explained by differences in sampling seasons between this study and other studies, since our study included specimens only from the winter, while studies from Greece, Bulgaria and Iran included the entire year. Detecting whether the diversity of intestinal helminths also shows seasonal variation is left for future research. The other possible reason for the differences in prevalence and diversity between other studies and ours is that the jackal population in Serbia has developed only recently. It is known that species introduced to a new area are usually less heavily parasitized, with a lower prevalence than the original population from which they have descended (Torchin *et al.*, 2003).

When the diversity of intestinal helminths between Serbian and Iranian populations is compared, only seven species are mutual, and all of them are common parasites of canid species. The difference in diversity of intestinal parasites in the two countries could be explained by the rule that, in some host species, similarities in parasite communities decay exponentially with increasing distance (Poulin, 2003). The data in table 2 show that the diversity of intestinal parasites in Serbia differs from both Bulgaria and Greece despite their close proximity. Note that these studies considered only a small number of hosts and more data are therefore required in order to come to a proper conclusion.

In Serbia, as in most other countries of south-eastern Europe, the jackal is the second most numerous wild canid after the red fox. Almost all intestinal helminths recorded in the jackal are also found in the red fox, with only a nematode from the genus *Gongylonema* not yet found in the red fox (Pavlović, 1994). Like the jackal, the red fox is an autochthonous canid of medium size, and the niches of the two animals overlap to a great extent (Lanszki *et al.*, 2006; Lanszki & Heltai, 2010). From an ecological point of view, this could explain the high number of common intestinal parasites of these two species.

Considering the constant spreading of the jackal's range and the permanent increase of its populations both in Serbia and throughout south-eastern Europe (Arnold *et al.*, 2012), it seems that this animal is likely to have a great parasitological significance, both for animal and human health, as a reservoir of specific parasites. For example, *T. canis*, *M. lineatus* and *D. caninum* are transmittable to humans and other canids. Of these, *T. canis* could be the most important as it is reported to be a common parasite in domestic and wild canids worldwide. In light of this, the next (necessary) step should be better monitoring of jackal population dynamics, as well as the prevalence of their parasites, throughout Serbia and the whole of its European range.

Acknowledgements

The authors wish to express their gratitude to all collaborators in the field who helped in collecting the material for this study: Goran Blagojević (Surčin); Zoran Gubin, Ljubiša Branković and Milan Stefanović (Smederevo); Nenad Mirković, Sladan Marković, Goran Cvetković and Mladen Bogdanović (Veliko Gradište); Živorad Pavlović and Predrag Živanović (Velika Plana); Borko Dimitrijević, Boriša Dimitrijević and Bata Dimitrijević (Svilajnac); Zoran Tufarević-Moce and Slobodan Kastratović (Negotin). The authors give their thanks to three anonymous reviewers for their useful advice and suggestions that significantly improved the paper. They are also very thankful to Maria del Mar Mancha Cisneros (Arizona State University in Tempe, USA), Enrico Sandro Colizzi (Utrecht University, The Netherlands) and Linda McPhee for final editing of the English text.

Financial support

This study was supported by the Ministry of Education and Science of the Republic of Serbia (Contract TR 31009).

Conflict of interest

None.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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