IJP: Parasites and Wildlife 11 (2020) 108–113

Contents lists available at ScienceDirect

IJP: Parasites and Wildlife

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

Invited article

First report of *Giardia duodenalis* infection in the crested porcupine (*Hystrix cristata* L., 1758)

Francesca Coppola[a,1], Michela Maestrini[a,1], Federica Berrilli[b], Isabel Guadano Procesi[b], Antonio Felicioli[a], Stefania Perrucci[a,*]

[a] Department of Veterinary Sciences, University of Pisa, Viale delle Piagge, 2, Pisa, 56124, PI, Italy
[b] Department of Clinical Science and Translational Medicine, University of Rome "Tor Vergata", Via Montpellier 1, 00133, Rome, Italy

ARTICLE INFO

Keywords:
Hystrix cristata
Nematodes
Protozoa
Giardia duodenalis
Potential zoonotic assemblages

ABSTRACT

Italy is the only European country where the crested porcupine (*Hystrix cristata*) lives. A parasitological investigation was performed on faecal samples, aimed to evaluate *Giardia* and other parasites in a free-ranging crested porcupine population in Central Italy. Samples were collected from captured and road-killed individuals as well as from feeding areas and pathways. Collected faecal samples were examined by the Mini-FLOTAC technique and a rapid immunoassay for the search of *Giardia* and *Cryptosporidium* spp. faecal antigens. For the identification of *Giardia* species and genotypes, molecular analysis was performed on *Giardia*-positive samples, by using PCR protocols able to amplify glutamate dehydrogenase, triosephosphate isomerase and a fragment of the small subunit ribosomal RNA genes.

A total of 52 crested porcupine faecal samples were collected and analysed. At microscopical examination, 39 out of 52 samples were found positive for at least a single parasite species and six different parasite taxa were identified. Forty-eight percent (25/52) of faecal samples were positive for *Giardia* spp. and 1.9% (1/52) for *Cryptosporidium* spp. at the immunoassay. Among 12 faecal samples belonging to different individuals, 33.3% (4/12) were positive for *Giardia* spp. By using the Mini-FLOTAC technique, positivity for *Trichuris* spp. (32.7%, 17/52), gastrointestinal strongyles (32.7%, 17/52), capillariid eggs (3.8%, 2/52) and coccidian oocysts (1.9%; 1/52) was also evidenced. Molecular analysis was performed on 17 out of 25 *Giardia*-positive isolates. At the *SSU rDNA* locus, expected bands were achieved for 12 out of 17 isolates and all samples were assigned to *Giardia duodenalis* assemblage B. Sequencing at *tpi* locus revealed potentially zoonotic *G. duodenalis* assemblage AII (two isolates) and assemblage BIV (one isolate). The present study provides the first report of *G. duodenalis* infection in *H. cristata*. More in depth studies are needed on the impact and epidemiology of *G. duodenalis* and other identified parasites in crested porcupines.

1. Introduction

Flagellated protozoa of the genus *Giardia* are gastrointestinal parasites of domestic and wild animals (Ryan and Cacciò, 2013; Cacciò et al., 2018). Among species of this genus, *Giardia duodenalis* (syn. *Giardia lamblia*, *Giardia intestinalis*) infects the small intestinal tract of numerous mammals worldwide, including humans (Ryan and Cacciò, 2013; Cacciò et al., 2018). *G. duodenalis* is considered a complex of genetically different but morphologically identical organisms with varying zoonotic potential and host preferences, known as assemblages (identified from A to H), and some sub-assemblages (Feng and Xiao, 2011; Ryan and Cacciò, 2013; Cacciò et al., 2018; Robertson et al., 2019). In particular, assemblages A (sub-assemblages I and II) and B (sub-assemblages III and IV) are usually identified among humans, but are also found in a number of other mammalian hosts, being considered potentially zoonotic (Marangi et al., 2010; Ryan and Cacciò, 2013; Cacciò et al., 2018). Some authors consider the assemblages as representing distinct species (Feng and Xiao, 2011; Thompson and Ash, 2016).

The crested porcupine (*Hystrix cristata*) is a large semi-fossorial, mainly nocturnal and herbivorous rodent mammal species that is widespread in both wild woody areas as well as highly anthropic woody

* Corresponding author. Department of Veterinary Sciences, University of Pisa, 56124, Pisa, Italy.
E-mail addresses: coppolafrancesca90@gmail.com (F. Coppola), michela.maestrini@phd.unipi.it (M. Maestrini), berrilli@uniroma2.it (F. Berrilli), isabel.guadano@gmail.com (I.G. Procesi), antonio.felicioli@unipi.it (A. Felicioli), stefania.perrucci@unipi.it (S. Perrucci).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.ijppaw.2020.01.006
Received 6 December 2019; Received in revised form 13 January 2020; Accepted 13 January 2020

agro-ecosystems (Bruno and Riccardi, 1995; Corsini et al., 1995; Coppola et al., 2019). Italy is the only European country in which the crested porcupine lives, coexisting and sharing the settlements with two other borrowing mammals, the red fox (*Vulpes vulpes*) and the badger (*Meles meles*) (Pigozzi, 1986; Vecchio et al., 2018; Coppola et al., unpublished study). Although the crested porcupine is protected by European (Bern Convention, 1979) and Italian law (National Law n. 503/1981), this species is still poor investigated. Only a single study deal with ectoparasites of *H. cristata* (Mori et al., 2015) living in Italy, while no data are available on endoparasites. Among the genus *Hystrix*, *Giardia* spp. infection has been recently detected in the Indian crested porcupine (*Hystrix indica*) (Chakraborty et al., 2015), but species and genotype identification was not performed. Among rodents, *Giardia muris*, *Giardia microti*, *Giardia cricetidarum*, but also the rodent-specific *G. duodenalis* assemblage G and the potential zoonotic *G. duodenalis* assemblages A and B, have been identified (Ryan and Zahedi, 2019; Cacciò et al., 2018; Helmy et al., 2018; Lyu et al., 2018; Thompson et al., 2010). As part of a broader monitoring study on pathogens of a free-ranging crested porcupine population in central Italy, a parasitological investigation was performed aimed to evaluate *Giardia* and other parasite infections in faecal samples of *H. cristata*.

2. Materials and methods

2.1. Sampling

The sampling was performed is a hilly area (86 m a.s.l.) of 4.476 ha located in Crespina-Lorenzana and Lari-Casciana Terme (10.56815 N - 43.56796 E) in the province of Pisa (Tuscany, Central Italy) (Fig. 1) from November 2018 to May 2019. Faecal samples were collected along seven transects 1.8 ± 1.2 km average long. Each transect was embedded in a zone. The average distance between transects was 2.9 ± 1.14 km. The seven zones (Z1-Z7) were chosen in order to be at a distance compatible with the crested porcupine clan average home-range (100 ha) (Fig. 1) (Sonnino and Lovari, 1994; Lovari et al., 2013). Each transect was monitored every 48 h and all the faecal samples found were removed even if they were not used in this study, as an attempt to ensure faecal samples were relatively fresh (max 48 h from faecal deposition) when collected for parasitological analysis.

Faecal samples were also collected from road-killed and captured porcupines (Fig. 2). Faecal samples were considered belonging to single individuals if taken from captured and road-killed animals, and if only a single faecal sample was collected from a transect during the whole sampling period. Therefore, only a partial number of collected faecal samples were considered attributable to different individuals. The porcupines were captured within a much larger study concerning the porcupine biology and health status. The capture activity was approved by the Italian Institute for Environmental Protection and Research (Protocol number 22584 of the 8 May 2017) and by Tuscany Region (Decree n. 14235 of the 3 October 2017). Collected faecal samples were stored at 4 °C and analysed within 24 h.

2.2. Parasitological analysis

Faecal samples were examined by using a commercial rapid immunoassay to detect *Giardia* spp. and *Cryptosporidium* spp. faecal

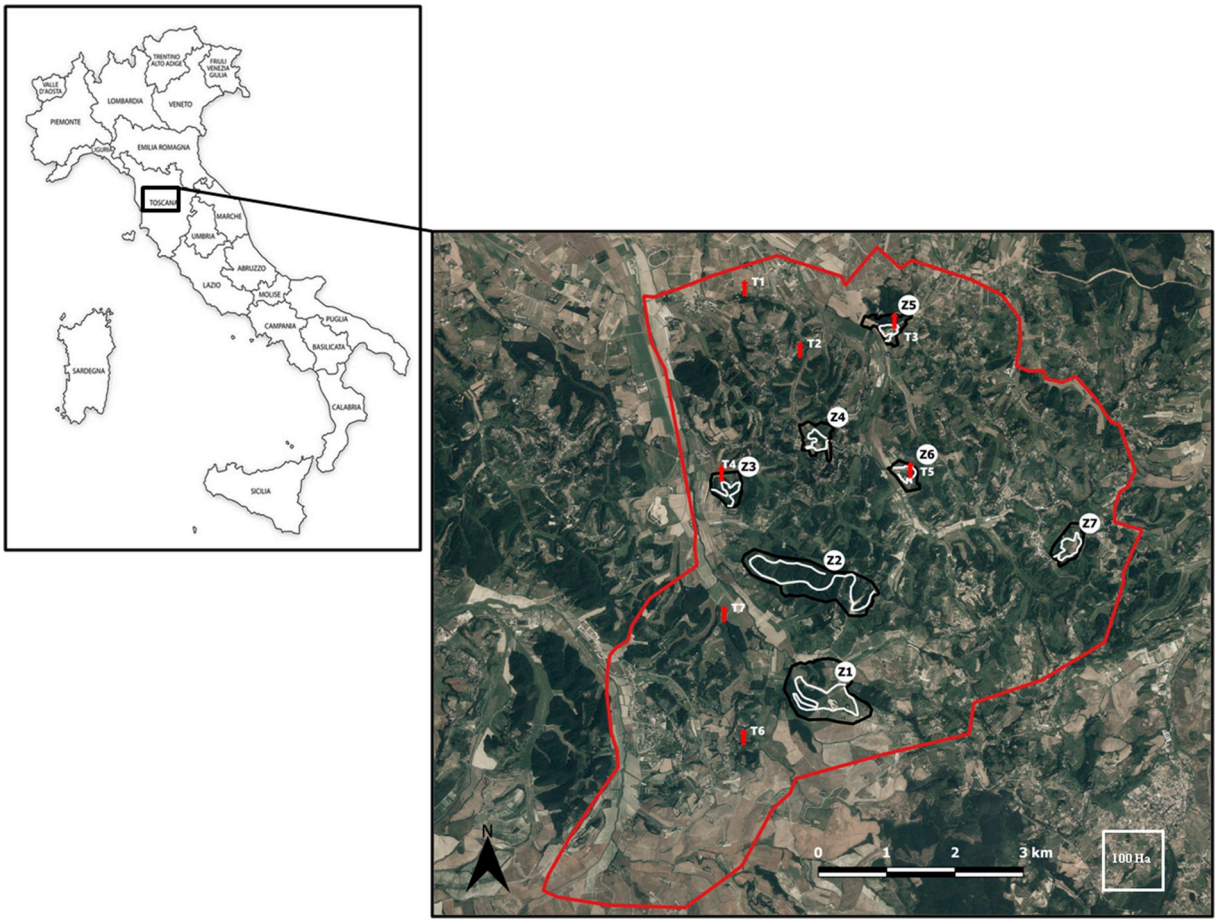

Fig. 1. Map of Italy: in the inset detail of the study area (border red line), zones (close areas in black, Z1-Z7), transects (white lines), and *Hystrix cristata* trap-sites (red arrows, T1-T7) where faecal samples were collected from November 2018 to May 2019. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

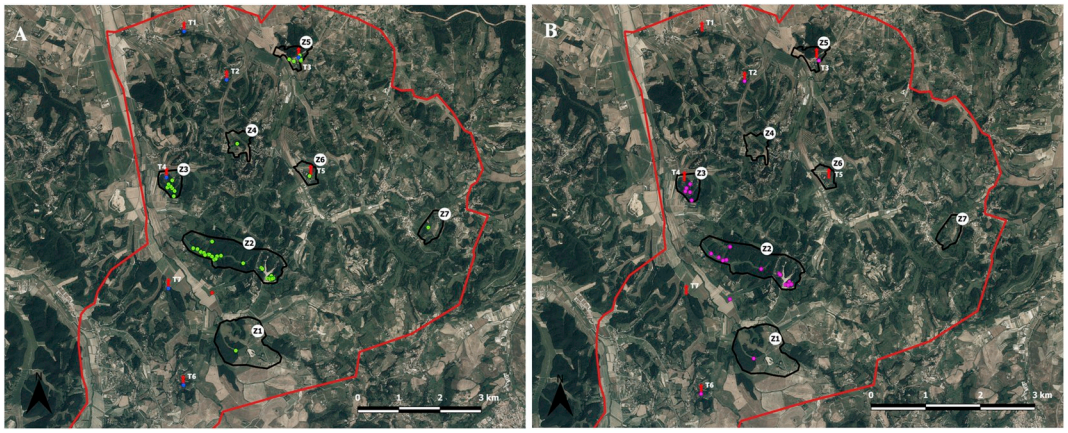

Fig. 2. Sampling area and location of *Hystrix cristata* faecal samples: **A**). Location of faecal samples collected in each zone (green dots), from captured (blue dots) and road-killed (red dot) animals; **B**). Location of *Giardia* spp. positive faecal samples (pink dots). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

antigens (RIDASCREEN® *Cryptosporidium/Giardia* Combi, R-Biopharm, Darmstadt, Germany). For the identification of helminthic eggs and protozoan cysts/oocysts, all faecal samples were analysed also microscopically by the Mini-FLOTAC technique (Cringoli et al., 2017).

2.3. Molecular analysis

Molecular investigation was performed to identify the species and genotypes of *Giardia* in samples found positive for *Giardia* spp. at parasitological analysis. For DNA extraction, samples were processed by a commercial kit (QIAamp DNA Stool Mini Kit, QIAGEN, Valencia, CA, USA). PCR protocols were applied to amplify a fragment of the small subunit ribosomal RNA (SSU rDNA, 130 bp, Read et al., 2002), of glutamate dehydrogenase (gdh, 432 bp, Read et al., 2004) and of triose phosphate isomerase (tpi, 530 bp, Sulaiman et al., 2003) genes. Due to the low specificity of all these primers, subsequent sequencing is necessary. Therefore, positive amplicons were purified using mi-PCR Purification Kit, Metabion International AG. Amplification products were sent to an external laboratory for sequencing (Bio-Fab Research, Rome, Italy). Forward and reverse sequences were manually checked using FinchTV. The obtained consensus sequences were then compared with those available in GenBank database by using the Standard Nucleotide BLAST search and aligned by Clustal Omega implemented in MEGA7.0 with representative sequences for the three loci and used as reference.

3. Results

A total of 52 porcupine faecal samples were collected and analysed. The 84.6% (n = 44) of the faecal samples were collected along transects of the seven different zones within the sampling area. In four of the seven zones, only a single faecal sample was found and collected during the whole investigation period. The 1.9% (n = 1, adult male) and the 13.5% (n = 7, 2 adult males, 3 sub-adult females and 2 female porcupettes) (Fig. 2, Table 1S) of the faecal samples were collected from road-killed and captured crested porcupines, respectively. Totally, 12/52 faecal samples resulted to be attributable to individual animals.

Overall, 39 out of 52 samples were found positive for at least one parasite taxa at parasitological analysis and six different parasites were identified (Table 1, Fig. 3). The 48% (25/52) of analysed faecal samples were found positive for *Giardia* spp. and the 1.9% (1/52) for *Cryptosporidium* spp. by the immunoassay only (Table 1). Among the 12 faecal samples belonging to different individuals, four samples (33.3%, 4/12) were positive for *Giardia* spp. (Table 1). At microscopical examination, positivity for *Trichuris* spp. eggs (32.7%, 17/52), gastrointestinal strongyle eggs (32.7%, 17/52), capillariid eggs (3.8%, 2/52) and coccidian oocysts (1.9%; 1/52) was also evidenced (Table 1, Fig. 3).

Table 1
Number of positive faecal samples collected from road killed and captured crested porcupines (*Hystrix cristata*) and sampled in transects for each taxa of identified parasites.

Parasites	N. positive (%)	Road killed	Captured	Faecal samples
Helminths				
GI[a] Strongyles	17/52 (32.7)	1	4	12
***Trichuris* sp.**	17/52 (32.7)	0	2	15
***Capillaria* sp.**	2/52 (3.8)	0	0	2
Protozoa				
***Giardia* sp.**	25/52 (48)	1	2	22
Coccidia	1/52 (1.9)	0	1	0
***Cryptosporidium* sp.**	1/52 (1.9)	0	0	1

[a] Gastrointestinal.

Multiple parasite infections were found in 20/39 positive samples (51.28%) (Table 2S). *Giardia* plus other parasites coinfections were detected in 15/39 (38.46%) positive samples (Table 2S).

Molecular analysis was performed on 17 out of the 25 *Giardia*-positive isolates. At the *SSU rDNA* locus, expected bands were achieved for 12/17 isolates. All isolates were assigned to assemblage B. Only 3 out of 17 samples were successfully amplified at the *tpi* locus. Sequencing confirmed assemblage BIV for one isolate and revealed assemblage AII for the other two samples. However, no heterozygous positions (double peaks) were detected during chromatogram inspection of these two isolates at both loci. Attempts to further subtype these samples at gdh locus were not successful.

4. Discussion

G. duodenalis is a common parasite of the small intestine and one of the most important and frequent causes of human and animal diarrheal disease worldwide (Cacciò et al., 2018). Long-term health consequences of giardiasis have also been documented in humans (Lanata et al., 2013).

Among parasites identified in 52 porcupine faecal samples collected within the study area, *Giardia* spp. was the most prevalent. Almost half of the samples examined (48%) were found positive to *Giardia* by the immunoassay and *G. duodenalis* was identified in 12 out of 17 isolates examined at molecular analysis. However, the possibility that some of the analysed faecal samples were belonging to the same animals is high, especially for those samples collected within transects where more than one sample was found.

Nevertheless, within the 25 *Giardia* spp. positive faecal samples to

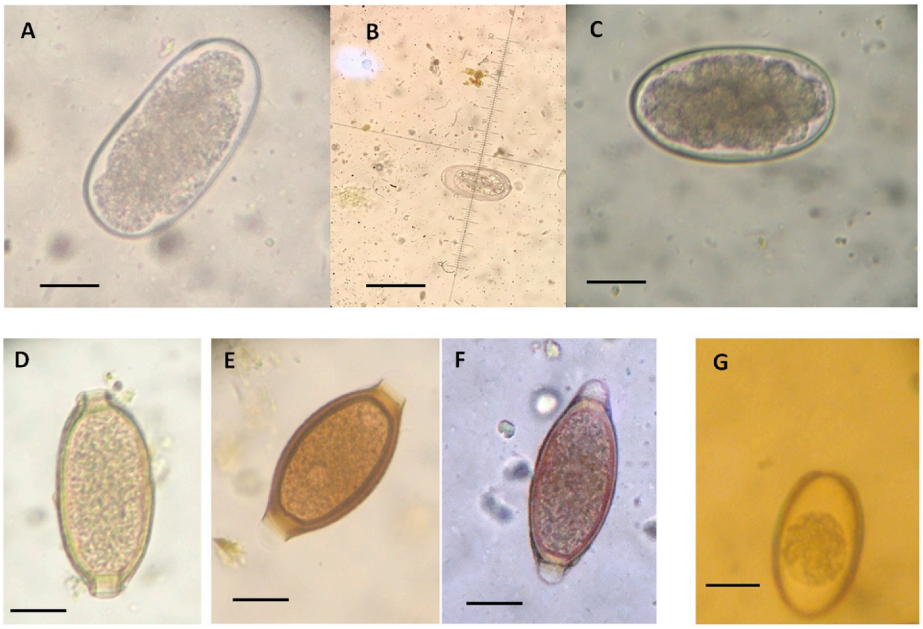

Fig. 3. Parasites identified in 52 crested porcupine faecal samples: **A-C**). Gastrointestinal strongyle eggs (59.8–78 μm x 28.6–44.2 μm) (A and C 400X, scale bar 15 μm; B 100X, scale bar 60 μm); **D**). Capillariid egg measuring 59.8 μm × 26 μm (400X, scale bar 15 μm); **E, F**). *Trichuris* spp. eggs of 60 x 28.6–40 μm in dimensions (400X, scale bar 15 μm); **G**). Coccidian oocyst measuring 36.4 μm × 23.4 μm (400X, scale bar 15 μm).

the immunoassay also 4 out of 12 (33.3%) individual faecal samples were included. These four samples were belonging to two captured animals, one road-killed specimen and the single faecal sample collected in Z1.

For the detection of *G. duodenalis*, *Cryptosporidium parvum* and *Cryptosporidium hominis*, a sensitivity and a specificity of 85–96% and 89%, 80-85% and ≥98%, 96% and 99% has been reported for the rapid immunoassay used in this study compared to conventional microscopy, direct fluorescent-antibody and PCR, respectively (Weitzel et al., 2006; Regnath et al., 2006; Chalmers et al., 2011). However, false negative results may be possible due to intermittent faecal excretion of these protozoans or if the amount of protozoan antigens in the examined samples is too small (https://clinical.r-biopharm.com/wp-content/uploads/sites/3/2017/05/C1121-RIDASCREEN-Cryptosporidium_Giardia-Combi_lang-2017-04-20_EN.pdf). Moreover, the sensitivity and the specificity of this commercial kit for the detection of other *Giardia* and *Cryptosporidium* species is unknown. Therefore, it is possible that some samples tested negative to these protozoa in the present study were instead positive.

Results from the immunoassay were not confirmed by PCR in all cases. As previously described, the difference between immunological and molecular results may be due to the presence of PCR inhibitors in faecal samples, with the amplification turning out unsuccessful due to the loss of pre-treatment methods for removal of specific PCR inhibitors (Schrader et al., 2012).

Nevertheless, this study is the first report of *G. duodenalis* infection in the crested porcupine. Indeed, *Giardia* spp. infection was detected also by Chakraborty et al. (2015) in the Indian crested porcupine (*H. indica*), but species, assemblage and subtype identification were not performed.

In the present study, different combinations of *G. duodenalis* assemblages were identified in two isolates at *SSU rDNA* and *tpi* loci. The genotyping lack of concordance is quite recurrent in *G. duodenalis*; these discordant results can derive from mixed infections, subsequent infections or heterogeneity of allelic sequences (Sprong et al., 2009; Ryan and Cacciò, 2013; Fahmy et al., 2015; Adell-Aledón et al., 2018). However, due to the small number of samples, it was difficult to draw any conclusion concerning the genotyping discordance.

The habitat diversity and environmental fragmentation linked to anthropic activities are key factors for the presence of crested porcupines (Toschi, 1965). In the Italian agro-ecosystem, the crested porcupine is considered as a pest and it benefits from the increase in the production of agricultural crops (Santini, 1980; Laurenzi et al., 2016). Therefore, contaminated anthropogenic settlements may represent a risk factor for the acquisition of *G. duodenalis* infection by crested porcupines in the examined area, as anthropogenic spread of *G. duodenalis* is well documented in other wildlife species and environments worldwide (Hillman et al., 2019; Thompson, 2013). Conversely, land-use changes by humans for agricultural development and urbanization have been associated with higher risks of zoonotic pathogen transmission by potential reservoir animals, and the presence of crested porcupines in habitat characterised by anthropic activities may enhance the possibility for the spread of crested porcupine parasite infections that could affect also humans (Chakraborty et al., 2015; Otranto and Deplazes, 2019; Mendoza et al., 2020). As assemblages AII and BIV are likely associated with human infections, results here obtained may suggest that *H. cristata* can act as source of *G. duodenalis* cysts potentially infectious to humans (Robertson et al., 2019; Cacciò et al., 2018). In Europe, *G. duodenalis* infection has been reported in badgers, red foxes and wild boars (Hamnes et al., 2007; Cacciò et al., 2008; Barlow et al., 2010; Beck et al., 2011; Onac et al., 2015; Stojecki et al., 2015; Robertson et al., 2019). Interestingly, the majority of identifiable *G. duodenalis* DNA isolated from red fox and wild boar samples in previous European studies was found to be assemblage A or B, suggesting that these wild animals may play a role in the zoonotic transmission of *G. duodenalis* (Stojecki et al., 2015; Robertson et al., 2019). In the area here examined, wild boars, red foxes and badgers share the same porcupine habitat, with red foxes and badgers occasionally sharing and cohabiting the same settlements (Coppola et al., unpublished study). Moreover, the ingestion of porcupine carrions by red foxes may represent another source of infection in these animals (Fais, 1991; Coppola et al., unpublished study). Therefore, it is possible to hypothesise a putative circulation and transmission of potentially zoonotic *G. duodenalis* assemblages among crested porcupines, red foxes, wild boars and badgers in the study area with a potential impact on the population health of these wild animal species. Moreover, these wild species may act as reservoirs of *G. duodenalis* infections for humans (Hillman et al., 2019; Thompson, 2013).

Concerning other parasites identified in this study, eggs of *Trichuris* spp. and of gastrointestinal strongyles were found in about 33% of examined samples, while capillariids, coccidia and *Cryptosporidium* spp. were much less frequent (about 2–4%). However, it should be

considered that the Mini-FLOTAC technique used for the detection of most of these parasites has a sensitivity of 10 eggs-oo(cysts)/gram of faeces (Cringoli et al., 2017). Therefore, it is possible that positivity was not evidenced for all those parasites present at lower quantities in examined faecal samples.

In previous studies, *Trichuris infundibulum* and *Trichuris hystricis* have been reported from *H. cristata* (Pavlov, 1957), and *Trichuris landak* from *H. javanica* (Purwaningsih, 2013), while gastrointestinal strongyles (*Trichostrongylus colubriformis*) and unidentified *Trichuris* and *Capillaria* species have been reported from *H. indica* (Wertheim and Durette-Desset, 1975; Mir et al., 2016). However, to the best of our knowledge coccidian and *Cryptosporidium* spp. infections have been never reported before in crested porcupines. About *Cryptosporidium*, it would be extremely interesting to perform further and more in-depth studies aimed to the identification of *Cryptosporidium* species infecting crested porcupines and to assess the molecular epidemiology of this protozoan species in the examined area. More specifically, it would be very interesting to know whether in the study area are involved species/genotypes shared with other wild mammals cohabiting with crested porcupines or specific for *H. cristata*, as host-adapted species/genotypes are frequently observed among wildlife (Thompson and Ash, 2019). It would be also very interesting to know whether in the study area are involved zoonotic species, considering the recent detection of potentially zoonotic *Cryptosporidium* species in European red foxes and badgers (Mateo et al., 2017).

Further studies aimed to know the impact on the health of crested porcupines of the remaining parasites identified in this study are also needed, since data are lacking.

In conclusion, results obtained in this investigation improved the knowledge on the parasite-fauna of crested porcupines in Italy and showed for the first time that *H. cristata* can be infected by *G. duodenalis*. More studies are needed on the epidemiology of *Giardia* and other parasite species in crested porcupine populations, especially on *G. duodenalis* assemblages to better evaluate the zoonotic potential of *Giardia* in *H. cristata*.

Financial support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors contributions

Conceived the study: SP, AF. Designed the experiment: SP, AF, MM, FC, FB. Performed field activity and sampling: AF, FC. Performed parasitological analysis: SP, MM. Performed molecular analysis: BF, GPI. Analysis and interpretation of data: SP, AF, MM, FC, BF, GPI. Wrote the first draft of the manuscript: SP, AF, MM, FC, FB. All authors wrote and revised the final version of the manuscript.

Declaration of competing interest

None.

Acknowledgements

Authors thank the Institute for Environmental Protection and Research (ISPRA) and the Tuscany Region for providing authorization for capture activity. Authors wish to thank also Samuele Baldanti for helping in faecal sampling and Dr. Andrea Agresti for helping in parasitological analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2020.01.006o.

References

Adell-Aledón, M., Köster, P.C., de Lucio, A., Puente, P., Hernández-de-Mingo, M., Sánchez-Thevenet, P., Dea-Ayuela, M.A., Carmena, D., 2018. Occurrence and molecular epidemiology of *Giardia duodenalis* infection in dog populations in eastern Spain. BMC Vet. Res. 14, 26. https://doi.org/10.1186/s12917-018-1353-z.

Barlow, A.M., Mullineaux, E., Wood, R., Taweenan, W., Wastling, J.M., 2010. Giardiosis in Eurasian badgers (*Meles meles*). Vet. Rec. 167, 1017. https://doi.org/10.1136/vr.c7346.

Beck, R., Sprong, H., Lucinger, S., Pozio, E., Cacciò, S.M., 2011. A large survey of Croatian wild mammals for *Giardia duodenalis* reveals a low prevalence and limited zoonotic potential. Vector Borne Zoonotic Dis. 11, 1049–1055. https://doi.org/10.1089/vbz.2010.0113.

Bern Convention, 1979. Treaty No.104: Convention on the Conservation of European Wildlife and Natural Habitats. Council of Europe. https://www.coe.int/en/web/bern-convention/.

Bruno, E., Riccardi, C., 1995. The diet of the crested porcupine *Hystrix cristata* L., 1758 in a Mediterranean rural area. Z. Saugetierkunde 60, 226–236.

Cacciò, S.M., Beck, R., Lalle, M., Marinculic, A., Pozio, E., 2008. Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. Int. J. Parasitol. 38, 1523–1531.

Cacciò, S.M., Lalle, M., Svärd, S.G., 2018. Host specificity in the *Giardia duodenalis* species complex. Infect. Genet. Evol. 66, 335–345.

Chakraborty, D., Hussain, S., Reddy, D.M., Raut, S., Tiwari, S., Kumar, V., Umapathy, G., 2015. Mammalian gastrointestinal parasites in rainforest remnants of Anamalai hills, Western Ghats, India. J. Biosci. 40, 399–406.

Chalmers, R.M., Campbell, B.M., Crouch, N., Charlett, A., Davies, A.P., 2011. Comparison of diagnostic sensitivity and specificity of seven *Cryptosporidium* assays used in the UK. J. Med. Microbiol. 60, 1598–1604.

Coppola, F., Vecchio, G., Felicioli, A., 2019. Diurnal motor activity and "sunbathing" behaviour in crested porcupine (*Hystrix cristata* L.,1758). Sci. Rep. 9, 14283. https://doi.org/10.1038/s41598-019-50784-y.

Coppola, F., Dari, C., Vecchio, G., Scarselli, D., Felicioli, A. Co-habitation of Settlements between Crested Porcupines (*Hystrix cristata*), Red Foxes (*Vulpes vulpes*) and European Badgers (*Meles meles*). Unpublished study.

Corsini, M.T., Lovari, S., Sonnino, S., 1995. Temporal activity patterns of crested porcupine *Hystrix cristata*. J. Zool. 236, 43–54.

Cringoli, G., Maurelli, M.P., Levecke, B., Bosco, A., Vercruysse, J., Utzinger, J., Utzinger, J., Rinaldi, L., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat. Protoc. 12, 1723–1732. https://doi.org/10.1038/nprot.2017.067.PMid:28771238.

Fahmy, H.M., El-Serougi, A.O., El Deeb, H.K., Hussein, H.M., Abou-Seri, H.M., Klotz, C., Aebischer, T., El Sayed Khalifa Mohamed, K., 2015. *Giardia duodenalis* assemblages in Egyptian children with diarrhea. Eur. J. Clin. Microbiol. Infect. Dis. 34, 1573–1581.

Fais, I., Costanzo, M., Massa, B., 1991. First data on the feeding habits of the red fox (*Vulpes vulpes* L.) in Sicily. Hystrix 3, 105–112.

Feng, Y., Xiao, L., 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin. Microbiol. Rev. 24, 110–140.

Hamnes, I.S., Gjerde, B.K., Forberg, T., Robertson, L.J., 2007. Occurrence of *Giardia* and *Cryptosporidium* in Norwegian red foxes (*Vulpes vulpes*). Vet. Parasitol. 143, 347–353.

Helmy, Y.A., Spierling, N.G., Schmidt, S., Rosenfeld, U.M., Reil, D., Imholt, C., Jacob, J., Ulrich, R.G., Aebischer, T., Klotz, C., 2018. Occurrence and distribution of *Giardia* species in wild rodents in Germany. Parasites Vectors 11, 213. https://doi.org/10.1186/s13071-018-2802-z.

Hillman, A.E., Ash, A.L., Lymbery, A.J., Thompson, R.C.A., 2019. Anthropozoonotic significance, risk factors and spatial distribution of *Giardia* spp. infections in quenda (*Isoodon obesulus*) in the greater Perth region, Western Australia. Int. J. Parasitol. Parasites Wildl. 9, 42–48.

Lanata, C.F., Fischer-Walker, C.L., Olascoaga, A.C., Torres, C.X., Aryee, M.J., Black, R.E., 2013. Global causes of diarrheal disease mortality in children < 5 years of age: a systematic review. PLoS One 8, e72788. https://doi.org/10.1371/journal.pone.0072788.

Laurenzi, A., Bodino, N., Mori, E., 2016. Much ado about nothing: assessing the impact of a problematic rodent on agriculture and native trees. Mamm. Res. 61, 65–72.

Lovari, S., Sforzi, A., Mori, M., 2013. Habitat richness affects home range size in a monogamous large rodent. Behav. Process. 99, 42–46.

Lyu, Z., Shao, J., Xue, M., Ye, Q., Chen, B., Qin, Y., Wen, J., 2018. A new species of *Giardia* Künstler, 1882 (Sarcomastigophora: hexamitidae) in hamsters. Parasites Vectors 11, 202. https://doi.org/10.1186/s13071-018-2786-8.

Marangi, M., Berrilli, F., Otranto, D., Giangaspero, A., 2010. Genotyping of *Giardia duodenalis* among children and dogs in a closed socially deprived community from Italy. Zoonoses Public Health 57, 54–58.

Mateo, M., de Mingo, M.H., de Lucio, A., Morales, L., Balseiro, A., Espí, A., Barral, M., Lima Barbero, J.F., Habela, M.Á., Fernández-García, J.L., Bernal, R.C., Köster, P.C., Cardona, G.A., Carmena, D., 2017. Occurrence and molecular genotyping of *Giardia duodenalis* and *Cryptosporidium* spp. in wild mesocarnivores in Spain. Vet. Parasitol. 235, 86–93. https://doi.org/10.1016/j.vetpar.2017.01.016.

Mendoza, H., Rubio, A.V., García-Peña, G.E., Suzán, G., Simonetti, J.A., 2020. Does land-use change increase the abundance of zoonotic reservoirs? Rodents say yes. Eur. J. Wildl. Res. 66, 6. https://doi.org/10.1007/s10344-019-1344-9.

Mir, A.Q., Dua, K., Singla, L.D., Sharma, S., Singh, M.P., 2016. Prevalence of parasitic infection in captive wild animals in Bir Moti Bagh minizoo (deer park), Patiala, Punjab. Vet. World 9, 540–543. https://doi.org/10.14202/vetworld.2016.540-543.

Mori, E., Sforzi, A., Menchetti, M., Mazza, G., Lovari, S., Pisanu, B., 2015. Ectoparasite load in the crested porcupine *Hystrix cristata* Linnaeus, 1758 in Central Italy.

Parasitol. Res. 114, 2223–2229. https://doi.org/10.1007/s00436-015-4413-3.
Onac, D., Oltean, M., Mircean, V., Jarca, A., Cozma, V., 2015. Occurrence of *Giardia duodenalis* zoonotic assemblages in red foxes from Romania. Sci. Parasitol. 16, 177–180.
Otranto, D., Deplazes, P., 2019. Zoonotic nematodes of wild carnivores. Int. J. Parasitol. Parasites Wildl. 9, 370–383.
Pavlov, A., 1957. Trichocephalidae and Capillariidae of animals and man and the diseases caused by them. In: Skrjabin, K.I., Shikhobalova, N.P., Orlov, I.V. (Eds.), Essentials of Nematodology. Izdatelstvo Akademii Nauk SSSR, Russia, pp. 261.
Pigozzi, G., 1986. Crested porcupines (*Hystrix cristata*) within badger setts (*Meles meles*) in the Maremma natural park, Italy. Saugetierk. Mitt. 33, 261–263.
Purwaningsih, E., 2013. The first report of new species: *Trichuris landak* n. sp. Asian Pac. J. Trop. Biomed. 3, 85–88. https://doi.org/10.1016/S2221-1691(13)60029-5.
Read, C., Walters, J., Robertson, I.D., Thompson, R.C.A., 2002. Correlation between genotype of *Giardia duodenalis* and diarrhea. Int. J. Parasitol. 32, 229–231.
Read, C.M., Monis, P.T., Thompson, R.C., 2004. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect. Genet. Evol. 4, 125–130.
Regnath, T., Klemm, T., Ignatius, R., 2006. Rapid and accurate detection of *Giardia lamblia* and *Cryptosporidium* spp. antigens in human fecal specimens by new commercially available qualitative immunochromatographic assays. Eur. J. Clin. Microbiol. Infect. Dis. 25, 807–809.
RIDASCREEN® Cryptosporidium/Giardia Combi. https://clinical.r-biopharm.com/wp-content/uploads/sites/3/2017/05/C1121-RIDASCREEN-Cryptosporidium_Giardia-Combi_lang-2017-04-20_EN.pdf. Last access 29 December 2019.
Robertson, L.J., Clark, C.G., Debenham, J.J., Dubey, J.P., Kváč, M., Li, J., Ponce-Gordo, F., Ryan, U., Schares, G., Su, C., Tsaousis, A.D., 2019. Are molecular tools clarifying or confusing our understanding of the public health threat from zoonotic enteric protozoa in wildlife? Int. J. Parasitol. Parasites Wildl. 9, 323–341. https://doi.org/10.1016/j.ijppaw.2019.01.010.
Ryan, U., Cacciò, S.M., 2013. Zoonotic potential of *Giardia*. Int. J. Parasitol. 43, 943–956. https://doi.org/10.1016/j.ijpara.2013.06.001.
Ryan, U., Zahedi, A., 2019. Molecular epidemiology of giardiasis from a veterinary perspective. Adv. Parasitol. 106, 209–254. https://doi.org/10.1016/bs.apar.2019.07.002.
Santini, L., 1980. The habits and influence on the environment of the old world porcupine *Hystrix cristata* L. in the northernmost part of its range. In: Proceedings of the 9th Vertebrate Pest Conference. 34. pp. 149–153. University of Nebraska – Lincoln. https://pdfs.semanticscholar.org/4238/ed55c3aed9081b71bddb67ed7ac36c4a7459.pdf, Accessed date: 19 November 2019.
Schrader, C., Schielke, A., Ellerbroek, L., Johne, R., 2012. PCR inhibitors - occurrence, properties and removal. J. Appl. Microbiol. 113, 1014–1026.
Sonnino, S., Lovari, S., 1994. Home-range and activity of crested porcupines in relation to some environmental variable. Ethol. Ecol. Evol. 6, 385–452.
Sprong, H., Cacciò, S.M., van der Giessen, J.W., 2009. Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Neglected Trop. Dis. 3, e558.
Stojecki, K., Sroka, J., Cacciò, S.M., Cencek, T., Dutkiewicz, J., Kusyk, P., 2015. Prevalence and molecular typing of *Giardia duodenalis* in wildlife from eastern Poland (Praha). Folia Parasitol. 62https://doi.org/10.14411/fp.2015.042. pii: 2015.042.
Sulaiman, I.M., Fayer, R., Bern, C., Gilman, R.H., Trout, J.M., Schantz, P.M., Das, P., Lal, A.A., Xiao, L., 2003. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg. Infect. Dis. 9, 1444–1452.
Thompson, R.C., Lymbery, A.J., Smith, A., 2010. Parasites, emerging disease and wildlife conservation. Int. J. Parasitol. 40, 1163–1170.
Thompson, R.C.A., 2013. Parasite zoonoses and wildlife: one health, spillover and human activity. Int. J. Parasitol. 43, 1079–1088.
Thompson, R.C.A., Ash, A., 2016. Molecular epidemiology of *Giardia* and *Cryptosporidium* infections. Infect. Genet. Evol. 40, 315–323.
Thompson, R.C.A., Ash, A., 2019. Molecular epidemiology of *Giardia* and *Cryptosporidium* infections – what's new? Infect. Genet. Evol. 75, 103951. https://doi.org/10.1016/j.meegid.2019.103951.
Toschi, A., 1965. Fauna d'Italia. Mammalia: Lagomorpha, Rodentia, Carnivora, Artiodactyla e Cetacea. Edizione Calderini, Bologna, Italy.
Vecchio, G., Coppola, F., Scarselli, D., Giannini, F., Felicioli, A., 2018. Crested porcupine in the Island of Elba, Italy: native or alien? Curr. Sci. 114, 246–247.
Weitzel, T., Dittrich, S., Möhl, I., Adusu, E., Jelinek, T., 2006. Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples. Clin. Microbiol. Infect. 12, 656–659.
Wertheim, G., Durette-Desset, M., 1975. Helminths of birds and mammals from Israel. VI. The taxonomy and ecology of Trichostrongylid Nematodes. Ann. Parasitol. Hum. Comp. 50, 735–762 Abstract.