



Short Communication

**GIARDIA AND CRYPTOSPORIDIUM IN RED FOXES (*VULPES VULPES*):
SCREENING FOR COPROANTIGENS IN A POPULATION OF CENTRAL
ITALY AND MINI-REVIEW OF THE LITERATURE**Roberto Amerigo Papini¹, Ranieri Verin²¹*Department of Veterinary Sciences, University of Pisa,
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ABSTRACT

Giardia and *Cryptosporidium* are common protozoan parasites affecting several animal species and humans. The aim of this survey was to investigate, for the first time, their prevalence in red fox (*Vulpes vulpes*) faecal samples in central Italy. Seventy-one red foxes of different ages and sexes were examined for antigenic detection of *Giardia* and *Cryptosporidium* in fecal samples by means of a commercial rapid immunochromatographic test. The sample was randomly selected from foxes culled during a population control program. They were divided into groups based on sex and age (≤ 1 -year-old and > 1 -year-old). Five (7%) and one (1.4%) out of 71 fecal samples were positive for the *Giardia* or *Cryptosporidium* antigens by immunochromatographic assay, respectively, and no coinfections were observed. The present prevalence rates of *Giardia* and *Cryptosporidium* antigens in faeces from *V. vulpes* suggest that this host species is likely to play only a limited role in the spread of the two protozoa in the study area. A concise review of the literature related to *Giardia* and *Cryptosporidium* in *V. vulpes* is presented.

Key words: *Giardia*, *Cryptosporidium*, coproantigens, red fox, *Vulpes vulpes***INTRODUCTION**

The protozoa *Giardia duodenalis* (Sarcomastigophora: Hexamitidae) and *Cryptosporidium* spp (Apicomplexa: Cryptosporidiidae) are among the most common intestinal parasites in humans and many other animal species, including pets, livestock, and wildlife (1, 2). They have a direct life cycle. Infected hosts shed environmentally resistant (oo)cysts in their faeces. (Oo)cysts are immediately infectious and may retain their infectivity for long periods.

Transmission occurs via the faecal-oral route by the ingestion of (oo)cysts from contaminated water and food. Human-to-human and animal-to-human transmissions are also frequent (1, 2). Giardiasis and cryptosporidiosis are usually asymptomatic. Clinical manifestations in humans, when present, can vary from acute self-limiting diarrhoea to chronic gastrointestinal disease with persistent diarrhea, nausea, vomiting, fever, abdominal pain, malabsorption, malnutrition, and delayed growth in children (1, 2). Cryptosporidiosis can be a potentially life-threatening disease in immunocompromised individuals (2), as well as in calves (3) because of the rapid loss of fluids from profuse watery diarrhoea.

Red foxes (*Vulpes vulpes*) are widespread worldwide (4). Following successful oral anti-rabies vaccination programs, their number has increased throughout Europe (5), including in Italy (6), reaching high population densities in urban areas (7). Red foxes have been previously reported

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as potential reservoirs of serious zoonotic parasites (8), including *Giardia* and *Cryptosporidium* (9 - 18), and may be a source for transmission of pathogens to humans and animals (19). Because of the growing population of red foxes in urban areas (7), humans and domestic animals may be increasingly exposed to the risk of infection with *Giardia* and *Cryptosporidium* from environments contaminated by (oo)cysts shed in the faeces of infected red foxes (19).

In the present work, samples from red foxes culled in Italy were screened by a commercial immunochromatographic rapid diagnostic test for the occurrence of *Giardia* and *Cryptosporidium* coproantigens, in order to investigate, for the first time in Italy, the prevalence of the two protozoa in this host species. This paper presents the results as well as a short review of the literature worldwide for species and genotypes of *Giardia* and *Cryptosporidium* in *V. vulpes*.

MATERIAL AND METHODS

Study area, animals examined, and collection of faecal samples

Between September 2016 and January 2017, fresh faecal specimens (at least 3 gr) were collected from the rectums of 71 red foxes legally shot by hunters, during a population control program in the province of Pisa (43°43'N 10°24'E), Tuscany, central Italy. Woods and hilly sites characterize the sampling areas with a high density of red foxes (1.2-1.6 foxes/km²). The sex of the animals was determined and their age was assessed by crystalline lens weight (20). Foxes were classified as juveniles (≤ 1 -year-old) or adults (> 1 -year-old). Overall, fecal

samples from 41 males, 30 females, 46 adults, and 25 juveniles were collected. Samples were directly placed in plastic containers, refrigerated at +4°C, and tested within 48 hours.

Immunochromatographic tests

Each faecal sample was tested separately by the Xspect® *Giardia/Cryptosporidium* test (Remel Thermo Scientific, Santa Fe Drive, Lenexa, KS, USA) according to the manufacturer's guidelines. The Xspect® *Giardia/Cryptosporidium* kit is a commercially available qualitative immunoassay. It is licensed to simultaneously detect the presence of the *Giardia* cyst antigen and *Cryptosporidium* oocyst antigen in preserved and unpreserved fecal specimens, and is intended for use in humans. Performance characteristics provided by the manufacturer report sensitivity values of 95.8% and 96.4% with a specificity of 98.5% for *Giardia* and *Cryptosporidium*, respectively, in comparison to microscopy (<https://www.thermofisher.com/order/catalog/product/R2450520>). The test is easy to use and requires minimal equipment and training. Results are visually read after a relatively short incubation time of 15 minutes. It is thus suitable for use in field situations where there is a need for rapid diagnostic information with limited time, technical support, and resources.

Statistical analysis

Prevalence values were determined as the number of positive animals/number of examined animals X 100 with the corresponding 95% confidence intervals (95% CI). Different prevalence values according to sex and age groups were compared with the use of the Fisher's exact test. Differences were considered significant when $P < 0.05$.

Table 1. Number of positive samples, prevalence, and 95% confidence intervals for the occurrence of *Giardia* and *Cryptosporidium* in faeces from red foxes (*Vulpes vulpes*) in Italy as determined by coproantigen detection

Subjects		Giardia			Cryptosporidium			Total		
		Positive No.	Prevalence	95% CI	Positive No.	Prevalence	95% CI	Positive No.	Prevalence	95% CI
Sex	Males (n=41)	2 ^a	4.9%	0.0-11.5%	1 ^c	2.4%	0.0-7.2%	3	7.3%	0.0-15.3%
	Females (n=30)	3 ^a	10%	0.0-20.7%	0 ^c	0.0%	0.0%	3	10%	0.0-20.7%
Age	Adults (n=46)	2 ^b	4.3%	0.0-10.2%	1 ^d	2.2%	0.0-6.4%	3	6.5%	0.0-13.7%
	Juveniles (n=25)	3 ^b	12%	0.0-24.7%	0 ^d	0.0%	0.0%	3	12%	0.0-24.7%
Total (n=71)		5	7%	1.1-13%	1	1.4%	0.0-4.1%	6	8.4%	2-14.9%

^{a, b, c, d} No statistically significant differences by Fisher's exact test: ^a $P = 0.64$; ^b $P = 0.33$; ^{c, d} $P = 1.00$

Table 2. Summary of literature data on the molecular characterization of *Giardia duodenalis* and *Cryptosporidium* species/genotypes in free-ranging red foxes (*Vulpes vulpes*)

Country	No. of samples examined	<i>Giardia duodenalis</i>			<i>Cryptosporidium</i>	Reference no.
		Positive samples	Assemblages	Positive samples	Species/genotypes	
USA	76	ND	ND	6	<i>Cryptosporidium canis</i> fox genotype (n=4) <i>Cryptosporidium canis</i> dog genotype (n=1) Muskrat genotype I (n=1)	9
Norway	269	13	A (n=5) B (n=2)	6	ND	10
UK	124	ND	ND	10	<i>Cryptosporidium parvum</i> (n=2)	11
Croatia	66	3	A (n=1)	ND	ND	12
Australia	19	6	D (n=1) E (n=1) A/D (n=1) A/E (n=3)	2	<i>Cryptosporidium canis</i> (n=1) <i>Cryptosporidium macropodum</i> (n=1)	13
UK	30	ND	ND	4	<i>Cryptosporidium bovis</i> (n=1) <i>Cryptosporidium parvum</i> (n=1) Muskrat genotype II (n=1)	14
Australia	23	0	ND	1	<i>Cryptosporidium canis</i> (n=1)	16
Sweden	104	14	B (n=4)	ND	ND	17
Spain	87	7	ND	7	<i>Cryptosporidium parvum</i> (n=3) <i>Cryptosporidium canis</i> (n=2) <i>Cryptosporidium felis</i> (n=1) <i>Cryptosporidium ubiquitum</i> (n=1)	18

ND = Not determined

RESULTS

Overall, six (8.4%, 95% CI: 2-14.9%) samples were positive for coproantigens, with a moderate prevalence (7%, 95% CI: 1.1-13%) for *Giardia* and low prevalence (1.4%, 95% CI: 0-4.1%) for *Cryptosporidium*.

Among the five *Giardia* positive red foxes, three were juveniles (two females and one male) and two were adults (one male and one female). The only *Cryptosporidium* positive red fox was an adult male. Coinfections with both parasites were not detected. Table 1 shows the distribution of positive samples by age and sex. No significant differences related to age and sex groups were found. All the foxes appeared to have been in good body condition, as determined by fat deposits and body weight.

DISCUSSION

Variations in the prevalence of *Giardia* and *Cryptosporidium* in red foxes from different geographical areas have been widely reported. There are considerable differences from country to country and sometimes even between different regions

within the same country. The present prevalence rate for *Giardia* (7%) falls in the prevalence range previously reported in red foxes, varying from 0% in Australia (16) to 44% Sweden (17). With respect to *Cryptosporidium*, the present prevalence rate (1.2%) is the lowest detected among those reported for red foxes to date, varying from 2% in Norway (10) to 38.7% in the Slovak Republic (15).

It is difficult to compare prevalence data from different studies, as the results may vary depending on the diagnostic performance of the different tests used, the geographical features of the study area, the animal population examined, and the sampling method used. In concordance with the results of other studies (10, 13), the present prevalence of *Cryptosporidium* was consistently lower than that found for *Giardia* (1.4% vs. 7%). According to some authors (10), the spontaneous recovery of *Cryptosporidium* infection in red foxes may be complete within relatively short time periods. Conversely, *Giardia* infection usually takes longer to achieve complete recovery and thus may be more frequently identified in single faecal samples.

Although marketed for humans, the Xspect® *Giardia/Cryptosporidium* test has previously been used in veterinary medicine for the detection of

Giardia and *Cryptosporidium* in diarrheic or non-diarrheic sheltered cats (21) and pet squirrels (22). When compared with direct immunofluorescence in fecal specimens from naturally-infected cats, this rapid test showed sensitivities of 79.4% (95% CI: 62.1-91.3%) for *Giardia* and 42.9% (95% CI: 17.7-71.1%) for *Cryptosporidium*. These values were offset by specificities as high as 99% (95% CI: 97.2-99.8%) and 99.1% (95% CI: 97.4-99.8%), respectively (21). At present, it is unknown whether or not the test works in stool specimens from red foxes similarly to those from cats.

Regarding results in cats, the test may be highly effective in identifying uninfected animals but moderately or fairly effective in identifying infected ones. In addition, immunoassays commercialized for the detection of *Giardia* and *Cryptosporidium* in humans are validated for use with human isolates. Red foxes have been reported to be frequently infected with *G. duodenalis* assemblages and *Cryptosporidium* species other than those most commonly found in humans (Table 2). Genetic heterogeneity of *Giardia* and *Cryptosporidium* isolates between humans and animals could affect the performance characteristics of immunoassays for human specimens when used in animals. These factors do not necessarily mean that the Xspect® *Giardia/Cryptosporidium* test should not be used in veterinary medicine, but rather that results in animals should be interpreted more cautiously, and a more sensitive additional method may be advisable to confirm negative results.

In the present survey, we found a moderate prevalence of *Giardia* and a very low prevalence of *Cryptosporidium* compared with most of the published findings from other red fox populations. This could in part be the result of under-diagnosis because the immunochromatographic test might have missed some true positive results. Thus, we cannot rule out that the actual prevalence of *Giardia* and *Cryptosporidium* was higher than reported here.

Some studies have used molecular analyses and sequencing to identify *G. duodenalis* genotypes or/and *Cryptosporidium* species and genotypes in faecal samples from red foxes. The results have demonstrated that red foxes may shed a wide variety of both host-adapted and potentially zoonotic isolates. A summary of these results is presented in Table 2. Since no genetic studies have assessed the species and genotypes of the two protozoa occurring in the examined red fox population, the public health implications, if any, of the findings presented here remain undetermined.

It is worth noting that red foxes may ingest (oo)cysts by eating infected prey. For instance, *Giardia* and *Cryptosporidium* are routinely found in small rodents (23), which are a common part of the fox diet. Because it is difficult to distinguish between a mechanical passage of ingested (oo)cysts and an actual infection of the host, the question may arise as to whether foxes are actually infected and subsequently excrete (oo)cysts or whether (oo)cysts are merely passing through their digestive tract (15, 19). We believe that it is likely that at least some of the previously reported findings were not due to natural, true infections.

CONCLUSION

The results of the present study show that red foxes culled in an area of central Italy shed *Giardia* cysts and *Cryptosporidium* oocysts in their faeces with moderate and low prevalence rates, respectively. Red foxes are therefore unlikely to play an important role as a source for environmental contamination with (oo)cysts and for transmission of these parasites to other wildlife, domestic animals, and humans in the study area. The present findings complement and expand previous knowledge on the presence of *Giardia* and *Cryptosporidium* in free-ranging red foxes. To the best of our knowledge, this is the first report on the occurrence of *Cryptosporidium* and *Giardia* in a red fox population in Italy.

CONFLICT OF INTEREST

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

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