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Endoparasitic fauna of red foxes (*Vulpes vulpes*) and golden jackals (*Canis aureus*) in Serbia

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Abstract

Wild canides have a high epizootiological – epidemiological significance, considering that they are hosts for some parasites which spread vector born diseases. Increased frequency of certain interactions between domestic and wild canides increases the risk of occurrence, spreading and maintaining the infection of parasitic etiology in domestic canides. The research was conducted in 232 wild canides (172 red foxes and 60 golden jackals). The examined material was sampled from foxes and jackals, which were hunted down between 2010 and 2014, from 8 epizootiological areas of Serbia (North-Bačka, West-Bačka, Southern-Banat, Moravički, Zlatiborski, Raški, Rasinski and Zaječarski district). On completing the parasitological dissection and the coprological diagnostics, in wild canides protozoa from the genus *Isospora* were identified, 3 species of trematoda (*Alaria alata*, *Pseudamphistomum truncatum* and *Metagonimus yokogawai*), cestods from the genus *Taenia* and 5 species of nematodes (*Toxocara canis*, *Ancylostomatidae*, *Trichuris vulpis* and *Capillaria aerophila*). The finding of *M. yokogawai* in golden jackals were, to the best of our knowledge, one of the first diagnosed cases of metagonimosis in golden jackals in Serbia. The continued monitoring of the parasitic fauna of wild canides is needed to establish the widespread of the zoonoses in different regions of Serbia, because they present the reservoirs and/or sources of these infections.

Keywords

Red foxes, golden jackals, endoparasites, epizootiology, Serbia

Introduction

Fox (*Vulpes vulpes* Linnaeus, 1758) and jackal (*Canis aureus* Linnaeus, 1758) belong to the order of *Carnivora*, in the *Canidae* family and they are very adaptabile to the influence of people, also to the changes of the ecosystem. The fox is widespread in North-America (primarily in the northern forests of Canada and Alaska), in Asia and in Europe (Aubry *et al.* 2009; Teacher *et al.* 2011). The jackal inhabits the dry parts of Africa, the southeast part of Asia and Little Asia, the Middle East, the countries of the Persian Gulf and some countries in Europe (northern Italy, Slovakia, Austria, Hungary, northern Poland, Slovenia, Croatia, Bosnia and Herzegovina, Albania, Montenegro, Serbia, Greece, Romania) with the biggest concentration on the Balkan peninsula (Krystufek *et al.* 1997; Krystufek and Tvrtkovic 1990; Arnold *et al.* 2012). After the Second World War it has practically vanished from the territory of Yugoslavia, as a collateral damage to the wolf poisoning campaign. The increase of their numbers in Serbia started near the end of the ’70 in the last century, when as a particularly fertile animal it begun conquering new terrain. In the last decades its numbers have increased in its traditional habitats – the Carpats, southeastern Serbia, south Banat and Srem (Milenković *et al.* 2006; Zachos *et al.* 2009).

The population of wild canides can endure serious epizooty, caused by species that parasite in pet dogs. Foxes and jackals have a great epizootical – epidemiological importance, considering that some agents of vector borne diseases parasite in them, from which the most important are: leishmaniosis, ehrlichiosis, babesiosis, boreliosis, dirofilariosis, bartonellosis and hepatozoonosis. The increased interactions between pets and wild dogs, increases the possibility of the occurrence, spread-

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ing and uphold of the disease in the populations of pet dogs (Ilić *et al.* 2012).

Since the results of previous studies of parasitic infections of foxes and jackals in Serbia are scarce, systematic research have been carried out in order to identify reservoirs of parasitic infections in wild canide populations and to identify susceptible hosts. The results represent a directive in the process of the protection of jackals and foxes, which would lead to the survival of these carnivores and to the balance in biocenosis.

Materials and Methods

Study period and area

The research was conducted on 232 wild carnivores, in the period between 2010-2014. The animals were shot during the hunting season, on 8 epizootiological areas of Serbia (North-Bačka, West-Bačka, Southern-Banat, Moravički, Zlatiborski, Raški, Rasinski and Zaječarski district). The biggest part of Serbia has moderate continental climate, with the exception of the southwest part, which is on the border with the subtropical Mediterranean and continental climate. The coordinates of the geographical centre of Serbia are: 44°01´ and 20°49´, and the average amount of rainfall for the whole country is 896 mm.

Sampling

The research was performed on 172 foxes (90 females and 82 males) and 60 jackals (32 females and 28 males). The fresh corpses were collected in plastic bags, (were) kept in a transportable fridge and brought to laboratories no later than 12 hours from their collecting. A parasitological dissection of the foxes and jackals was carried out, and the specimens were classified as young specimens (up to 1 year old) and mature specimens (older than 1 year) based on their tooth records and body mass. After opening up the bodies a systematical exam was carried out to establish any pathological findings or to register the presence of parasites in them. For the detection of adult and larvae form of parasites in the digestive system, some of the segments were opened up, the mucosal surfaces were rasped and were washed through parasitological sieve. The isolated cestodes were conservated in 5% formalin, while nematodes and trematodes in 70% ethanol.

In order to determine trematodes, cestodes and nematodes, morphological identification of the parasites was done after parasitological section of foxes and jackals. An initial diagnosis of taeniid worms was given at the genus level, based on the shape, size and number of rostellar hooks of the parasites (Verster, 1969).

The feces (5–10 g) was examined by qualitative methods of diagnostics for the presence of helminth eggs and coccidia oocysts. The method applied was flotation with saturated aqueous NaCl solution (specific gravity of 1.200 at 20°C) and saturated aqueous $ZnSO_4$ solution (specific gravity 1.300 to 1.400 at 20°C).

In order to diagnose developmental and adult forms of *C. aerophila*, the trachea, bronchi and lungs were examined macroscopically; 25 g of lung parenchyma was examined by the method of Baermann's and a tracheal lavage was performed, too. On the autopsy the trachea was examined throughout its length with the larynx until the bifurcation. The tracheas were opened throughout their length and were observed under binocular microscope at 50 times magnification. The material was examined fresh or preserved in 30% ethyl alcohol. A detailed morphometric and morphologic analysis of diagnosed trichurid eggs types were conducted, which was done also on the adult forms of the found parasites, and the characteristics confirmed the findings of the nematode *C. aerophila*.

Table I. Prevalence of endoparasitoses of foxes of different age

ENDOPARASITES SPECIES	THE NUMBER OF POSITIVE FOXES (PREVALENCE %)							
	Age category							
	YOUNG ANIMALS (n = 69)		ADULT ANIMALS (n = 103)		TOTAL (n = 172)		χ^2	P
	No.	%	No.	%	No.	%		
Isospora spp.	23	33.33	10	10.75	33	19.18	14.87	0.00***
Alaria alata	32	46.37	53	56.98	85	49.41	0.43	0.51
Taenia spp.	1	1.45	7	7.53	8	4.65	2.66	0.10
Toxocara canis	58	84.05	27	29.03	85	49.41	55.31	0.00***
Ancylostomatidae	41	59.42	29	31.18	70	40.69	16.74	0.00***
Trichuris vulpis	33	47.82	62	66.66	95	55.23	2.55	0.11
Capillaria aerophila	0	0	22	23.65	22	12.79	16.90	0.00***
Pseudamphistomum truncatum	0	0	2	2.15	2	1.16	1.37	0.24

*** p< 0.001

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Table II. Prevalence of endoparasitoses of jackals of different age

ENDOPARASITES SPECIES	THE NUMBER OF POSITIVE JACKALS (PREVALENCE %)							
	Age category							
	YOUNG ANIMALS (n = 22)		ADULT ANIMALS (n = 38)		TOTAL (n = 60)		χ^2	P
	No	%	No	%	No.	%		
Isospora spp.	4	18.18	0	0	4	6.66	7.40	0.00***
Alaria alata	5	22.72	13	34.21	18	30.00	0.86	0.35
Taenia spp.	0	0	0	0	0	0		
Toxocara canis	8	36.36	6	15.78	14	23.33	3.30	0.07
Ancylostomatidae	9	40.90	11	28.94	20	33.33	0.90	0.34
Trichuris vulpis	1	4.54	6	15.78	7	11.66	1.71	0.19
Capillaria aerophila	0	0	0	0	0	0	/	/
Pseudamphistomum truncatum	1	4.54	1	2.63	2	3.33	0.16	0.69
Metagonimus yokogawai	0	0	1	2.63	1	1.66	0.59	0.44

***p<0.001

Table III. Prevalence of mixed infections of foxes and jackals with 2 types of endoparasites

ENDOPARASITES SPECIES	NUMBER OF WILD CARNIVORES (PREVALENCE %)					
	FOXES (n = 172)		JACKALS (n = 60)		χ^2	P
	No.	%	No.	%		
Toxocara canis *Alaria alata*	22	12.79	1	1.66	6.16	0.01*
Toxocara canis *Ancylostomatidae*	2	1.16	2	3.33	1.24	0.27
Toxocara canis *Isospora* spp.	1	0.58	0	0	0.35	0.55
Toxocara canis *Trichuris vulpis*	4	2.32	0	0	1.42	0.23
Toxocara canis *Capillaria aerophila*	2	1.16	0	0	0.70	0.40
Ancylostomatidae *Trichuris vulpis*	13	7.56	0	0	4.80	0.03*
Ancylostomatidae *Isospora* spp.	8	4.65	2	3.33	0.19	0.67
Ancylostomatidae *Alaria alata*	3	1.74	1	1.66	0.002	0.96
Trichuris vulpis *Alaria alata*	7	4.07	0	0	2.52	0.11
Trichuris vulpis *Capillaria aerophila*	2	1.16	0	0	0.70	0.40

*p< 0.05

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Table IV. Prevalence of mixed infections of foxes and jackals with 3 or more types of endoparasites

TYPE OF MIXED	INFECTIONS NUMBER OF WILD CARNIVORES (PREVALENCE %)					
	FOXES (n = 172)		JACKALS (n = 60)		χ^2	P
	No.	%	No.	%		
3 species of endoparasites	53	30.81	6	10	10.16	0.00***
4 species of endoparasites	10	5.81	0	0	3.65	0.06
5 species of endoparasites	2	1.16	2	3.33	1.24	0.27

***p< 0.001

Statistics

The statistical analysis was done in Graph Pad Prism software. For determining a statistically significant difference between age groups of foxes and jackals that tested positive for certain endoparasites we used the Hi-square (χ^2) test.

Results

In the examined foxes the biggest prevalence of nematode infections were: *T. vulpis* (55, 23%–95/172), *T. canis* (49,41% – 85/172), ancylostomatidosis (*Ancylostoma caninum* and *Uncinaria stenocephala*) (40,69%– 0/172). The trematode *A. alata* was (49,41%–85/172) present. A statistically very significant difference was established (p<0.001) in the prevalence of *Isospora* spp., *T. canis*, ancylostomatidae and *C. aerophila* compared to the age category of the foxes (Table I).

The largest number of examined jackals were infected with ancylostomatidae (*Ancylostoma caninum* and *Uncinaria stenocephala*) (33.33%–20/60), with the trematode *A. alata* (30.00%–18/60) and nematodes *T. canis* (23.33%–14/60). There was a statistically highly significant difference (p <0.001) in the prevalence of *Isospora* spp. in relation to the age category of jackals (Table II).

In the tested foxes there were 10 types of mixed infections, caused by 2 parasites, and the *T. canis – A. alata* was the dominant (12,79%–22/172). In jackals there were 4 types of mixed infections with 2 species of parasites, all with low prevalence. The most distributed were the *T. canis – Ancylostomatidae* and *Isospora* spp. – *Ancylostomatidae* (3,33%–2/60). A statistically significant difference was determined (p<0.05) in the prevalence of the mixed infections with *T. canis – A. alata* and *Ancylostomatidae – T. vulpis* comparing foxes and jackals (Table III).

Considering the other types of mixed infections, in 30,81% of the foxes (17/53) and 10,00% jackals (6/60) had a mixed infection with 3 types of endoparasites. A much smaller number of animals were diagnosed with mixed infection with 4 and 5 species of endoparasites. A statistically highly significant difference (p <0.001) was established in the prevalence of mixed infections with three kinds of endoparasites between foxes and jackals (Table IV).

Discussion

As a result of a synanthrope lifestyle foxes have a crucial role in the relationship between the populations of wild canides, household pets and humans, which can lead to the spreading of vector borne diseases and zoonoses (Duscher *et al.* 2005; Torina *et al.* 2013).

There are insuficient data on the distribution of golden jackals in Serbia, but it is assumed that these animals migrate from Western Europe to the eastern latitudes. Thus, twenty years ago, through the Carpathians and the Danube Basin, the jackal first appeared in eastern Serbia, in order to then spread to Belgrade and beyond the territory of Vojvodina (Petrović *et al.* 2012). In Croatia, the jackals came from the direction of Hungary over Drava river, and also rivers Morava, Timok, Danube, Nera, Sava and Tisa, which are natural corridors (Krystufek *et al.* 1997). It is suspected that as a result of such migration, jackals may affect the transfer, dissemination and uphold of new parasitic species which are receptive to domestic dogs. If we take into account the close phylogenetic relationship between the jackal and the domestic dog, then the listed assumption is fully justified (Arnold *et al.* 2012).

In addition to the significant role in the epizootic chain of parasitic infections, one should not ignore the impact of these wild carnivores on the ecosystem. The increased population of the golden jackal means that the foxes have a competitor. The reasons are the similarity and competitiveness in their carnivorous diet, which is why foxes are moving to other habitats and thus contribute to the further spreading of the parasitic pathogen (Majláthová *et al.* 2007; Duscher *et al.* 2013).

The first detailed surveys on helminth determination of foxes in Serbia dates from more than 50 years ago. Parasitic fauna of foxes were analised and over 29 species of helminths were detected in tested foxes in period between 1957-1962 (Lozanić, 1965). Later, in period between 1988-1992, it was shown that the prevalence of trematodosis in the tested foxes were 49,44%, cestodosis 62,45%, while nematodosis reached 88,10%. That survey, for the first time in foxes in Serbia, determined some new species of helminths, such as *Euryhelmis squamosa*, *Taenia multiceps*, *Taenia serialis*, *Mesocestoides litteratus*, *Diphyllobothrium latum*, *Echinococcus granulosus* and *Toxocara mystax* (Pavlović, 1993). In our research, in

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young foxes toxocarosis (84,05%) and ancylostomatidosis (59,42%) were the dominant findings, while in adults trichuriosis (66,66%) and alariosis (56,98%) were mostly detected. In young jackals, ancylostomatidosis had the highest prevalence (40,90%) followed by toxocarosis (36,36%), while in adults alariosis (34.21%) and ancylostomatidosis (28,94%) were detected.

From protozoa only species of the genus *Isospora* were diagnosed in this survey (19,18% in foxes and 6,66% in jackals), which parasites mostly in young and weak individuals, causing acute disease. Older individuals are infected rarely due to the existence of acquired immunity (Abdulazizis *et al.* 2011). In adult jackals coccidiosis was not diagnosed during this research.

The role of foxes in epizootiology of trematodal parasitic zoonoses was analized by Pavlović *et al*. (1998) in period 1988-1998. This results showed that the prevalence of trematodosis of tested foxes in the region of Belgrade was 48,56% (Pavlović *et al.* 1998). In comparation with these earlier data, in our survey *Alaria alata* was detected (in 49,41% of foxes and 30,00% in jackals), *Pseudamphistomum truncatum* (in 1,16% of foxes and in 1,66% in jackals) and *Metagonimus yokogawai* (in 1,66% of jackals). The finding of a high prevalence of alariosis is important, since the paratenic hosts for this zoonosis can also be snakes and wild boars, which insufficiently heat-treated meat poses a potential risk to human health (Moehl *et al.* 2009; Duscher *et al.* 2014). Foxes and jackals are infected with metacercariae through the intermediate host (snails and amphibians). Development forms of alariosis have so far been established in muscle, glandular and fatty tissue of wild boars in several European countries (Jaksić *et al.* 2002; Moehl *et al.* 2009; Portier *et al.* 2011).

The opistorhid *P. truncatum* is a typical common agent, which parasites in wild carnivores in Central, Southern and Eastern Europe, Russia, Denmark, Ireland, the UK, Germany and Poland (Hildebrand *et al.* 2011). The first finding of *P. truncatum* in red foxes in Serbia, was described by Lozanić (1965) within the sistemic survey of helminths of foxes in the Belgrade district.

The finding of *M. yokogawai* in golden jackals in this study is an important epizootiological data, as this is, to the best of our knowledge, one of the first diagnosed cases of metagonimosis in golden jackals in Serbia. Ćirović *et al*. (2013) have diagnosed in jackals in Serbia *P. truncatum* with a prevalence of 10.30%. As a part of the life cycle of this trematode is in snails and freshwater fish, the possibility of infection and risk are for all consumers of fish, including humans, (Cakić *et al.* 2007; Sherrard-Smith *et al.* 2009).

Foxes are reservoirs for many nematode infections, and are important for their uphold in populations of dogs. Ancylostomatidosis is one of the most common and most pathogen intestinal helminthosis of wild carnivores, diagnosed in 40.69% of foxes and in 33.33% of jackals. The finding of ancylostomatidae is important given that the larvae of *A. caninum* (and rarely *U. stenocephala*), can persist in the tissues of more than a year, causing cutaneous larva migrans (CLM) in humans (Razmjoo *et al.* 2014).

The ascaridae *Toxocara canis* was diagnosed in 49.41% of foxes and in 23.33% jackals tested. Data from various studies indicate that the prevalence of this nematodosis varies in foxes in some parts of Europe, and the results are consistent with the findings of some authors (Manke and Stoya, 1998; Hofer *et al.* 2000; Smith *et al.* 2003). According to the findings of Pavlović and Kulišić (1994), ascarididae were widespread in foxes in the teritory of Belgrade. They revealed the prevalence of *Toxocara canis* in 46.09% of adult foxes and in 81.81% pupies, *T mystax* in 9.66% and *T. leonina* in 5.20% tested foxes. The relatively high prevalence of *T. canis* in foxes, highly influenced by the accumulation of ascaridide type eggs in the soil and the ability to maintain infective larvae in a paratenic host (Saeed and Kapel, 2006). This nematode is responsible for the development of various forms of human toxocarosis, such as the visceral larva migrans (VLM), ocular larva migrans (OLM) or neurotoxocarosis (Macpherson, 2013). According to serological studies in Austria this disease is found in 6.30% of people (Poeppl *et al.* 2013), but it is estimated that there are hundreds of undiagnosed cases per year (Auer, 2011). In Serbia, there is a hight risk of these zoonoses, considering the revealed high prevalence of toxocarosis in dogs and a high level of soil contamination by *T. canis* eggs and in the recent years several hundred children had proven infections (Považan *et al.* 2011; Čolović-Čalovski *et al.* 2014).

In the examined foxes trichuriosis was the most common endoparasitosis (55.23%), while in jackals it was established with the prevalence of 11.66%. Due to their free range, wild carnivores represent an important source of eggs of *T. vulpis*, which contaminate the environment and expose domestic carnivores to the possibility of infection (Kirkova *et al.* 2006). This nematode was detected in foxes in other countries in Southeast Europe (Di Cerbo *et al.* 2008; Borecka *et al.* 2013; Vergles Rataj *et al.* 2013) and it has been proven that it can cause visceral larva migrans (VLM) in humans (Masuda *et al.* 1987; Márquez-Navarro *et al.* 2012). Because of the zoonotic potential of this species and the role of dogs, foxes and jackals as the source of infection, the most important is to set the accurate diagnosis, in order to prevent further infections or reinfections.

In the examined areas of Serbia respiratory capilariosis was established with 12.79% prevalence, and only in adult foxes (23.65%). It is assumed that one of the reservoirs of this zoonosis for domestic carnivores in the suburbs of Serbia are foxes from the territory of Vojvodina, in particular from the West Backa district that is presented in the study. In support of this assumption are the results of certain authors, who diagnosed in 84.00% the nematode *C. aerophila* in the trachea of foxes, from different parts of Vojvodina (Lalošević *et al.* 2013). Because of the close contact with people, pets pose a risk to the health of the human population.

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The role of foxes in epizootiology and epidemiology of nematode parasitic zoonoses in Serbia was examned in earlier decades. Pavlović *et al*. (1997) were diagnosed high occurance of nematodes in 82.31% of adult foxes and 71.57% in young foxes.

In our survey, teniosis was the only cestodosis detected in 4.65% of the tested foxes. Some previous studies in Serbia revealed higher occurance of cestodes in foxes, with prevalence of 49.02%. The most frequent were from *Mesocestoides* genus. *Mesocestoides lineatus* was found in 37.98% and *M. litteratus* in 10.95% of tested foxes (Pavlović *et al.* 2008). In this study for the first time was announced detection of *M. litteratus* in Vojvodina. Isolation of the *Mesocestoides* have a high epizootiological importance because of possible human infections. In different countries of Europe there are different types of parasites of the genus *Taenia* in red foxes (*T. serialis*, *T. teniaeformis*, *T. hydatigena*, *T. pisiformis*, *T. crassiceps* i *T. polyacantha*), which cause serious diseases in aberrant and intermediate hosts (Al-Sabi *et al.* 2014), including humans (OIE 2005). The possibility of differential diagnosis of the genus *Taenia* species exist, based on the application of molecular diagnostic methods (Al-Sabi *et al.* 2011; Armua-Fernandez *et al.* 2011) and as an alternative is not yet practiced in epidemiological studies in foxes (Al-Sabi *et al.* 2014).

In Europe, foxes are the main definitive hosts of the cestode *Echinoccocus multilocularis* which causes alveolar echinococcosis, which can be potentially lethal for humans (Duscher *et al.* 2006; Al-Sabi *et al.* 2014). Due to the habits of foxes in marking their territory (defecating on elevated spots), the distribution of the eggs of this cestode is eased and the contact with the intermediate (rodents) and a random host (people) is more possible, too (Duscher, 2011).

Red foxes and golden jackals are the main reservoirs of sylvatic trichinella (*Trichinella spiralis* and *T. britovi*) in Serbia, which have an important role in the cycle of the spread of zoonotic diseases (Cvetković *et al.* 2011). Petrović *et al*. (2012) established trichinelosis in 5.00% of the tested foxes, and in 8.33% of the jackals on the territory of Vojvodina. They can also play an important role in maintaining the sylvatic cycle of *Toxoplasma gondii* (Karbowiak *et al.* 2010). Wild canides are host for many other parasites (*Ehrlichia canis*, *Leishmania donovani*, *Hepatozoon canis*, *Dirofilaria* spp., *A. caninum*, *E. granulosus*) (Duscher *et al.* 2014; Gavrilović *et al.* 2015).

Parasitic zoonoses of wild carnivores are becoming a more actual public health problem around the world, which is why the importance and role of these species of carnivores as reservoirs of infection, only further increases. In order to predict the extent of certain zoonoses in certain regions of Serbia, for which foxes and jackals are the sources and/or reservoirs of infection, it is necessary to conduct monitoring of the parasites of these carnivores.

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