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Wild carnivores as source of zoonotic helminths in north-eastern Italy

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Summary

Two hundreds and sixty red foxes, eighteen badgers and eight stone martens from north-eastern Italy were examined for zoonotic parasites by SCT, with particular attention to *Trichinella* sp. and *Echinococcus multilocularis*. No adult worms of *E. multilocularis* were observed in the intestine of red foxes or mustelids. Out of 223 fox faecal samples analysed by a commercial CA-ELISA, 5.8 % was positive to *Echinococcus* coproantigens. *Trichinella* sp. was detected by digestion of muscle in 1.2 % of examined foxes (2/172), but not in mustelids (0/11). *Toxocara canis*, responsible for human toxocarosis, resulted to be common in fox population (48.5 %). This parasite was found both in young (53.3 % of cubs and 61.9 % of sub-adults) and in adult animals (42.7 %). Other zoonotic parasites as *Uncinaria stenocephala* (52.3 %) and *Trichuris vulpis* (0.4 %) were detected in fox populations.

Key words: red fox; badger; stone marten; helminths; zoonoses; Italy

Introduction

Domestic animals were historically considered as potential sources of zoonoses. Yet in the last few years it has become clear that free-ranging animals and birds are a major source of emerging human pathogens (Daszak *et al.*, 2000; Deplazes & Eckert, 2001). Particularly, wild carnivores harbour a great variety of viruses, protozoans and helminths that could be transmitted to both domestic animals and humans. Among wild carnivores, mustelids and canids are well-known reservoirs of important pathogens. Thanks to their large vagility wild carnivores play a main role in the diffusion of zoonoses, which should not to be understated.
Yet only sporadic records on helminths of these carnivores have been available in Italy so far (Iori *et al*., 1990; Magi *et al.*, 1999; Millan & Ferroglio, 2001; Ribas *et al.*, 2004; Rossi *et al.*, 1983; Stancampiano *et al.*, 1998), and mostly referring to populations of central and southern regions. Red fox (*Vulpes vulpes*), badger (*Meles meles*) and stone marten (*Martes foina*) are widespread species in the whole of Italy (Boitani *et al.*, 2003); they can colonise different habitats, including anthropogenetic ones.
Among them, the red fox contributes to a human infection supported by *Echinococcus multilocularis*, a tapeworm causing at its larval stage a serious human liver disease followed by a tumour-like proliferation, the alveolar echinococcosis (AE). The infection can be due to (i) accidental ingestion of parasite eggs, (ii) oral contact with hands contaminated by infected soil or plants, or (iii) handling of carnivores, their final hosts. AE shows a widening distribution area in Europe, mainly due to the changing ecological pattern of the major definitive host, *Vulpes vulpes*. Recently *E. multilcularis* was reported in foxes from Italy (Manfredi *et al.*, 2002) where the parasite was found in the northern region of Trentino Alto Adige. There it seems to be mainly distributed in the province of Bozen, close to the Austrian border. Though, other foxes found in the inner province of Trento resulted positive to the modified nested PCR analysis (Casulli *et al*., 2005).
The aims of this paper are to: (i) identify parasites of wild carnivores causing human infections with particular attention to *E. multilocularis* and *Trichinella* sp., and (ii) update data on the composition of gastrointestinal helminth fauna in wild carnivores from the eastern Italian Alps.

Material and methods

Sample collection
Between 1997 and 2003, two hundreds and sixty foxes, eighteen badgers and eight stone martens were collected in the provinces of Trento and Belluno, respectively in the north-eastern Italian regions of Trentino Alto Adige and Veneto. The animals collected were found naturally dead

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or were hunted according to a national law, L. 157/92. Their carcasses were transported to the provincial sections of Zooprophylactic Institutes. There the intestine and faeces from *Vulpes vulpes*, the gastrointestinal tract from *Meles meles* and from *Martes foina,* and the lungs from only six stone martens, were sampled and sent to the Faculty of Veterinary Medicine of Milan. Then, other parts - such as diaphragm, masseter, and the anterior tibial muscles - were taken from 172 red foxes and 11 mustelids.
Out of 174 foxes, 15 were classified as very young (cubs), 42 as young and 117 as adult considering their individual dentition and teeth wear together with their body size. As to their habitual altitude, 76 % of the foxes was from areas up to 1000 m a.s.l., while 24 % resulted from even higher environs up to 2200 m a.s.l.

Parasitological examination
The faecal samples of 223 foxes were detected by CA-ELISA searching the coproantigens released by *Echinococcus.* CHEKIT-Echinotest by Bommeli Diagnostic, Switzerland was used; according to its instructions, the results obtained, expressed by percentage, should be considered positive when they showed > 40%.
Then, the whole sediment on intestinal contents was analyzed by sedimentation and counting technique or SCT, which proved high sensitivity and specificity to *Echinococcus multilocularis*, as described by Hofer *et al.* (2000).
The trachea and bronchi from the lungs of stone martens were cut open; the entire bronchial tree was washed in saline solution to examine its sediment; the parenchyma was minced, washed in saline solution and its sediment was checked under a dissecting microscope (MAFF, 1986).
The parasites were preserved in alcohol 70 % before being clarified or stained through procedures previously described by Euzeby (1982), and identified using a Zeiss Axioscop microscope. They were classified according to the morphological keys proposed by Kozlov (1977), Durette-Desset & Pesson (1987), and Jancev (1986). Among the collected specimens only the scoleces could be counted while some others - like the cestodes from the mustelids - proved to be damaged and unidentifiable at a major taxonomical level. Prevalence (P) and mean abundance (A) of parasites were calculated for each host species, according to Bush *et al.* (1997).
Last, the *Trichinella* sp. infection was diagnosed by the enzymatic digestion of muscles.

Results

Out of 223 fox faecal samples analysed by CA-ELISA, 210 resulted to be negative (94.2 %, 95 % CI: 90.0 % – 96.7 %) to *Echinococcus* coproantigens. None of the positive samples (P = 5.8 %, 95 % CI: 3.3 % – 10.0 %) were confirmed by necroscopy.
On the whole, eleven different taxa of helminths (at species or genus level) were recorded in the fox intestines (Table 1). The prevalence of parasites in the considered animals showed to be high (P = 85.8 %; 95 % CI: 80.0 % – 89.7 %). Nematodes were found in the majority of hosts (P = 77.0 %, 95 % CI: 71.2 % – 81.8 %) and the most common species were *Uncinaria stenocephala* and *Toxocara canis* (P = 52.3 %, 95 % CI: 46.1 % – 58.5 %, and P = 48.5 %, 95 % CI: 42.3 % – 54.7 %, respectively), (Fig. 1). Among cestodes (P = 45.0 %, 95 % CI: 38.9 – 51.3), only *Mesocestoides lineatus* and *Taenia* sp. were recorded; no *Echinococcus multilocularis* adult worm was detected in our animal sample. Other parasites, as the nematodes *Trichuris vulpis*, *Oxynema crassispiculum* and *Capillari*a sp., and the trematodes *Metorchis vulpis* and *Plagiorchis elegans* proved rare and with very low abundance (Table 1).

Table 1. Intestinal helminths recovered in red fox (n = 260) by direct parasitological examination

Class	Parasite species	P (%; 95 % CI)	A ± SD
Cestoda	*Mesocestoides lineatus*	27.7 (22.4 – 33.6)	6.53 ± 29.59
	Taenia spp.	24.2 (19.2 – 29.9)	5.93 ± 27.45
Nematoda	*Toxocara canis*	48.5 (42.3 – 54.7)	3.64 ± 8.93
	Uncinaria stenocephala	52.3 (46.1 – 58.5)	8.56 ± 28.91
	Pterigodermatites affinis	24.2 (19.2 – 29.9)	1.62 ± 5.88
	Molineus legerae	5.8 (3.4 – 9.5)	0.13 ± 0.75
	Oxynema crassispiculum	0.4 (0.02 – 2.4)	0.02 ± 0.31
	Trichuris vulpis	0.4 (0.02 – 2.4)	0.00 ± 0.06
	Capillaria sp.	0.4 (0.02 – 2.4)	0.00 ± 0.06
Trematoda	*Metorchis vulpis*	0.8 (0.01 – 3.0)	0.01 ± 0.09
	Plagiorchis elegans	0.8 (0.01 – 3.0)	0.02 ± 0.22
TOTAL		85.8 (80.8 – 89.7)	26.46 ± 60.26

A: mean abundance; SD: standard deviation; P: prevalence; CI: confidence interval

As to the different altitudes where the foxes had been found, significant differences in parasite burdens were found only for cestodes (Kruskal Wallis test: $P < 0.001$). In fact, they were more abundant when considering altitudes over 1000 m a.s.l. where small mammals, the intermediate host, are more prevalent in the fox diet.
As to the gender of foxes, no difference was found among their parasite burdens (U-Mann test: each species $P > 0.05$).
When considering the age of the hosts, statistically supported differences emerged in the total parasite burdens and for *U. stenocephala* (Kruskal Wallis test: $P < 0.01$ and $P < 0.05$, respectively). Particularly, young animals were infected by the ancylostomid at a higher level than adults and cubs (A = 16.4 ± 51.9 *vs* A = 7.1 ± 24.2 and A = 2.7 ± 4.2, respectively). In contrast, *T. canis* was distributed uniformly among the three classes of age (Table 2).
Out of 172 foxes examined for *Trichinella* sp. only two were positive (1.2 %). Both animals were from Val di Sole (Trento), and were found respectively at altitudes of about 900 and 1000 m a.s.l.

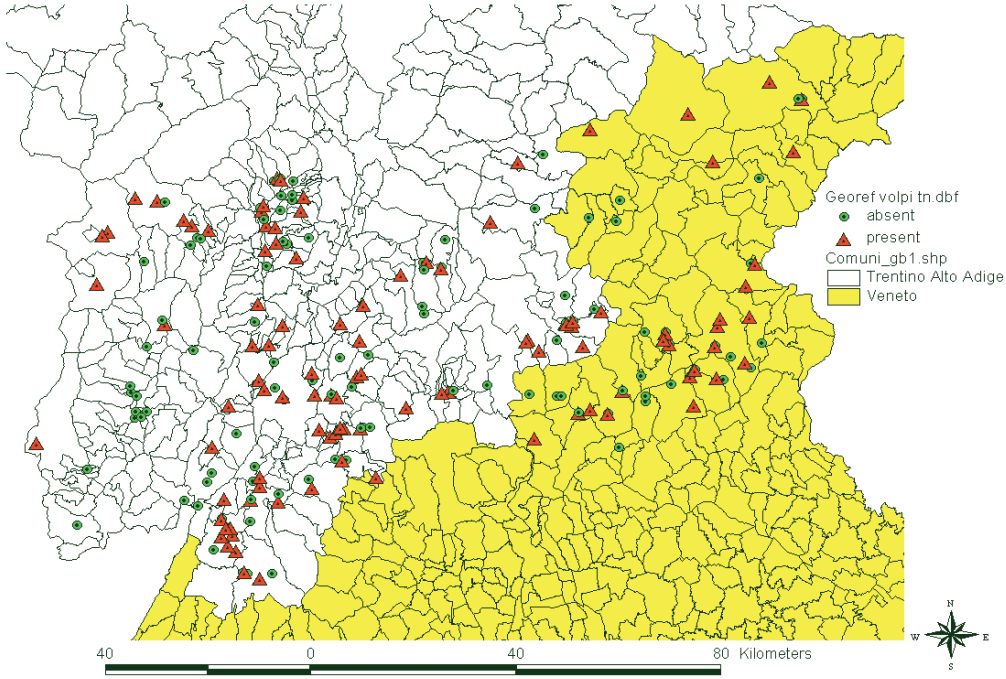

Fig. 1. Distribution of *Toxocara canis* in the study area

The majority of badgers (P = 94.4 %, 95 % CI: 70.6 % – 99.7 %) and stone martens (P = 75.0 %, 95 % CI: 35.6 % – 95.5 %) were positive to gastrointestinal parasites. Cesto-des were recorded in both hosts (badger: P = 22.2 %, 95 % CI: 7.4 % – 48.1 %; stone martens: P = 12.5 %, 95 % CI: 0.6 % – 53.3 %), all the identified worms belonged to *Mesocestoides* sp. and *Taenia* sp.; no *Echinococcus* specimens were found. Nematoda were identified, such as *Uncinaria criniformis, Molineus patens* and *Aonchotheca putorii* (Table 3). *Uncinaria criniformis* was found in the majority of badgers, and a significantly supported difference was found between the two hosts regarding the parasite abundance (U Mann-Whitney test: $P = 0.001$). All the lungs of stone martens were parasitized by one or more species of the parasites *Eucoleus aerophilus* (P = 88.9 %; A = 2.4 ± 1.3), *Crenosoma petrowi* (P = 66.7 %; A = 7.3 ± 11.1), and *Sobolevingylus petrowi* (P = 16.7 %; A = 1 ± 2.4). None of the mustelids were positive to *Trichinella* sp.

Table 2. Intestinal helminths recovered in red fox (n= 174), according to the three age classes

Class	**Parasite species**	**Cub (n = 15)** **P (%; 95 % CI)** **A ± SD**	**Young (n = 42)** **P (%; 95 % CI)** **A ± SD**	**Adult (n = 117)** **P (%; 95 % CI)** **A ± SD**
Cestoda	*Mesocestoides lineatus*	0 0	21.4 (10.8 – 37.2) 3.7 ± 12.4	29.1 (21.2 – 38.3) 9.4 ± 41.8
	Taenia spp.	6.7 (0.03 – 33.9) 1.1 ± 4.4	19.1 (9.1 – 34.6) 3.5 ± 12.4	22.2 (15.3 – 31.0) 6.7 ± 34.7
Nematoda	*Toxocara canis*	53.3 (24.7 – 77.7) 3.3 ± 4.5	61.9 (45.6 – 76.0) 5.2 ± 15.1	42.7 (33.7 – 52.2) 2.9 ± 7.4
	Uncinaria stenocephala	53.3 (824.7 – 77.7) 2.7 ± 4.2	76.2 (60.2 – 87.4) 16.4 ± 51.9	52.1 (42.7 – 61.4) 7.1 ± 24.2
	Pterigodermatites affinis	13.3 (2.3 – 41.6) 0.2 ± 0.6	26.2 (14.4 – 42.3) 2.9 ± 7.6	30.8 (22.7 – 40.1) 1.8 ± 6.8
	Molineus legerae	6.7 (0.03 – 33.97) 0.1 ± 0.2	14.3 (5.9 – 29.2) 2.9 ± 7.6	5.1 (2.1 – 11.3) 0.1 ± 0.9
	Oxynema crassispiculum	0 0	2.3 (0.1 – 14.1) 0.2 ± 0.5	0 0
	Capillaria sp.	0 0	0 0	0.9 (0.04 – 5.4) 0
Trematoda	*Metorchis vulpis*	0 0	0 0	0.9 (0.04 – 5.4) 0
	Plagiorchis elegans	0 0	2.9 (0.2 – 16.6) 0.1 ± 0.4	0.9 (0.04 – 5.4) 0
TOTAL		**80.0 (51.4 – 94.7)** **7.4 ± 7.8**	**100 (89.6 – 100)** **32.1 ±5 7.8**	**83.8 (75.5 – 89.4)** **28.1 ± 75.9**

Table 3. Gastrointestinal helminths recovered in badger (n=18) and stone marten (n=8) by direct parasitological examination

Class	Parasite species	Host species	P (%; 95% CI)	A ± SD
Nematoda	*Uncinaria criniformis*	badger	88.9 (63.9-98.0)	33.61±28.24
		stone marten	25.0 (4.4-64.4)	1.75±3.88
	Molineus patens	badger	38.9 (18.3-63.9)	7.78±14.98
		stone marten	25.0 (4.4-64.4)	0.38±0.74
	Aonchotheca putorii	badger	22.2 (7.4-48.1)	23.56±81.42
		stone marten	37.5 (10.2-74.1)	8.00±21.43
Cestoda	*Taenia sp.*	badger	11.1 (0.2-3.6)	2.1±6.6
		stone marten	12.5 (0.6-53.3)	0.13±0.35
	Mesocestoides sp.	badger	11.1 (0.2-3.6)	9.7±38.1
		stone marten	0	0
TOTAL		badger	94.4 (70.6-99.7)	76.72±105.89
		stone marten	75.0 (35.6-95.5)	10.25±21.21

A: mean abundance; SD: standard deviation; P: prevalence; CI: confidence interval

Discussion

The results of this study showed a high prevalence of intestinal helminth infection in north-eastern Italian fox populations and mustelids. Adults of *Echinococcus multilocularis* were absent in all hosts; nevertheless, CA-ELISA revealed a positive value in the 5.8 % of the examined faecal samples, which appears to be consistent with the low prevalence in foxes previously reported in Italy (Manfredi *et al.*, 2002; Casulli *et al.*, 2005). The discrepancies between the results of ELISA and SCT can be related to the absence of *E. multilocularis* adults in intestines. In a previous survey, infected foxes from Trentino Alto Adige showed a very low worm burden (Manfredi *et al*., 2004). It should also be considered that *Echinococcus* coproantigens can be detected in faeces up to five days following the tapeworm expulsion from the intestine (Eckert *et al*., 2001). Nevertheless, a cross-reaction with other cestodes might not be excluded (e.g. Eckert, 2003); in fact, cestodes were found in the intestine of 10/13 positive samples to *Echinococcus* sp. coproantigens.

Generally speaking, wild canids can be considered a major potential source of human infection with *Echinococcus;* other parasites seem to be of lower concern being their effects less dramatic. Thus, the role of wild canids as reservoir of zoonotic parasites should not to be understated for several reasons. Firstly, the high prevalence values of *Toxocara canis* and *Uncinaria stenocephala* in our foxes suggest that this carnivore may constitute an important source of selected parasites of veterinary and sanitary relevance. Particularly, nowadays the first nematode species is recognized as responsible for several forms of toxocarosis, while the latter is one of the ancylostomids causing the cutaneous larva migrans (Acha & Szyfres, 1989; Deutz *et al.*, 2005). Other studies have proved that these nematodes are both highly diffuse in red foxes and recently prevalence values similar to those found in the foxes from north eastern Italy have been reported (Suchentrunk & Sattmann, 1994; Steinbach *et al.*, 1994; Saeed *et al.*, 2006). Further, the prevalence of *T. canis* and *U. stenocephala* is higher in red foxes than in domestic dogs as observed in different study areas (Veneziano *et al.*, 2006; Martinez-Moreno *et al.*, 2007). In our opinion, foxes can act mainly as reservoir of parasites towards other canids, especially dogs. Besides, a high risk for the dissemination of parasite eggs can be supported by roaming animals. Particularly, the tenacity of *Toxocara* eggs towards environmental conditions might produce high level of contamination locally (Deutz *et al.*, 2005). Dogs can acquire *Toxocara* infection by ingestion of larvated eggs from fox faeces deposited in the environment or by ingestion of paratenic hosts (Antolová *et al.*, 2004). In particular, as stray dogs are exposed to natural infection more than owned dogs, they could be an important source of infection for humans, as observed by Martinez-Moreno *et al.* in 2007. However, a recent seroepidemiological study among people belonging to occupational groups at high risk, such as farmers and veterinarians, highlighted a high *Toxocara* seroprevalence in hunters (14.8 %) (Deutz *et al.*, 2005). In this case it could be assumed that the infection may occur via contaminated hands after handling infected definitive hosts, i.e. fox carcasses, because *Toxocara canis* eggs have frequently been traced in the pelage of mammals (Wolfe & Wright, 2004). Yet, human infection can also occur from wildlife environmental contamination. And for parasitic zoonoses acquired from wildlife, human intrusions into many wildlife habitats provide significant opportunities for parasite transmission (Daszak *et al.*, 2001).

In our study, T*oxocara canis* resulted distributed in the whole red fox population (Fig. 1), with no difference in

infection rate between young and adult animals. This is in agreement with the observations by Richards & Lewis (2001) in naturally infected foxes and with their opinion that adult foxes are highly involved in the dissemination of eggs into the environment.
And among zoonotic parasites, also the nematode previously named, *Trichuris vulpis,* should be included. In fact, it is responsible for trichurosis in humans and it is reported as having a role in the ethiology of the Larva migrans syndrome (Masuda *et al.*, 1987; Kirkova *et al.*, 2006).
Recently, other species of canids - like jackals and raccoon dogs - rapidly increased in Europe to a density similar to that of foxes. These canids showed to be able to spread into new territories, Italy included. In the last twenty years, the golden jackal, *Canis aureus*, has been recorded in north eastern Italy (Boitani *et al.*, 2003), and very recently also the raccoon dog was signalled (Lapini, 2006). Both canids can host many species of parasites infecting foxes. The raccoon dog is a vector of rabies (Holmala & Kauhala 2006) and parasites particularly of *Echinococcus multilocularis*, as showed by some infected raccoon dogs found in Germany, of *Sarcoptes scabiei* (Shibata & Kawamichi 1999; Ninomiya & Ogata 2005), and of *Trichinella* spp. (Oksanen *et al*., 1998; Oivanen *et al*., 2002). Epidemiologically these canids could be significant as fostering environmental contamination by parasite eggs, thus becoming a potential health hazard. Finally, previous records of *Trichinella* sp. in northern Italy mainly concerned carnivores from western regions (Di Matteo *et al.*, 1991; Remonti *et al.*, 2005). Therefore, the detection of *Trichinella* sp. in the present study updates the data related to the spreading of this parasite in the north-eastern area, confirming the absence of *Trichinella* at low altitudes (Pozio, 1998).
In Italy red foxes seem to be the main reservoir of *T. britovi,* an encapsulated species showing hosts with cannibalistic or scavenger behaviour. The parasite spreads among populations of wild carnivores living in natural ecosystems, not altered by humans. In these habitats cannibalistic and scavenger behaviour occurs more frequently than in anthropic ones (Balestrieri *et al.*, 2007; Pozio, 1998). In the present study, most of the foxes (76 %) were found dead in localities up to 1000 m a.s.l., where urbanisation is denser than in any other territory above.
In particular, the two infected animals were found in localities close to the Stelvio National Park at altitudes where sylvatic trichinellosis has been considered most prevalent among foxes.
As to other parasites we could record, the composition of gastrointestinal helminth fauna in the studied carnivores reveals the presence of some target species to host, like *Pterigodermatitis affinis*, *Molineus legerae* and *Oxynema crassispiculum* in red foxes; *Uncinaria criniformis*, *Molineus patens* and *Aonchotheca putorii* in mustelids. *Molineus legerae* has been recorded only in France and Spain. However its presence should not be considered exceptional; in fact, the nematodes belonging to this genus are strictly associated with wild canids and show a species-specific degree higher than *M. patens*, rather specific for mustelids even if recordable in foxes (Durette-Desset & Pesson, 1987; Segovia *et al.*, 2004; Sato *et al.*, 2006).
The nematode *Oxynema crassispiculum* together with the trematodes *Metorchis vulpis* and *Plagiorchis elegans* were very rare components of fox helminth fauna (Di Cerbo & Manfredi, unpublished data). Further, *O. crassispiculum* was recorded very few times in red foxes and this seems to be the first record in a free ranging European fox (El-Shehabi *et al.*, 1999). As to the gastrointestinal helminth fauna of mustelids, the presence of *U. criniformis* has been reconfirmed in Italy, which allows us to update its geographical distribution now reaching the Alpine host populations (Magi *et al.*, 1999). As far as we know, no data on the occurrence of *U. criniformis* in Italian *M. foina* were available previously. The parasite, which has a direct cycle (geohelminth), was found both in badgers and stone martens, but occurred more frequently in the former species; this according to Rosalino *et al.* (2006), who reported the badger as the main and definitive host of *U. criniformis*.
As to another parasite, *A. putorii*, no differences were found between the two hosts. This capillarid nematode can have both a direct and an indirect cycle. Earthworms, on which badgers and stone martens base their diet, are its intermediate hosts (Biancardi & Marassi, 2002; De Marinis & Asprea, 2003). Moreover, bronchopulmonary helminths of stone marten are parasites with an indirect cycle too, with molluscs and earthworms as intermediate hosts. Thus, the homogeneity of helminth fauna recorded in mustelids could be associated to a partial overlap of both habitats and trophic sources, i.e. earthworms and snails, between badgers and stone martens alpine populations.

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