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Endoparasites of Red Fox (*Vulpes vulpes*) in Central Italy

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ABSTRACT: A parasitologic study on 129 red foxes (*Vulpes vulpes*) from Tuscany (central Italy) was carried out in 2004–2006. Five intestinal species were found at necropsy: *Dipylidium caninum* (prevalence 57.3%), *Mesocestoides lineatus* (45.4%), *Uncinaria stenocephala* (39.1%), *Toxocara canis* (9.1%), and *Toxascaris leonina* (5.4%). Other parasites not associated with the intestine included *Crenosoma vulpis* (14.7%), *Capillaria aerophila* (7.0%), *Angiostrongylus vasorum* (7.0%), and filarial parasites (17.8%). Coprologic tests were less sensitive and less specific in identifying parasites than direct examinations at necropsy. *Trichinella* larvae were not found in muscles submitted to artificial digestion. By immunologic assay, antigens of *Echinococcus* spp. were detected in fecal samples of 20 foxes, but results could not be confirmed by fecal examination or molecular tests.

Key words: Helminths, Italy, red fox, *Vulpes vulpes*.

The red fox (*Vulpes vulpes*) is the most widespread wild carnivore in Italy and represents a possible reservoir for domestic animal and zoonotic parasites, such as species of *Toxocara*, *Trichinella*, and *Echinococcus* (Enemark et al., 2000; Smith et al., 2003). A survey of red fox from the Tuscany region (central Italy) reported a high prevalence of intestinal parasites (82%); *Uncinaria stenocephala*, *Toxocara canis*, and *Mesocestoides* sp. were dominant species, but *Toxascaris leonina* and *Dipilydium caninum* also were present (Capelli et al., 2003). The aim of the present study was to further investigate the intestinal parasites of the fox population of this region and to extend the analysis to several tissue parasites, particularly zoonotic parasites.

In total, 129 foxes, killed during the hunting seasons 2004–2005 in Tuscany (within 42°30′–43°40′N and 10°25′–11°40′E; elevation 0–900 m above sea level) were examined. Foxes originated from plain areas and hills around Grosseto ($n=37$), Cecina ($n=50$), Pisa ($n=26$), and Siena ($n=16$). Carcasses were stored in sealed plastic bags at −20 C, and intestines were kept at −80 C for at least 7 days before necropsy to inactivate infective material. Data were collected on sex, weight, and location. Foxes were divided into two age classes, ≤2 yr and >2 yr, based on tooth wear (Harris, 1978).

To detect *Trichinella* larvae, samples of diaphragmatic, lingual, and tibial muscles (10 g) were digested in a Stomacher with pepsin (1:10,000 international units) and 17.5% HCl for 25 min at 41 C according to the Commission Regulation (EC) N. 2075/2005. Fecal samples were divided in three aliquots that were analyzed by microscopic, immunologic, and molecular techniques.

Intestines were examined for adult worms by stereomicroscopy. The entire contents of the intestinal lumen and the scraping of the internal intestinal wall were treated using the sediment and counting technique according to Eckert (2003) to detect eggs, cysts, and smaller parasites from concentrated material. The sediment was partly examined by microscopy, and partly stored in 70% ethanol for molecular analyses.

Lungs, heart, and main vessels were dissected, and adult parasites, larvae, eggs, or a combination were directly collected or detected in lung washes or pulmonary section smears. Nematodes were clarified in lactophenol, identified in accordance

TABLE 1. Intestinal helminths found in red fox (*Vulpes vulpes*) by necropsy. Pr = prevalence. A = abundance; M = mean intensity; R = range; I = index of importance.

	n	Pr (%)	A	M	R	I (%)
Dipylidium caninum	63	57.3	45.8	80.0	1–1,000	58.24
Mesocestoides lineatus	50	45.4	37.3	82.1	1–350	37.68
Uncinaria stenocephala	43	39.1	4.5	11.5	1–55	3.89
Toxocara canis	10	9.1	0.5	6.1	1–20	0.11
Toxascaris leonina	6	5.4	0.7	12.7	1–64	0.08

with Anderson et al. (1992). Cestodes were identified in accordance with Schmidt (1986) and were stored in 70% ethanol for molecular analyses. Microfilariae species identification was based on morphology, morphometry, and Barka staining technique as described previously (Magi et al., 2008). The immunologic assay to detect antigens of *Echinococcus* in feces was carried out by using the Chekit Echinotest Biphasic (Bomelli Diagnostic, Bonn, Switzerland).

A polymerase chain reaction (PCR) protocol based on *Echinococcus* mitochondrial 12S rRNA and described by Dinkel et al. (1998) was used to detect and identify *Echinococcus* species. Genomic DNA was extracted from 200 mg of each fecal sample (QIAamp DNA stool kit, QIAGEN, Milan, Italy) and from 300 µl of the sediment obtained from intestinal scrapes (Wizard SV Genomic DNA purification kit, Promega, Madison, Wisconsin, USA) following the manufacturers' protocols. The *Echinococcus* mitochondrial 12S rRNA target sequence has been used in phylogenetic studies (von Nickisch-Rosenegk et al., 1999), and the PCR yields a 373-bp fragment common to at least 12 cestode species (*E. multilocularis*, *E. granulosus*, *Taenia hydatigena*, *T. martis*, *T. taeniformis*, *T. crassiceps*, *T. mustelae*, *T. ovis*, *T. pisiformis*, *T. polyacantha*, *T. serialis*, and *Mesocestoides* spp.). Sequencing of the amplicons and sequence comparison by CLUSTAL W analysis were also performed to identify species.

The following epidemiologic indices were computed for each intestinal parasite found in foxes: Pr = prevalence, M = mean intensity, A = abundance, R = range (Bush et al., 1997), and I = index of importance (Thul et al., 1985). Test results and helminth counts were compared using McNemar's test (Armitage at al., 2002). Sensitivity, specificity, predictive values, and accuracy of the qualitative antigen test were calculated (Armitage at al., 2002), using necropsy results as a reference. Other statistical analyses required the use of the following tests: χ^2 test and Fisher's exact test to compare percentages of animals harboring parasites and Kruskal-Wallis test and Wilcoxon rank sum test to compare parasite burdens (Armitage et al., 2002). The analysis was carried out using R 2.5.1 (R Development Core Team, 2007). Results were considered statistically significant if $P<0.05$.

No *Trichinella* larvae were detected in muscles submitted to in vitro digestion. At necropsy, 92 of 110 (83.6%) foxes had intestinal parasites (Table 1). *Dipylidium caninum* was most prevalent with a high mean intensity (80); this was the dominant species ($I=58\%$). *Mesocestoides lineatus* was the second most prevalent parasite ($I=38\%$). *Uncinaria stenocephala* was the most prevalent nematode and seemed as subordinate species ($I=4\%$). The frequency distributions of these species in the fox population did not differ significantly from negative binomials distributions ($P=0.22$, 0.19, and 0.74 respectively). A relatively small number of foxes were infected by *Toxocara canis* (9.1%) and *Toxascaris leonina* (5.4%). No helminths were found in 18 foxes (16.4%); 36 specimens (32.7%) had one helminth species; 35 foxes (31.8%) had two species, 18 animals

TABLE 2. Comparisons of qualitative coprologic tests and necropsy results for red fox (*Vulpes vulpes*); s = sensitivity; s′ = specificity; PPV = positive predictive value; NPV = negative predictive value; AC = accuracy of the coprologic test assuming necropsy as reference test.

Necropsy/coprology	+/+	+/−	−/+	−/−	s	s′	PPV	NPV	AC
Dipylidium caninum	0	63	0	47	0.00	1.00	—	0.43	0.43
Mesocestoides lineatus	0	50	0	60	0.00	1.00	—	0.55	0.55
Uncinaria stenocephala	23	20	26	41	0.53	0.61	0.47	0.67	0.58
Ascarides	10	5	0	95	0.67	1.00	1.00	0.95	0.95
Capillaria aerophila	2	7	14	87	0.22	0.86	0.13	0.93	0.81

(16.4%) had three species, and three (2.7%) had four species.

The prevalence of *D. caninum* was significantly lower in the area of Pisa ($P=0.007$). Prevalence was significantly higher in the hills of Grosseto ($P=0.004$), whereas the prevalence of *M. lineatus* was significantly higher in the area of Siena ($P=0.045$).

The Echinotest indicated that 20 samples were positive to *Echinococcus* antigens. Fecal samples and intestinal scrapings for these 20 samples were negative for *Echinococcus* by microscopic examination and PCR. Sequenced amplicons showed the best homology to *M. lineatus* DNA (accession L49450; von Nickisch-Rosenegk et al., 1999).

Crenosoma vulpis and *C. aerophila* were found in trachea and lungs. The first species was identified in 14.7% of the animals. The infection rates were significantly different in the four areas ($P=0.007$), being higher in the area of Pisa, and according to sex (27% in males versus 6% in females; $P=0.001$). *Capillaria aerophila* was present in 7.0% of the foxes.

Angiostrongylus vasorum was found in the cardiopulmonary system of 7.0% of the specimens. Moreover, 23 foxes (17.8%) were positive for filarial parasites. Adult *Dirofilaria immitis* was found in the heart of eight animals; two of these also had microfilariae, and in one case *D. repens* also was present. Finally, 12 foxes had microfilariae of *Acanthocheilonema reconditum,* and three animals had *A. dracunculoides* (Magi et al., 2008). All filarial species and all stages were found only in foxes killed at an elevation of 0–200 m above sea level (a.s.l.).

Fecal examinations on 110 foxes identified eggs of *U. stenocephala/Ancylostoma caninum* (44.5%), *C. aerophila* (14.5%), *T. canis* (6.4%), *T. leonina* (2.7%), *Trichuris vulpis* (2.7%), larvae of *Strongyloides* spp. (2.7%) and oocysts of *Eimeria* spp (9.1%). The results from necropsy and fecal examination for *U. stenocephala*, *C. aerophila, T. canis* and *T. leonina* are consistent, but sensitivity and specificity for fecal examination were low compared with necropsy results (Table 2).

The present study confirms that red foxes in Tuscany host many parasite species reported previously in foxes in Italy. The fox population in this region, however, seems to be *Trichinella*- and *Echinococcus*-free.

A comparison of our results with those reported in other surveys is difficult, because most of the previous published studies report on parasite prevalence; the different techniques applied to recover the parasites may have some influence on prevalence estimates. However, several observations can be made from our data. The intestinal tapeworm *D. caninum* had a prevalence (57.3%) and a mean intensity (80) much higher than reported previously in Italy (<15%; Guberti and Poglayen, 1991; Capelli et al., 2003), whereas *M. lineatus* showed values comparable with published reports (Capelli et al., 2003). The prevalence (39.1%) and mean intensity (11.5) of *U. stenocephala* are in agreement with previous reports from Tuscany (Capelli et al., 2003). For other

helminths, *T. leonina* had a mean intensity of the infection of 12.7, which is higher than normally observed (<5). On the contrary, prevalence of *T. canis* (9.1) was lower than recorded in other surveys (Iori et al., 1990; Capelli et al., 2003; Manfredi et al., 2003).

Crenosoma vulpis was the most prevalent pulmonary parasite (14.7%), in agreement with studies carried out in Italy (Poli et al., 1985; Iori et al., 1990; Manfredi et al., 2003). The prevalence of *A. vasorum* was 7% and in European countries it ranges from 0% to 48%, whereas the prevalence of *C. aerophila* (7%) was much lower than usually reported (>30%; Davidson et al., 2006). *Acanthocheilonema* species were more widespread than *Dirofilaria* species, as expected due to the different vectors used by these parasites and their dissimilar presence at the various elevations. Ticks involved in the transmission of *Acanthocheilonema* are abundant on the study areas. In areas at 0–200 m. a.s.l., *Culex pipiens* and *Aedes albopictus*, the natural vectors for dirofilarial parasites in Italy (Cancrini et al., 2003, 2006), also are abundant and can feed 24 hours a day.

Coprologic methods detected fewer nematode infections than necropsy. This result could be due to small amount of feces sometimes recovered, and to the fact that each sample was divided into three aliquots. The immunologic tests for detection of *Echinococcus* coproantigens were nonspecific and lacked sensitivity. In fact, both microscopy and PCR failed to detect eggs and the adults/proglottids from feces and intestinal scrapings. These techniques may have application to detect *Echinococcus* in fecal samples where direct examination of the animal is not possible and to monitor the presence of *E. multilocularis* and *E. granulosus* in areas at risk, but they are not as reliable and cannot replace traditional methods.

Numerous parasites found in this study suggest that urban foxes could be a potential source of infestation to domestic pets and occasionally to humans. As such, further surveys of parasites of red foxes of Tuscany may be warranted in the future.

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