



Short Communication

OCCURRENCE OF *CRYPTOSPORIDIUM SPP.* AND *GIARDIA DUODENALIS* IN RED FOXES (*VULPES VULPES*) IN BOSNIA AND HERZEGOVINAAdnan Hodžić¹, Amer Alić², Jasmin Omeragić¹¹*Department of Parasitology and Invasive Diseases, Veterinary Faculty, University of Sarajevo, Zmaja od Bosne 90, 71 000 Sarajevo, Bosnia and Herzegovina*²*Department of Pathology, Veterinary Faculty, University of Sarajevo, Zmaja od Bosne 90, 71 000 Sarajevo, Bosnia and Herzegovina*

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ABSTRACT

The aim of this study was to determine the occurrence and distribution of *Cryptosporidium spp.* and *Giardia duodenalis* among free-living red foxes (*Vulpes vulpes*) in Bosnia and Herzegovina. For this purpose, a total of 123 fecal samples from red foxes, shot during hunting seasons between January 2011 and March 2012 were examined using immunofluorescent microscopy. Overall, observed prevalences of *Cryptosporidium spp.* and *Giardia duodenalis* were 3.2 % (4/123) and 7.3% (9/123), respectively. The results show that foxes might play the role of potential reservoirs of *Cryptosporidium* and *Giardia* parasites, but further molecular analysis are necessary to elucidate the source of infection, routes of transmission and zoonotic potential of these two pathogens.

Key words: Bosnia and Herzegovina, *Cryptosporidium spp.*, *Giardia duodenalis*, immunofluorescence, red fox**INTRODUCTION**

Cryptosporidium spp. and *Giardia duodenalis* are ubiquitous intestinal protozoan parasites that are recognised as prevalent and widespread pathogens of humans and domestic animals. They are being increasingly recognised as parasites of a diverse range of wildlife species (1, 2, 3, 4). Their life cycles are completed within an individual host, with transmission by the direct fecal–oral route or through ingestion of contaminated food or water (1, 2, 5). The red fox (*Vulpes vulpes*) is the most abundant wild canid species living in Bosnia and Herzegovina and is considered to be an important source of potentially zoonotic *Cryptosporidium* and

Giardia parasites, for both humans and domestic animals (6, 7, 8). Despite their hunting, the population density and distribution of red foxes has increased throughout Europe as a consequence of intensive oral anti-rabies vaccination programs (9). In Bosnia and Herzegovina, according to the State Veterinary Office, the total number of red foxes is about 21,000. Their number has been estimated to grow since the oral vaccination program started in October 2011. The growing population of red foxes, which have become synanthropic animals in some cases, increases the risk of human and domestic animal contact with (oo)cysts, via feces contaminated pastures, water and recreational areas and has important implications for the control and prevention of these pathogens in rural and periurban areas. However, very few studies (3, 6, 7, 8, 10) have been carried out and little information is available on the prevalence, distribution and genetic characterization of *Cryptosporidium spp.* and *Giardia duodenalis* in red foxes. The aim of this study was to determine the occurrence and distribution of *Cryptosporidium spp.* and *Giardia duodenalis* among free-living red foxes in Bosnia

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and Herzegovina, since no studies have been conducted to date in this area.

MATERIALS AND METHODS

The study area was the entire territory of Bosnia and Herzegovina which covers 51,209.2 km² and it is located in the western part of the Balkan Peninsula (43°52' N, 28°25' E). The geography is characterized by major differences which determine variations in climate, vegetation and landscape. The central and eastern parts of the country are mountainous (from 500 to over 2,000 m above the sea level) and are characterized by continental mountain climate and average annual precipitation ranging from 1,000 to 1,200 l/m². The northwest is moderately hilly, while the northeast is predominantly flatland, with moderate continental climate and an average annual precipitation ranging from 700 to 1,300 l/m². The southern part of the country (Herzegovina) has a mediterranean climate with very high precipitation (from 1,000 to 1,800 l/m²) and dominant karst and plain topography. Absolute minimum temperature during the winter seasons is about -35°C, while in the summer periods the absolute maximum temperature can reach 45°C. About 43% of territory is forested and most of the forest areas are in the central, eastern and western parts of the country.

Fecal samples from 123 red foxes (63 males, 43 females, 17 unknown), mostly originating from mixed and coniferous forests in six different regions (Table 1), were collected during the hunting season, between January 2011 and March 2012.

The samples were analyzed for the presence of *Cryptosporidium* spp. and *Giardia duodenalis*

(oo)cysts by a sucrose flotation concentration and immunofluorescent staining method, using FITC-conjugated (oo)cysts wall-specific monoclonal antibodies (MeriFluor *Cryptosporidium*/*Giardia*, Meridian Bioscience, Cincinnati, OH, USA) as previously described (11, 12).

RESULTS

Cryptosporidium spp. and *Giardia duodenalis* were detected in 4 (3.2%) and 9 (7.3%) samples, respectively. Co-infection with both parasites was not detected. Prevalences and geographical distribution of infected animals are shown in Table 1 and Figure 1.

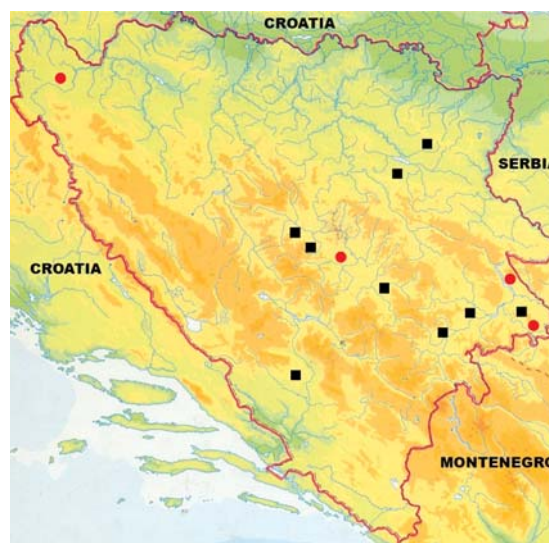


Figure 1. Geographical distribution of *Cryptosporidium* spp. (●) and *Giardia duodenalis* (■) infected foxes in Bosnia and Herzegovina

Table 1. Prevalences of *Cryptosporidium* spp. and *Giardia duodenalis* in Bosnia and Herzegovina

Region	Examined foxes	<i>Cryptosporidium</i> spp.		<i>Giardia duodenalis</i>	
		n	%	n	%
Central Bosnia	41	1	2.4	3	7.3
North Bosnia	5	0	0.0	0	0.0
North East Bosnia	26	0	0.0	2	7.7
East Bosnia	19	2	10.5	3	15.7
North West Bosnia	12	1	8.3	0	0.0
Herzegovina	20	0	0.0	1	5.0
Total	123	4	3.2	9	7.3

n - number of infected animals

DISCUSSION

Based on our results, the prevalences of *Cryptosporidium* spp. and *Giardia duodenalis* in the red fox population, were found to be relatively low but widespread. All infected foxes inhabit areas ranging from 126 to 1.201 m above sea level. Among all the regions surveyed in this study, the highest prevalences were detected in the region of East Bosnia (10.5% and 15.7%). The possible explanation for this observation and the main factor favoring infection with these two water-borne pathogens, could be the existence of large floodplain areas around the Drina river (length: 346 km) and its tributaries which flood the habitats, leaving numerous swamps after abundant rainfall. In contrast, much lower prevalences were recorded in Hercegovina (0.0% and 5.0%), most likely due to the high permeability of karst soil and the lack of surface waters.

Lower prevalence of 1,6% (2/124) of *Cryptosporidium* spp. was found in red foxes from 19 counties in Ireland (3), while the prevalence found by Zhou et al. (6) in Maryland, USA was 8.0% (6/76). Ravaszova et al. (10) examined fecal samples of 62 red foxes from the Slovak Republic using two different diagnostic methods, *Cryptosporidium* sandwich ELISA and modified Kinyoun's acid-fast stain and found a very high prevalence of 38.7% (24/62) and 20.96% (13/62), respectively. Hamnes et al. (7) detected *Cryptosporidium* oocyst in 6 (2.2%) and *Giardia* cysts in 13 (4.8%) fecal samples from the total of 269 Norwegian red foxes, while Beck et al. (8) found the same prevalence (4.5%) of *Giardia duodenalis* in Croatia. The results of molecular genetic studies demonstrated that foxes can be infected with the *Cryptosporidium canis* fox genotype, the *Cryptosporidium muskarat* genotype and the *Cryptosporidium canis* dog genotype, which is the only known human pathogen (6). Nucleotide sequence analysis of the *gdh*, *SSU-rDNA* and β -giardin genes of *Giardia duodenalis*, demonstrated a high degree of heterogeneity and the genotyping information showed the occurrence of zoonotic Assemblages A and B in red foxes, suggesting a potential role of these animals as a source of infections for humans or other animals (7, 8).

CONCLUSION

In conclusion, the results of the present study show that foxes might be potential reservoirs of *Cryptosporidium* spp. and *Giardia duodenalis*, but further molecular analysis are necessary to elucidate the source of infection, the routes of transmission and the zoonotic potential of these two pathogens. This is the first report on the occurrence of *Cryptosporidium* spp. and *Giardia duodenalis* in the red fox population from Bosnia and Herzegovina.

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