International Journal for Parasitology: Parasites and Wildlife 6 (2017) 1–7

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Italian wolves (*Canis lupus italicus* Altobello, 1921) and molecular detection of taeniids in the Foreste Casentinesi National Park, Northern Italian Apennines

Giovanni Poglayen [a, *], Francesca Gori [b], Benedetto Morandi [a], Roberta Galuppi [a], Elena Fabbri [c], Romolo Caniglia [c], Pietro Milanesi [c], Marco Galaverni [c], Ettore Randi [c], Barbara Marchesi [a], Peter Deplazes [b]

[a] Alma Mater Studiorum, Department of Veterinary Medical Science, Via Tolara di Sopra, 50-40064, Ozzano dell'Emilia, Bologna, Italy
[b] Institute of Parasitology, University of Zurich, Winterthurerstrasse 266a, CH-8057 Zurich, Switzerland
[c] Genetics Laboratory, Institute for Environmental Protection and Research (ISPRA), Ozzano dell'Emilia, Bologna, Italy

ARTICLE INFO

Article history:
Received 5 October 2016
Received in revised form
21 December 2016
Accepted 16 January 2017

Keywords:
Canis lupus italicus
National park
Non-invasive genetics
Molecular identification
Parasites
Taeniids

ABSTRACT

After centuries of massive decline, the recovery of the wolf (*Canis lupus italicus*) in Italy is a typical conservation success story. To learn more about the possible role of parasites in the wolves' individual and population health and conservation we used non-invasive molecular approaches on fecal samples to identify individual wolves, pack membership, and the taeniids present, some of which are zoonotic. A total of 130 specimens belonging to 54 wolves from eight packs were collected and examined. Taeniid eggs were isolated using a sieving/flotation technique, and the species level was identified by PCR (gene target: 12S rRNA and *nad1*). Taeniid prevalence was 40.7% for *Taenia hydatigena*, 22.2% for *T. krabbei*, 1.8% for *T. polyacanta* and 5.5% for *Echinococcus granulosus*. The prevalence of *E. granulosus* is discussed. Our results show that the taeniid fauna found in wolves from the Foreste Casentinesi National Park is comparable to that described for other domestic and wild Italian canids and provides insights into the wolves' diet and their relationship with the environment.

1. Introduction

After centuries of massive decline, several populations of large carnivores (brown bear, wolf, lynx, and wolverine) are now recolonizing parts of their historical ranges in many European countries thanks to the implementation of active adaptive conservation efforts (Chapron et al., 2014). The wolf in Italy is a typical conservation success story (Randi, 2011).

At the end of the Second World War, Italian wolves were close to extinction, surviving at their historical minimum population size in two isolated areas in the Southern Apennines (Zimen and Boitani, 1975; Boitani, 1984, 1992). However, since the late eighties socio-ecological changes and the increase in wild ungulates in natural areas have favored a spontaneous re-expansion of Italian wolves along the Apennines to the Western Italian and French Alps (Breitenmoser, 1998; Boitani, 2000; Valière et al., 2003; Fabbri et al., 2007; Marucco and McIntire, 2010). On one hand, the impact of this rapid recovery can increase conflicts with hunters seeking the same prey, livestock breeders suffering economic losses caused by wolf predation on domestic herds (Milanesi et al., 2015), and the general public many of whom have a historical fear of wolves, which are still perceived as a potential threat to human safety (Linnell and Boitani, 2011; Glikman et al., 2012). On the other, the wolf arouses positive harmonies as a flagship species whose biology, ecology and population dynamics remain poorly known in the Italian ecological context.

During the last 40 years, many studies have investigated the distribution and expansion of the Italian wolf population (Zimen and Boitani, 1975; Fabbri et al., 2007), its abundance (Marucco et al., 2009; Caniglia et al., 2012; Galaverni et al., 2016), composition and home ranges of packs (Ciucci et al., 1997; Apollonio et al., 2004; Scandura et al., 2011; Caniglia et al., 2014), its genetic variability (Randi et al., 2000; Randi and Lucchini, 2002; Lucchini et al., 2004), the threat posed by hybridization with domestic dogs

* Corresponding author.
E-mail address: giovanni.poglayen@unibo.it (G. Poglayen).

http://dx.doi.org/10.1016/j.ijppaw.2017.01.001

(Caniglia et al., 2013; Randi et al., 2014) and the impact on wild and domestic ungulates (Gazzola et al., 2005). A number of studies have investigated Italian wolf parasites (Arru et al., 1988; Guberti et al., 1991, 1993, 1998, 2004, 2005Gori et al., 2015) because of the recognized role of wildlife parasites in shaping individual host fitness (Hudson, 2002) and their public health significance as zoonoses (Thompson, 2013). All these studies paid particular attention to *Echinococcus granulosus* sensu stricto (sheep strain genotype 1), an important emerging and re-emerging zoonotic agent, above all in the Mediterranean basin (Sadjjadi, 2006). *E. granulosus* is a small tapeworm approximately 3 mm in length, endemic in this region since the appearance of sheep farming and hence a close relationship has developed over the centuries between the domestic dog (definitive host) and small ruminants as the main intermediate hosts. The official data of sheep cystic echinococcosis (CE) in Italy is summarized in Deplazes et al. (in press), while the prevalence in adult sheep is at least around 40% (Poglayen et al., 2008a, b). The low prevalence of cystic echinococcosis (CE) in wild ruminants, the main wolf prey, has prevented the establishment of a purely wild animal cycle so far (Guberti et al., 2004). The low number of wolves (n = 1300–1800) (Galaverni et al., 2016), a high prevalence of infected sheep (40%), and many positive dogs, allow the wolf to be considered in a parallel epidemiological context, closely linked to the domestic cycle (Guberti et al., 2004).

The other species of tapeworm give rise to speculation in attempts to understand and know more about the wolf diet, as the larval stage of each cestode has a specific host range (i.e. *Taenia hydatigena*: wild and domestic ungulates; *T. krabbei*: only wild ungulates; *T. polyacantha*: micromammals).

The aim of this molecular study was to evaluate the presence of taeniid tapeworms in the wolves of the Foreste Casentinesi, Monte Falterona e Campigna National Park (FCNP), Northern Italy. This area provides opportunities to better understand the ongoing expansion of the Italian wolf population as some (Cagnolaro et al., 1974; Apollonio et al., 2004) claim that the wolf never disappeared from the FCNP, which acted as a natural ecological corridor along the Apennines guaranteeing the link between wolves from Central Italy and those of the Western Alps (Fabbri et al., 2007; Caniglia et al., 2014).

Most of the studies on wildlife intestinal parasites depend on standard methodologies based on post-mortem examination (Wobeser, 2007). As the wolf is a protected and elusive species these techniques are not a feasible option, so we used fecal analyses (Carbonell and Rodriguez, 1998) combining parasitological analysis with individual host genotyping based on fecal DNA (e.g. Zhang et al., 2011). This approach allowed us to identify each fecal sample's taxonomic affiliation (e.g. wolf, dog or hybrid), genetic profile, sex and, thanks to the pedigree reconstruction, the family group to which it belonged (Lucchini et al., 2002; Fabbri et al., 2007; Marucco et al., 2012; Caniglia et al., 2014).

2. Materials and methods

2.1. Study area

The study area includes the Foreste Casentinesi, Monte Falterona e Campigna National Park (FCNP) located in the Northern Italian Apennines (43°51′34.26″N; 11°44′38.39″E) and covers a surface of about 36,000ha, ranging from 400 to 1658 m a.s.l. (Fig. 1). Much of the area is woodland, characterized by some of the oldest European secular forests of silver fir (*Abies alba* Miller, 1759) and deciduous mixed woods of oak (*Quercus* spp.), beech (*Fagus sylvatica* L.), sycamore (*Acer pseudoplatanus* L.) and chestnut (*Castanea sativa* Miller). The area is densely populated by wild ungulates, including wild boar (*Sus scrofa* L., 1758), red deer (*Cervus elaphus* L., 1758), roe deer (*Capreolus capreolus* L., 1758), fallow deer (*Dama dama* L., 1758) and mouflon (*Ovis musimon* Pallas, 1762). The park lies between two regions, Emilia-Romagna and Tuscany. The protected area includes roads and 13 villages, with an average human density of 41.05 people/km^2. Few domestic ungulates (cattle, sheep and goats) are reared inside the park and hunting is strictly forbidden.

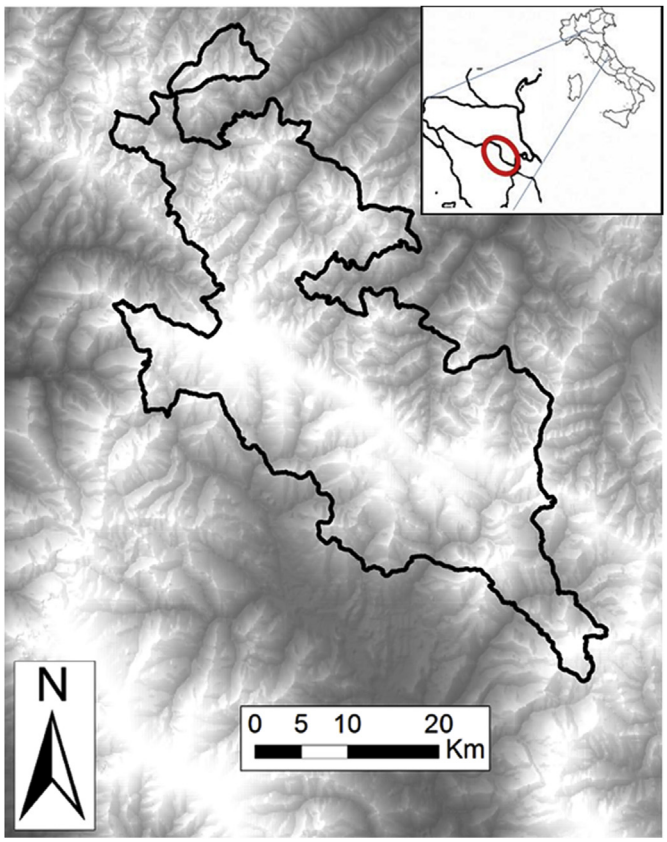

Fig. 1. The study area is located on the two sides of the Apennine watershed between Romagna and Tuscany, including the whole territory of the Foreste Casentinesi, Monte Falterona and Campigna National Park (FCNP).

2.2. Sample collection and individual genotyping analysis

From 2001 to 2008 the Environmental Section (CTA) of the Italian Forestry Corp (CFS) and Institute for Environmental Protection and Research (ISPRA) started an intensive genetic monitoring program based on the non-invasive collection of scat samples to investigate the presence, status and distribution of the wolf population in the FCNP. The project was carried out in the framework of a wider regional study, whose results are reported in Caniglia et al. (2014).

During the genetic monitoring project, 1433 non-invasive presumed wolf biological samples were collected in the FCNP and analyzed at the ISPRA Genetic Laboratory to identify the genetic profile of individual wolves. Feces were collected along trails or country roads chosen opportunistically to maximize the probability of finding fresh samples and covering the entire study area. Roads and trails were surveyed at least once per month and the geographic coordinates of every sample were recorded by GPS.

Small samples from the external portions of scats were individually stored in 10 vials of 95% ethanol. Before any manipulation,

as a safety precaution they were stored for 10 days at −80 °C (Eckert et al., 2001), then at −20 °C until DNA extraction.

DNA was extracted using the MultiPROBE IIex robotic liquid handling system (Conquer Scientific, San Diego, CA, USA) and the QIAGEN QIAmp DNA stool extraction kit (QIAGEN Inc., Hilden Germany). The individual genotype of each sample was identified using a multiple-tube approach (Taberlet et al., 1997) at 12 unlinked autosomal canine microsatellites (short tandem repeats, STRs) selected for their polymorphism and reliable scorability, and a restriction fragment length polymorphism to the ZFX gene to gender identification. Maternal haplotypes were identified by sequencing 350 base pairs of the mitochondrial DNA (mtDNA) control region and paternal haplotypes by typing 4 Y-linked microsatellites (Y-STR). DNA sequences and microsatellites were analyzed in a 3130XL ABI automated sequencer (Applied Biosystems, Foster City CA, USA), using the ABI software SEQSCAPE 2.5 for sequences and GENEMAPPER 4.0 for microsatellites (Applied Biosystems). GIMLET was used to reconstruct the consensus genotypes for each sample, compare them and control the good attribution of several samples to the same individual. The reliability of the reconstructed multilocus genetic profile was assessed using RELIOTYPE (Miller et al., 2002) and a threshold of 0.95. Only genotypes with a probability of reliability to ≥0.95 were retained. For details on PCR conditions and primer references, multi-tube protocol, reliability and match tests, see Caniglia et al. (2014).

2.3. Species identification from genetic profiles

We used STRUCTURE v.2.3 (Falush et al., 2003) to assign the individual genotypes as wolves, dogs, or wolf *x* dog hybrids. Reference wolf (n = 168) and dog (n = 160) genotypes were randomly selected from the ISPRA *Canis* database. We ran STRUCTURE with five replicates of 10^4 burn-in followed by 10^5 iterations of Markov chain Monte Carlo sampling, with the ADMIXTURE model and assuming independent allele frequencies. According to previous studies (Caniglia et al., 2014), the optimal number of populations was set at $K = 2$ (the value that maximized the posterior probability of the data). At $K = 2$, we assessed the average proportion of membership (Q_i) of the sampled populations to the inferred clusters. Then we assigned genotypes to the Italian wolf or dog clusters at threshold $q_i = 0.95$ (individual proportion of membership; Randi, 2008), or identified them as admixed if their q_i values were intermediate.

2.4. Pack identification and pedigree

We determined the spatial distributions by 95% kernel analysis using the ADEHABITATHR package for R (Calenge, 2006) for all the genotypes sampled in restricted ranges (<100 km^2) at least four times and for periods longer than 24 months, and mapped them in ARCGIS 10.0. We performed parentage analyses considering candidate parents all the individuals sampled in the first year of sampling and more than four times in the same area, and candidate offspring all the individuals collected within the 95% kernel spatial distribution of each pack and in a surrounding buffer area of approximately 17-km radius from the kernel center (for details, see Caniglia et al., 2014).

2.5. Taeniidae identification

From the ISPRA genetic bank 130 specimens belonging to 54 wolves, chosen according to the genetic profile (not dog or wolf x dog hybrid) and to abundance of material, were examined for taeniidae eggs.

Up to 2 g of feces were sieved in a filter (mesh 150 μm) and washed several times in a cup. The filtrate was centrifuged (1600 × g) for ten minutes and the pellet collected. Taeniid eggs were isolated from the pellet using the flotation and sieving method described by Mathis et al. (1996) and subjected to morphological identification under an inverted microscope. In egg positive samples and in 14 of the negative samples, DNA extraction was carried out with the complete sieving fraction as described by Štefanić et al. (2004). The 14 negative samples were included as negative controls. A total of 69 samples were analyzed using a multiplex-PCR to discriminate between *E. granulosus* and *E. multilocularis* and other cestodes including *Taenia* spp. (Trachsel et al., 2007). To obtain clear sequences of the *E. granulosus* positive samples, the PCR was repeated but only using *E. granulosus* primers (Cest5 and Cest4) keeping the same conditions as described above, and therefore amplicons were sequenced. Another PCR targeting *nad1* gene (Armua-Fernandez et al., 2011) was used in samples without clear sequencing results to confirm the species of those samples. The amplicons were directly sequenced after purification of the PCR products using the MinElute® PCR purification kit (Qiagen). Sequencing was performed by Synergene Biotech GmbH, Biotech Center Zurich, Switzerland (http://www.synergene-biotech.com). Primer Cest5 and Cest5seq was used for non-*Echinococcus* cestodes including *Taenia* spp. and Cest5 for *E. granulosus*, while primer *nad1*T-Rv for *Taenia* spp. for amplicons obtained with multiplex-PCR and *nad1* PCR, respectively. Sequences were compared with the one present in GenBank using Blast tool (http://www.blast.ncbi.nlm.nih.gov).

3. Results

3.1. Genetic individual identification and pack reconstruction

From the 1433 analyzed samples, 544 were successfully genotyped (38%), belonging to 137 individuals: 117 wolves and 20 dogs (no wolf *x* dog hybrids were identified). The kinship and spatial analyses identified eight packs within the FCNP park (Fig. 2a), for which complete genealogies were reconstructed and are available in Caniglia et al. (2014).

3.2. Parasite identification

We examined 130 fecal samples from 54 different wolf individuals, of which 35 individuals (and 90 corresponding samples) belonged to the eight packs (Fig. 2) and 19 were not assigned to any known pack (40 samples). Fecal samples were examined only for Taeniidae eggs, and showed a 42.1% frequency (55/130) and a prevalence of 61.1% (33/54) (Tables 1 and 2, respectively). Among the five parasite species isolated, *T. hydatigena* was the most common in terms of frequency in all samples (23.8%) and prevalence in the population examined (40.7%), followed by *T. krabbei* with a frequency of 10.7% and a prevalence of 22.2%. One sample (0.7%) corresponding to one animal (1.8%) was positive for *T. polyacantha*. *E. granulosus* "*sensu stricto*" (G1-G3) was found in three samples (2.3%) belonging to three different wolves (5.5%).

The nucleotide sequence was not obtained from six positive samples, so the taeniids could only be identified as other cestodes including *Taenia* spp., while no sample was positive for *E. multilocularis*. Of the 33 positive wolves, 22 were sampled only once (66.6%), five twice (15.1%) and of these only one was positive for the same parasite (*T. krabbei*) in the second sampling. Four wolves were sampled three times (12.1%) and three of these maintained positivity for *T. hydatigena*. One wolf (3%) was sampled four times and another five times. Two samples were simultaneously positive for *T. hydatigena* and *E. granulosus* (Fig. 2b).

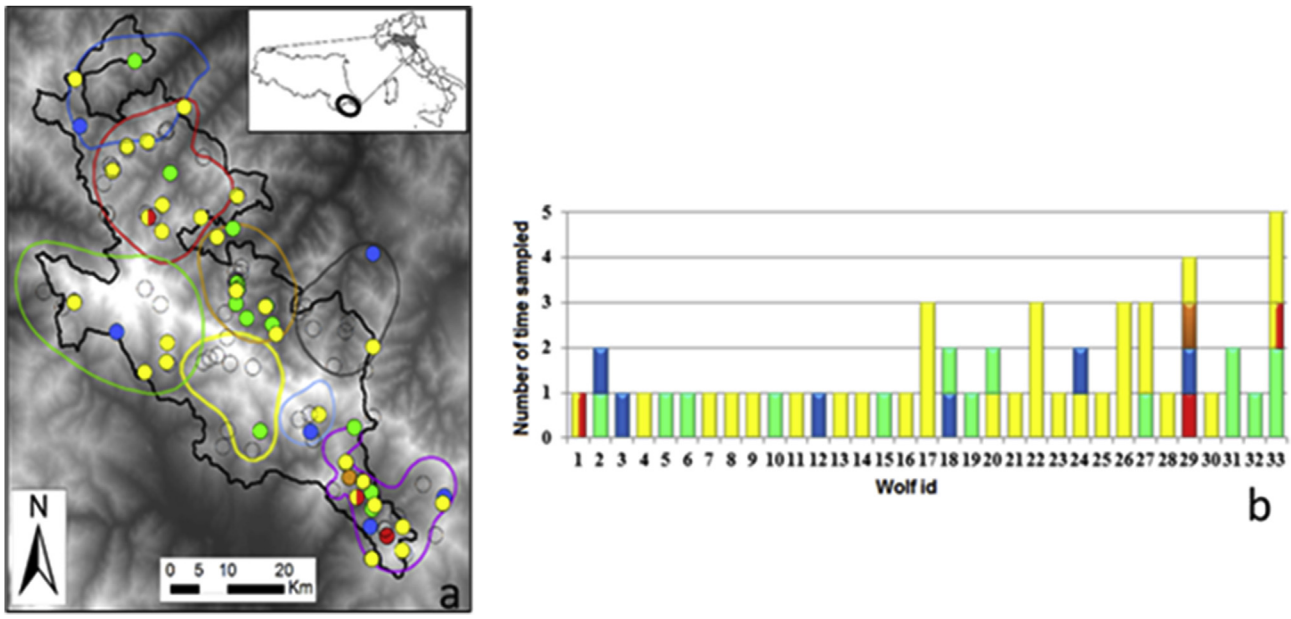

Fig. 2. a) Boundaries of eight wolf packs (see Caniglia et al., 2014) in blue, red, green, orange, gray, yellow, light blue and purple lines. Occurrence of species of parasites in wolf scats in red (*Echinococcus granulosus*), yellow (*Taenia hydatigena*), green (*T. krabbei*), orange (*T. polyacantha*) and blue (non-*Echinococcus* cestodes including *Taenia* spp. no sequence). Yellow-red dots indicate the occurrence of both *E. granulosus* and *T. hydatigena*. Empty dots indicate the absence of parasites. b) Frequency of sampling in positive animals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Frequency of taeniid species findings in fecal samples from Foreste Casentinesi National Park (Italy).

Total samples n = 130	Taeniid species	Number of positive samples (Frequency %)	Confidence interval (95%)
	Taenia hydatigena (Pallas, 1766)	31 (23.8)	16.5–31.1
	Taenia krabbei (Moniez, 1879)	14 (10.7)	5.4–16
	Taenia polyacantha (Leuckart, 1856)	1 (0.7)	0.0–2.1
	Echinococcus granulosus (G1-G3)	3 (2.3)	0.0–4.8
	non-*Echinococcus* cestodes including Taenia spp.	6 (4.6)	1–8.2
Total	4 Taeniid species	55 (42.1)	33.7–50.5

Table 2
Prevalence of different taeniid species found in the sampled population.

Total wolves n = 54	Taeniid species	Number of positive animals (Prevalence %)	Confidence interval (95%)
	Taenia hydatigena	22 (40.7)	27.6–53.8
	Taenia krabbei	12 (22.2)	11.2–33.2
	Taenia polyacantha	1 (1.8)	0.0–5.3
	Echinococcus granulosus (G1-G3)	3 (5.5)	0.0–11.5
	non-*Echinococcus* cestodes including *Taenia* spp.	6 (11.1)	2.8–19.4
Total		33 (61.1)	48.1–74.1

4. Discussion

Our 130 fecal wolf samples showed a taeniid prevalence close to 60%, the most common being *T. hydatigena* with a prevalence of 40.7%. None of the eight family packs presented the same composition of taeniid fauna, and only one had all four isolated species. As expected, since no sample was positive for *E. multilocularis*, the prevalence of the other zoonotic cestode, *E. granulosus* (G1-G3), is not surprising because of its wide diffusion in Italy. In fact, when slaughterhouse data were matched with the national ovine registry to identify the geographical origin of animals all over the country, CE prevalence was at least 40% in adult sheep (Poglayen et al., 2008a,b). The lower detection rate compared with Gori et al.'s findings (2015) should be ascribed to the particular environment of our wolves, a national park with a high wild prey density and virtually echinococcosis-free. No wild cycle of *E. granulosus* has been described in Italy and the *E. granulosus* prevalence in carnivores in this park is probably linked to predation on domestic animals. The information on wolf attacks stems from a Regional program to refund damaged breeders and thereby contribute to wolf conservation. These attacks appear very close to the park boundaries with three cases even inside a wolf pack (Fig. 3). The presence of *E. granulosus* in wolves far away from the reported attacks could be attributed to the wolves' mobility also to reduce energetic hunting efforts. According to Guberti et al. (2005), the low prevalence of *E. granulosus* is further confirmation of the absence of a wild cycle of this parasite. A deterministic model was adopted to simulate a purely theoretical sylvatic cycleand demonstrate that even if both the wolf and the wild ungulate population are increasing, the wolf is still part of the parasite's main dog/sheep

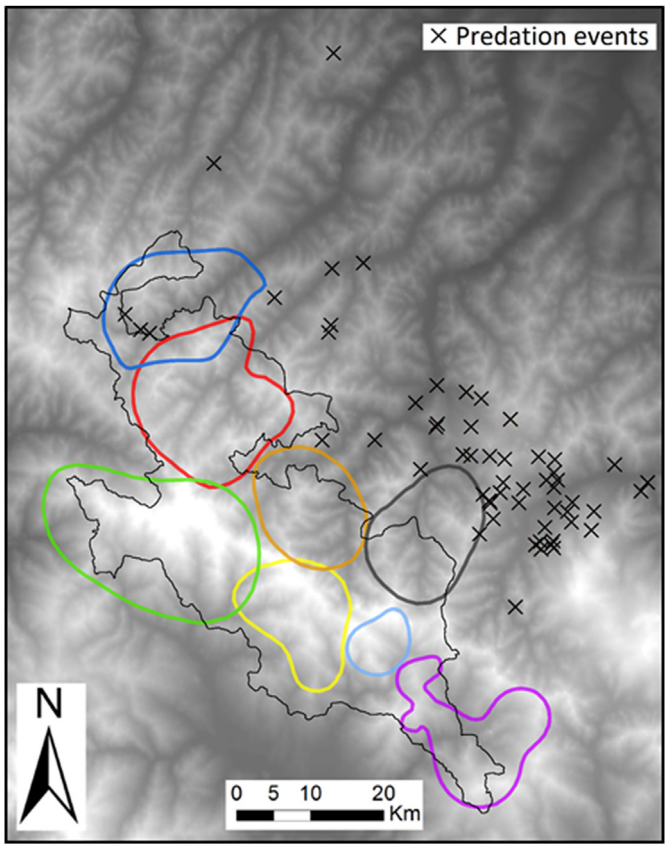

Fig. 3. Distribution of attacks on livestock in the Emilia-Romagna region, near and inside the FCNP wolf packs. (Data from Emilia-Romagna attacks control program).

cycle (Guberti et al., 2004). To confirm this stochastic model, an active surveillance program on wild fauna hunted or found dead in our region has been implemented by public research laboratories (Istituto Zooprofilattico Sperimentale) with the main aim to protect livestock. No CE has ever been detected in the wild ruminants of the area (Tosi pers comm).

The presence of taeniids is always related to the host diet. Although the wolf is a carnivore, its diet is varied and well suited to the different trophic niches offered in southern Europe where this canid has apparently adapted to feed on fruit, rubbish and livestock as well as small and medium-sized mammals (Meriggi and Lovari, 1996). The lack of specific surveys on parasite fauna in different potential prey means we can only speculate on the presence of metacestodes.

T. hydatigena was the most common species detected in stools and wolves in our survey. This parasite is also common in wolves in Europe (Craig and Craig, 2005; Moks et al., 2006; Bagrade et al., 2009; Ćirović et al., 2015) whose intermediate hosts belong to wild and domestic ungulates. The second commonest parasite species was *T. krabbei* whose lower presence should be linked to a strictly wild cycle as the main intermediate host and wolf prey in the FCNP is roe deer. Before the advent of molecular biology tools, *T. ovis* and *T. krabbei* were difficult to distinguish morphologically, Lavikainen et al. (2008) suggested possible identification mistakes in old studies which may have included the isolation of *T. ovis* in Italy (Guberti et al., 1993). *T. krabbei* is also common in Europe (Craig and Craig, 2005; Bagrade et al., 2009). *T. polyacantha* reflects the presence of intermediate hosts (micromammals), and its low prevalence (1.8%) suggests wolves make scarce use of these kinds of prey. According to Scaravelli (2001), 19 micromammal species are present in the FCNP.

Our study differs from the other six national studies on taeniids. One adopted an epidemiological approach (Gori et al., 2015), while the other five were both parasitological and epidemiological (Guberti et al., 1991, 1993, 1998, 2004, 2005) and referred to 119 dead wolves collected throughout the Apennines range of species distribution (Table 3). Therefore the results were obtained by total worm count followed by morphological identification of parasites. The only possible comparison between these and our data is in terms of taeniid presence/frequency. More recently, Gori et al. (2015) reported the molecular results for cestodes from 179 fecal samples attributed to wolf, evaluating size, shape, smell and composition according to Bassi et al. (2012) in the Liguria region. In this case, the same parasitological approach was adopted, but lacking the species identification from the genetic profile the stool recognition is less accurate: in fact, stray dogs in the Emilia-Romagna region are confined in kennels, whereas in Liguria many stray dogs are still free-ranging. This would create some bias on the actual species sampled. Furthermore, the Liguria study area is larger and included the whole region (540,000ha) where scattered protected and hunting areas are mixed, whereas our area includes only a small National Park (36,000ha). The comparison with last survey can be done only in term of taeniids frequency (Table 3). In our surveys, each scat is molecularly referred to a single wolf of a single pack (Fig. 2a).

In summary, the main differences between previous national experiences are by parasitological and epidemiological approach with Guberti et al, (1991, 1993, 1998, 2004 and 2005), while only epidemiological with Gori et al., (2015). All the differences are difficult to explain because of the different sampling (a whole region vs a small National Park) and the different stools identification.

The taeniids found are common parasites of Italian wolves with different prevalence rates (Guberti et al., 1991, 1993, 1998, 2004, 2005; Gori et al., 2015). The only data from the same area on carnivore taeniids, isolated by necropsy, referred to foxes in which taeniid species were the same (Poglayen et al., 1985, 1988; Fiocchi et al., 2016).

From a public health perspective, it is important to emphasize the absence of *E. multilocularis* in the Apennines. In recent years, this taeniid has become an important parasite in Northern Europe, also in an urban context. The only stable small focus is present in foxes of North-Eastern Italy with no human cases (Casulli et al., 2005; Dellamaria et al., 2014).

Among the taeniids detected, our study focused on *E. granulosus*. In Italy, CE is widely prevalent in livestock (Garippa, 2006; Deplazes et al., in press), making wolf infection a negligible aspect in the public health context. Efforts to combat CE target the domestic cycle using well-known tools of proven efficacy. National abattoirs ensure the destruction of positive offal so that no infectious material may enter the meat production cycle. The problem arises with frequent illegal home slaughter which favours the spread of the parasite among shepherds and farm dogs and contributes to

Table 3
Difference in taeniid frequency between our results and those of Gori et al. (2015).

Taeniids	Gori et al. (2015)	Present study
Taenia hydatigena	19.6	23.8
Taenia krabbei	4.5	10.7
Taenia ovis	2.2	Not found
Taenia crassiceps	0.6	Not found
Hydatigena taeniaeformis	0.6	Not found
Echinococcus granulosus s.l.	5.6	–
Echinococcus granulosus s.s.	–	2.3
Taenia polyachanta	Not found	0.7

maintain high infection rates in these dogs, ruminants and humans. The lack of a national CE control program is solely responsible for the Italian situation. Some local efforts are of no use in a general context. As *Canis lupus italicus* is a species subject to conservation, the involvement of wolves in Italy in *E. granulosus* transmission in the absence of a wild animal parasite cycle can be considered a downstream phenomenon of the domestic cycle.

Since Guberti et al.'s first paper (1991), the wolf population has increased to approximately 1800 heads and expanded to reach the North-Eastern Alps, but the taeniid fauna has remained the same. Therefore, these parasite species do not pose a risk for wolf conservation.

The combined non-invasive method adopted in this study confirms its importance in the study of ecology, behavior and parasitology without interfering with the sensitive population dynamics of Italy's most important carnivore. In addition, our global approach involved collaboration with theriologists and genetists expert in the Italian wolf population sharing our parasitological expertise. Nature is a complex mosaic and in-depth study of one tile alone will not shed light on the whole picture.

Acknowledgment

The authors are grateful to Anne Prudence Collins for the English revision and to Dr Maria Luisa Zanni for the predation on livestock regional data.

References

Apollonio, M., Mattioli, L., Scandura, M., Mauri, L., Gazzola, A., Avanzinelli, E., 2004. Wolves in the Casentinesi Forests: insights for wolf conservation in Italy from a protected area with a rich wild prey community. Biol. Conserv. 120, 249–260.

Armua-Fernandez, M.T., Nonaka, N., Sakurai, T., Nakamura, S., Gottstein, B., Deplazes, P., Phiri, I.G.K., Katakura, K., Oku, Y., 2011. Development of PCR/dot blot assay for specific detection and differentiation of taeniid cestode eggs in canids. Parasitol. Int. 60, 84–89.

Arru, E., Garippa, G., Fico, R., 1988. Sulla presenza di *Echinococcus granulosus* nella volpe (*Vulpes vulpes*) e nell'lupo (*Canis lupus italicus*). Soc. Ital. Sci. Veterinarie 42, 927–929.

Bassi, E., Donaggio, E., Marcon, A., Scandura, M., Apollonio, M., 2012. Trophic niche overlap and wild ungulate consumption by red fox and wolf in a mountain area in Italy. Mamm. Biol. 77, 369–376.

Bagrade, G., Kirjusina, M., Vismanis, K., Ozoliņs, J., 2009. Helminth parasites of the wolf *Canis lupus* from Latvia. J. Helminthol. 83, 63–68.

Boitani, L., 1984. 1984. Genetic considerations on wolf conservation in Italy. Bollettino Zool 51, 37–41.

Boitani, L., 1992. Wolf research and conservation in Italy. Biol. Conserv. 61, 125–132.

Boitani, L., 2000. Action Plan for the Conservation of Wolves in Europe (*Canis lupus*). Council of Europe Press, Strasbourg, France, pp. 18–113.

Breitenmoser, U., 1998. Large predators in the Alps: the fall and the rise of man's competitors. Biol. Conserv. 83, 279–289.

Cagnolaro, L., Rosso, D., Spagnesi, M., Venturi, B., 1974. Investigation on the wolf (*Canis lupus*) distribution in Italy, in canton ticino and canton grigioni (Switzerland). Ric. Biol. Selvag. 59, 1–75.

Calenge, C., 2006. The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. Ecol. Model 197, 516–519.

Caniglia, R., Fabbri, E., Cubaynes, S., Gimenez, O., Lebreton, J.D., Randi, E., 2012. An improved procedure to estimate wolf abundance using non-invasive genetic sampling and capture-recapture mixture models. Conserv. Genet. 13, 53–64.

Caniglia, R., Fabbri, E., Greco, C., Galaverni, M., Manghi, L., Boitani, L., Sforzi, A., Randi, E., 2013. Black coats in an admixed wolf × dog pack: is melanism an indicator of hybridization in wolves? Eur. J. Wildl. Res. 59, 543–555.

Caniglia, R., Fabbri, E., Galaverni, M., Milanesi, P., Randi, E., 2014. Noninvasive sampling and genetic variability, pack structure, and dynamics in an expanding wolf population. J. Mammal. 95, 41–59.

Carbonell, E., Rodriguez, A., 1998. Gastrointestinal parasites of the Iberian lynx and other wild carnivores from central Spain. Acta Parasitol. 43, 128–136.

Casulli, A., Manfredi, M.T., La Rosa, G., Di Cerbo, A.R., Dinkel, A., Romig, T., Deplazes, P., Genchi, C., Pozio, E., 2005. *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) of the Italian Alpine region: is there a focus of autochthonous transmission? Int. J. Parasitol. 35, 1079–1083.

Chapron, G., Kaczensky, P., Linnell, J.D.C., et al., 2014. Recovery of large carnivores in Europe's modern human-dominated landscapes. Science 346, 1517–1519.

Ćirović, D., Pavlović, I., Penezić, A., 2015. Intestinal helminth parasites of the grey wolf (*Canis lupus* L.) in Serbia. Acta Vet. Hung 63, 189–198.

Ciucci, P., Boitani, L., Francisci, F., Andreoli, G., 1997. Home range, activity and movements of a wolf pack in central Italy. J. Zool. 243, 803–819.

Craig, H.L., Craig, P.S., 2005. Helminth parasites of wolves (*Canis lupus*): a species list and an analysis of published prevalence studies in Nearctic and Palaearctic populations. J. Helminthol. 79, 95–103.

Dellamaria, D., Trevisiol, K., Francione, E., Citterio, C., Simonato, G., Cazzin, S., Casulli, A., Deplazes, P., Marangon, S., Capelli, G., 2014. A Focus of *Echinococcus Multilocularis* in Foxes of North-eastern Italy: after Ten Years Is Still There. XXVIII Congresso Nazionale SoIPa, p. 223.

Deplazes, P., Rinaldi, L., Alvarez-Rojas, C.A., Torgerson, P., Harandi, M.F., Romig, T., Antolova, D., Schurer, J., Lahmar, S., Cringoli, G., Magamb, J., Thompson, A., Jenkings, E., Global distribution of alveolar and cystic echinococcosis. Advances in Parasitology: In Press.

Eckert, J., Gottstein, B., Heath, D., Liu, F.J., 2001. Prevention of Echinococcosis in humans and safety precautions. In: Eckert, J., Gemmell, M.A., Meslin, F.X., Pawlowski, Z.S. (Eds.), Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. OIE and WHO, Paris, France, pp. 238–248.

Fabbri, E., Miquel, C., Lucchini, V., Santini, A., Caniglia, R., Duchamp, C., Weber, J.M., Lequette, B., Marucco, F., Boitani, L., Fumagalli, L., Taberlet, P., Randi, E., 2007. From the Apennines to the Alps: colonization genetics of the naturally expanding Italian wolf (*Canis lupus*) population. Mol. Ecol. 16, 1661–1671.

Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure: extensions to linked loci and correlated allele frequencies. Genetics 164, 1567–1587.

Fiocchi, A., Gustinelli, A., Gelmini, L., Rugna, G., Renzi, M., Fontana, M.C., Poglayen, G., 2016. Helminth parasites of the red fox *Vulpes vulpes* (L., 1758) and the wolf *Canis lupus italicus* Altobello, 1921 in Emilia-Romagna, Italy. Ital. J. Zool. 83, 1–17. http://dx.doi.org/10.1080/11250003.2016.1249966.

Galaverni, M., Caniglia, R., Fabbri, E., Milanesi, P., Randi, E., 2016. One, no one, or one hundred thousand: how many wolves are there currently in Italy? Mamm. Res. 61, 13–24.

Garippa, G., 2006. Updates on cystic echinococcosis (CE) in Italy. Parassitologia 48, 57–59.

Gazzola, A., Bertelli, I., Avanzinelli, E., Tolosano, A., Bertotto, P., Apollonio, M., 2005. Predation by wolves (*Canis lupus*) on wild and domestic ungulates of the Western Alps. Italy. J. Zool. 266, 205–213.

Glikman, J.A., Vaske, J.J., Bath, A.J., Ciucci, P., Boitani, L., 2012. Residents' support for wolf and bear conservation: the moderating influence of knowledge. Eur. J. Wildl. Res. 58, 295–302.

Gori, F., Armua-Fernandez, M.T., Milanesi, P., Serafini, M., Magi, M., Deplazes, P., Macchioni, F., 2015. The occurence of taeniids of wolves in Liguria (northern Italy). Int. J. Parasitol. Parasites. Wildl. 4, 252–255.

Guberti, V., Francisci, F., Andreatta, U., Andreoli, A., 1991. *Echinococcus Granulosus* in Wolf in Italy. XV Extraordinary Congress for the Celebration of the 50 Years of A.I.H. Rome. November 4–8, pp. 905–909.

Guberti, V., Stancampiano, L., Francisci, F., 1993. Intestinal helminth parasite community in wolves (*Canis lupus*) in Italy. Parassitologia 35, 59–65.

Guberti, V., Zaffaroni, E., Morabito, P., Lanfranchi, P., 1998. Epidemiologia di *Echinococcus granulosus* nel lupo in Italia. Parassitologia 40, 80.

Guberti, V., Bolognini, M., Lanfranchi, P., Battelli, G., 2004. *Echinococcus granulosus* in the wolf in Italy. Parassitologia 46, 425–427.

Guberti, V., Fenati, M., Bolognini, M., Lanfranchi, P., Battelli, G., 2005. Utilizzo di un modello matematico per lo studio dell'epidemiologia dell'echinococcosi nel lupo. ISTISAN Congressi-Istituto Superiore di Sanità, Rome, pp. 9–10. June, p.56.

Hudson, P.J., 2002. The Ecology of Wildlife Diseases. Oxford University Press, New York.

Lavikainen, A., Haukisalmi, V., Lehtinen, M.J., Henttonen, H., Oksanen, A., Meri, S., 2008. A phylogeny of members of the family Taeniide based on the mitochondrial *cox1* and *nad1* gene data. Parassitol 135, 1457–1467.

Linnell, J.D.C., Boitani, L., 2011. Building biological realism into wolf management policy: the development of the population approach in Europe. Hystrix 23, 80–91.

Lucchini, V., Fabbri, E., Marucco, F., Ricci, S., Boitani, L., Randi, E., 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. Mol. Ecol. 11, 857–868.

Lucchini, V., Galov, A., Randi, E., 2004. Evidence of genetic distinction and long-term population decline in wolves (*Canis lupus*) in the Italian Apennines. Mol. Ecol. 13, 523–536.

Marucco, F., Pletscher, D.H., Boitani, L., Schwartz, M.K., Pilgrim, K.L., Lebreton, J.D., 2009. Wolf survival and population trend using non-invasive capture-recapture techniques in the Western Alps. J. Appl. Ecol 46, 1003–1010.

Marucco, F., McIntire, E.J.B., 2010. Predicting spatio-temporal recolonization of large carnivore populations and livestock depredation risk: wolves in the Italian Alps. J. Appl. Ecol. 47, 789–798.

Marucco, F., Avanzinelli, E., Boitani, L., 2012. Non-invasive integrated sampling design to monitor the wolf population in piemonte, italian. Alps. Hystrix 23, 5–13.

Mathis, A., Deplazes, P., Eckert, J., 1996. An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. J. Helmintol. 70, 219–222.

Meriggi, A., Lovari, S., 1996. A review of wolf predation in southern Europe: does the wolf prefer wild prey to livestock? J. Appl. Ecol. 33, 1561–1571.

Milanesi, P., Caniglia, R., Fabbri, E., Galaverni, M., Meriggi, A., Randi, E., 2015. Non-invasive genetic sampling to predict wolf distribution and habitat suitability in the Northern Italian Apennines: implications for livestock depredation risk. Eur. J. Wildl. Res. 61, 681–689.

Miller, C., Joyce, P., Waits, L.P., 2002. Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160, 357–366.
Moks, E., Jõgisalu, I., Saarma, U., Talvik, H., Järvis, T., Valdmann, H., 2006. Helminthologic survey of the wolf (*Canis lupus*) in Estonia, with an emphasis on *Echinococcus granulosus*. J. Wildl. Dis. 42, 359–365.
Poglayen, G., Guberti, V., Leoni, B., 1985. Parassiti presenti in volpi (*Vulpes vulpes*) della provincia di Forlì. Parassitologia 27, 303–311.
Poglayen, G., Roda, R., Ravaioli, C., Leoni, B., Guberti, V., 1988. Aggiornamenti sulla diffusione dei parassiti di *Vulpes vulpes* in provincia di Forlì: implicazioni ecologiche e gestionali. Supplemento alle ricerche di biologia della selvaggina, vol. 14, pp. 441–446.
Poglayen, G., Stancampiano, L., Garippa, G., Varcasia, A., Pipia, A.P., Bio, C., Romanelli, C., 2008a. La diagnosi al mattatoio, un osservatorio epidemiologico privilegiato per l'echinococcosi cistica. X Congresso Nazionale SIDiLV. Alghero 22–24. Ottobre, pp.270–271.
Poglayen, G., Stancampiano, L., Varcasia, A., Pipia, A.P., Bio, C., Romanelli, C., 2008b. Updating on cystic echinococcosis in northern Italy. XthEuropean Multicolloquium Parasitol. 24–28. Paris, August.
Randi, E., Lucchini, V., Christensen, M.F., Mucci, N., Funk, S.M., Dolf, G., Loeschcke, V., 2000. Mitochondrial DNA variability in Italian and east European wolves: detecting the consequence of small population size and hybridization. Conserv. Biol. 14, 464–473.
Randi, E., Lucchini, V., 2002. Detecting rare introgression of domestic dog genes into wild wolf (*Canis lupus*) populations by Bayesian admixture analyses of microsatellite variation. Conserv. Genet. 3, 31–45.
Randi, E., 2008. Detecting hybridization between wild species and their domesticated relatives. Mol. Ecol. 17, 285–293.
Randi, E., 2011. Genetics and conservation of wolves *Canis lupus* in Europe. Mamm. Rev. 41, 99–111.
Randi, E., Hulva, P., Fabbri, E., Galaverni, M., Galov, A., Kusak, J., Bigi, D., Bolfikova, B.C., Smetanova, M., Caniglia, R., 2014. Multilocus detection of wolf x dog hybridization in Italy, and guidelines for marker selection. PLoS One 9, e86409.
Sadjjadi, S.M., 2006. Present situation of echinococcosis in the Middle East and arabic North africa. Parasitol. Int. 55, 197–202.
Scandura, M., Iacolina, L., Capitani, C., Gazzola, A., Mattioli, L., Apollonio, M., 2011. Fine-scale genetic structure suggests low levels of short-range gene flow in a wolf population of the Italian Apennines. Eur. J. Wildl. Res. 57, 949–958.
Scaravelli, D., 2001. Chirotteri, micromammiferi, mesomammiferi, pesci. In "I vertebrati del Parco Nazionale delle Foreste Casentinesi-Stato delle conoscenze e indicazioni per la conservazione e la gestione." ST.E.R.N.A, D.R.E.AM. Italia per il Parco Nazionale.
Štefanić, S., Shaikenov, B.S., Deplazes, P., Dinkel, A., Torgerson, P.R., Mathis, A., 2004. Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* ("sheep strain") in naturally infected dogs. Parasitol. Res. 92, 347–351.
Taberlet, P., Camarra, J., Griffin, S., Uhrès, E., Hanotte, O., Waits, L.P., Dubois-Paganon, C., Burke, T., Bouvet, J., 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Mol. Ecol. 6, 869–876.
Thompson, R.C.A., 2013. Parasite zoonoses and wildlife: one health, spillover and human activity. Int. J. Parasitol. 43, 1079–1088.
Trachsel, D., Deplazes, P., Mathis, A., 2007. Identification of taeniid eggs in the feces from carnivores based on multiplex-PCR using targets in mitochondrial DNA. Parasitol 134, 911–920.
Valière, N., Fumagalli, L., Gielly, L., Miquel, C., Lequette, B., Poulle, M.L., Weber, J.M., Arlettaz, R., Taberlet, P., 2003. Long distance wolf recolonization of France Switzerland inferred from non-invasive genetic sampling over a period of 10 years. Anim. Conserv. 6, 83–92.
Wobeser, G.A., 2007. Disease in Wild Animal: Investigation and Management, second ed. Springer, Berlin.
Zhang, L., Yang, X., Wu, H., Gu, X., Hu, Y., Wei, F., 2011. The parasites of giant pandas: individual-based measurement in wild animals. J. Wildl. Dis. 47, 164–171.
Zimen, E., Boitani, L., 1975. Number and distribution of wolves in Italy. Z fur Saugetierkd. 40, 102–112.