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Brief Report

The occurrence of taeniids of wolves in Liguria (northern Italy)

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ABSTRACT

Canids are definitive hosts of *Taenia* and *Echinococcus* species, which infect a variety of mammals as intermediate or accidental hosts including humans. Parasite transmission is based on domestic, semi-domestic and wildlife cycles; however, little is known of the epidemiological significance of wild large definitive hosts such as the wolf. In this study, 179 scats of wolves (*Canis lupus italicus*) collected throughout the Italian region of Liguria were analyzed for the detection of taeniid infection. Taeniid egg isolation was performed using a sieving/flotation technique, and the species level was identified by PCR (gene target: 12S rRNA and *nad 1*) followed by sequence analyses. Based on sequence homologies of ≥99%, *Taenia hydatigena* was identified in 19.6%, *Taenia krabbei* in 4.5%, *Taenia ovis* in 2.2%, *Taenia crassiceps* in 0.6%, *Hydatigera taeniaeformis* in 0.6% and *Echinococcus granulosus* in 5.6% of the samples. According to these results, *Canis lupus italicus* can be considered as involved in the wild (including cervids and rodents) and semi-domestic cycles (including sheep and goats) of taeniids in this area.

1. Introduction

The grey wolf *Canis lupus* (Linnaeus, 1758), a large carnivore of the Canidae family, is widely distributed throughout Eurasia and North America (Mech and Boitani, 2003). In Europe, at the end of the last century, the wolf survived in highly fragmented populations as a result of legal human persecution (Breitenmoser, 1998). The Italian wolf population *Canis lupus italicus* (Altobello, 1921) progressively increased after full legal protection and conservation action in 1976 (Boitani, 1992). From the Central Apennines, wolves recolonized the entire Apennine chain and the Western Alps, where they established stable packs (Fabbri et al., 2007; Marucco et al., 2012). Despite this positive trend, illegal killings are still one of the most important threats to the species (Boitani, 2000; Lovari et al., 2007), and thus it is still considered as endangered. Depredation on livestock is the main cause of conflict between human activities and the presence of wolves (Meriggi et al., 2011). These events have increased the probability of contact between wolves, domestic animals and humans, which has led to a higher risk of pathogen transmission (Daszak et al., 2000). However, due to the increase of human activity in wildlife habitats, a transmission of infectious agents from domestic animals to wild animals should be considered (Thompson, 2013). A European example for such scenario is the establishment of the Asian eye worm *Thelazia callipaeda* in fox populations (Malacrida et al., 2008).

In this study, we focused on Taeniidae (Cestoda), a family of tapeworms with a considerable medical and veterinary importance (Craig and Pawłowski, 2002; Hoberg, 2002). A previous study carried out on illegally killed wolves, from all over the Apennine chain (Guberti et al., 1993, 2004), identified the Italian wolf as a definitive host of many species of taeniids (*Taenia hydatigena*, *Taenia multiceps*, *Taenia pisiformis*, *Taenia ovis* and *Echinococcus granulosus sensu lato*). *E. granulosus* is the etiological agent of cystic echinococcosis (CE), an important parasitic zoonosis transmitted mainly in a domestic cycle, involving sheep and dog, in southern Europe (Eckert and Deplazes, 2004; Alvarez Rojas et al., 2014). The aim of this study was to evaluate with a non-invasive sampling the role of wolves as definitive hosts for the taeniid species.

2. Materials and methods

From 2011 to 2014, an investigation was carried out on six packs of wolves (approximately 66 individuals; Meriggi et al., 2013) living in the northern Apennines and southern Alps in Liguria (Italy).

The study area (5343 km^2) was subdivided into 100 km^2 sample units, each containing at least one transect (Fig. 1) randomly selected from among the existing footpaths for a total of 64 transects and 298 km. Every transect was covered four times a year (once

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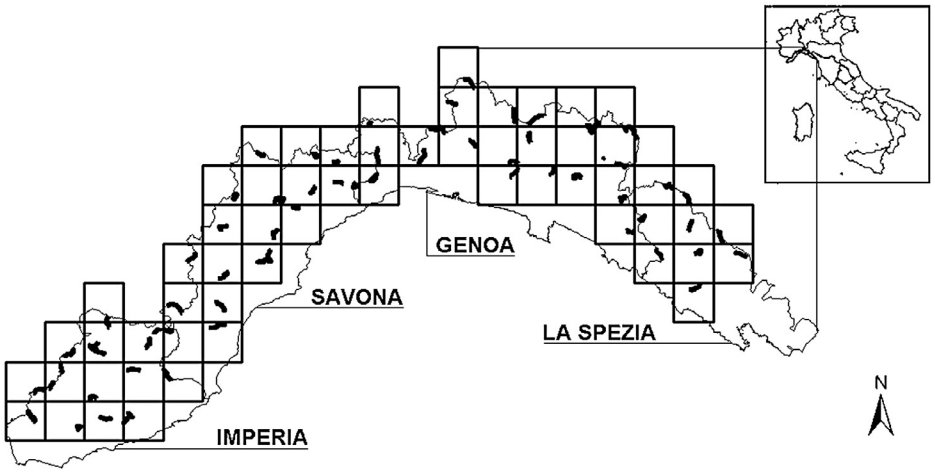

Fig. 1. Study area, Liguria (Italy). Dark areas represent transects investigated to sample wolf scats. Each square represents a sample unit of 100 km^2 over an area of 5343 km^2.

per season) in order to collect wolf scats, georeferenced in the UTM WGS84 32N-coordinate system using ARCGIS 10.0 (ESRI, http://www.esri.com/software/arcgis/).

A total of 179 fecal samples of wolves (44.75 ± 34.17 per year; 2.99 ± 5.39 per sample units) were collected from all over the region. Samples were stored at –80 °C for safety precautions (Eckert et al., 2001). In order to correctly discriminate wolf scats to those of dogs and foxes, we applied a critical analysis to reduce the probabilities of misinterpretation. Specifically, we evaluated size, shape, smell, composition and location (Mattioli et al., 1995; Bassi et al., 2012). Wolf adult droppings are 10–15 cm long and 3–5.5 cm thick, cylindrical, with sub-divisions, tapered at one of the extremities (Chame, 2003; Bang et al., 2007; Jedrzejewsky and Sidorovich, 2010). The secretion produced by the anal gland adheres to the feces during defecation, atrophied in most dog breeds, and give it an acrid and characteristic smell (Harrington and Asa, 2003). Moreover, wolves usually use feces in territorial marking. In the wild, feces are more common along trails and road, particularly at junctions, placed on conspicuous objects (Peters and Mech, 1975; Vilà et al., 1994). Finally, we also considered undigested prey remains (i.e., fur, snails and parts of bones), especially from large wild ungulates (i.e. wild boar and deer) (Meriggi et al., 2014) into wolf scats.

About 2 g of each fecal sample was placed in 15 ml tubes. Taeniid eggs were isolated using the flotation and sieving method described by Mathis et al. (1996), and morphological identification was carried out under an inverted microscope. DNA extraction of taeniid egg positive samples was performed as described by Štefanić et al. (2004), and species discrimination was carried out by multiplex PCR (for *Taenia* spp. and *E. granulosus* gene target: 12S rRNA, for *E. multilocularis* gene target: *nad 1*) and sequence analyses (Trachsel et al., 2007). Each *E. granulosus* positive sample was subsequently analyzed with *E. granulosus* "sheep strain" specific PCR (gene target: 12S rRNA) according to Štefanić et al. (2004).

In 8 out of 49 *Taenia* positive samples by multiplex PCR, the sequencing results were unclear and could not discriminate between *T. serialis/T. multiceps*. Therefore, further analyses of these samples using a PCR, targeting *nad1* gene, according to Armua-Fernandez et al. (2011) were performed, which resulted in a 500 bp amplicon which allows further species discrimination by sequence analysis.

The amplicons were directly sequenced after purification of the PCR products using the MinElute PCR purification kit (Qiagen, Hilden, Germany). Sequencing was performed by Synergene Biotech GmbH, Biotech Center Zurich, Switzerland (http://www.synergene-biotech.com), using primers Cest5seq and Cest4 for *Taenia* spp. and *E. granulosus* amplicons obtained with multiplex PCR (Trachsel et al., 2007), and *nad1*T-Rv for *Taenia* spp. amplicons obtained with *nad1* PCR (Armua-Fernandez et al., 2011).

Sequencing results were compared with entries in the GeneBank nucleotide database, using BLAST (http://www.blast.ncbi.nlm.nih.gov).

3. Results and discussion

Overall, 59 (33.0%) out of 179 samples were positive for eggs of taeniids (Table 1). *Taenia hydatigena, T. ovis, T. crassiceps, Hydatigera* (*Taenia*) *taeniaeformis* and *E. granulosus* identification was performed sequencing 12S amplicons obtained by multiplex PCR (Trachsel et al., 2007), while the 8 doubtful samples were correctly identified as *T. krabbei* by sequencing the amplicons obtained with *nad1* PCR. In both genes, the sequence homology of the amplicons was ≥ 99% identical to the corresponding sequences in the GeneBank. By multiplex PCR in 6 of 59 positive samples, multiple infections with *Taenia* spp. and *E. granulosus* were detected (*E. granulosus* with *T. hydatigena* (3 cases), with *T. krabbei* (2 cases) and with unspecified *Taenia* sp. (one case)).

The non-invasive sampling approach, based on collection of scats in the environment, is suitable for epidemiological studies on wild animals, and necessary when protected species are investigated without interfering in the existing structure of the population. The molecular identification of the parasite infections, obtained through the isolation of their eggs in feces, PCR analysis and sequencing, is a valuable tool to estimate the level of the environmental contamination, and it has been previously used for epidemiological investigation on wolves (Guerra et al., 2013). When stool are fresh, individual identification of the animal, using molecular analyses is possible (Galaverni et al., 2012). This study based on mostly old scats collected in the environment did not allow the individual animal

Table 1
Frequency of taeniid findings in wolf fecal samples from the Liguria region (Italy).

Taeniid species*	Frequency (%)	Confidence interval (95%)
Taenia hydatigena	19.6	14.1–26.0
Taenia krabbei	4.5	2.0–8.5
Taenia ovis	2.2	0.8–5.4
Taenia crassiceps	0.6	0.0–2.9
Hydatigera taeniaeformis	0.6	0.0–2.9
Echinococcus granulosus sensu lato	5.6	2.9–9.9
Total positive	33.0	26.1–40.1

* As determined by PCR (*nad 1* and 12S rRNA gene).