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Lepus corsicanus gastro-intestinal helminths: first report

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Summary

Italian hare *Lepus corsicanus* De Winton, 1898, is a true species living in simpatry with *Lepus europaeus* in mainland Italy and with *Oryctolagus cuniculus* in Sicily, where *L. europaeus* is absent. Up to date, nobody has studied the helminths of this endemic Italian Mammal. Therefore, in order to describe Italian hare gastro-intestinal helminths, gastro-intestinal tract of 29 Italian hares, coming from mainland Italy (#14) and from Sicily (#15) were collected between 1997 and 2009. Twentyfive hares were positive for at least one parasite (86 %). Six parasite species were isolated, 2 cestodes (*Cittotaenia pectinata*, prevalence 3 %) and *Paranoplocephala* sp., 3 %) and 4 nematodes (*Trichostrongylus retortaeformis*, 86 %, *Graphidium strigosum*, 14 %, *Trichuris* sp., 10 % and *Teladorsagia circumcincta*, 7 %). Both *Teladorsagia circumcincta circumcincta* and *Teladorsagia circumcincta trifurcata* morphotypes were identified. Comparison with available data regarding *L. europaeus* and *O. cuniculus* in Italy is provided. Being not *T. circumcincta* and *Paranoplocephala* sp. typical parasites of leporids, a description of the observed specimens is given.

Keywords: *Lepus corsicanus;* helminths; Italy

Introduction

Italian hare *Lepus corsicanus* De Winton, 1898, formerly considered a subspecies of European brown hare *Lepus europaeus* Pallas, 1778, is an italian endemic species rediscovered by means of morphological (Palacios *et al*., 1989; Palacios, 1996; Riga *et al.*, 2001) and molecular analyses (Pierpaoli *et al.*, 1999). In its natural range no morphological and genetic hybrids were found in areas where viable populations of *L. corsicanus* and *L. europaeus* coexist in simpatry. Phylogenetical analyses suggested that *L. corsicanus* and *L. europaeus* are not closely related sister *taxa*, but belong to distinct evolutionary lineages that dispersed in Western Europe in different periods during the early Pleistocene (Pierpaoli *et al.*, 1999).
The current distribution of *L. corsicanus* is still poor known. This threatened species is found in Central and Southern mainland Italy and in Sicily; its mainland *status* is classified as "vulnerable" (Angelici *et al*., 2008). In Sicily the species seems relatively more abundant but possibly in decline (Angelici & Luiselli, 2001). Populations of *L. corsicanus* in mainland Italy are in sympatry and syntopy with *L. europaeus*, while in Sicily, where European brown hare is absent, *L. corsicanus* lives in sympatry and syntopy with wild rabbit, *Oryctolagus cuniculus*.
Up to date, the only reports of parasites in Italian hare regard EBHS virus (Guberti *et al*., 2000) and ticks (Dantas-Torres *et al.*, 2011) while nobody has studied the helmints of this endemic Italian Mammal. Therefore, coherently with the National Action Plan (Trocchi & Riga, 2001) that emphasises the importance of scientific research on *L. corsicanus* diseases in natural areas, we carried out a preliminar analysis of Italian hare gastro-intestinal helminths biocenosis.

Materials and methods

Gastro-intestinal tract of 29 Italian hares have been collected from their whole living area, in Italy, between 1997 and 2008 by I.N.F.S. (National Institute for Wildlife, nowadays merged into I.S.P.R.A. – National Institute for Environmental Research). Age estimation has been attained on the basis of skull ossification and development (Cabon-Raczynska, 1964).
The gastro-intestinal tract was preserved in 10 % formalin (#19) or frozen at -20°C (#10), and examined between October 2008 and October 2009. Stomach and intestine were longitudinally opened, gently scraped with a microscope slide to allow the detaching of parasites, and washed with tap water. Their content was than collected in conical flasks and repeatedly washed in order to obtain the sediment

Table 1. Description of the 29 specimens of *Lepus corsicanus* examined for gastro-intestinal helminths collection

		Sicily (#15)	Continental Italy (#14)	total
sex				
	male	8	5	13
	female	7	9	16
age				
	young*	-	2	2
	sub-adult	2	2	3
	adult	13	12	25
cause of death	legally shot for study purpose	15	8	23
	found dead**	-	3	3
	dead in captivity***	-	3	3

* a male and a female 3 month old animals born in captivity
** 2 adult females knock down and an adult male found dead
*** comprise the two 3 month old animals

to be screened under a stereomicroscope for parasite collection. Collected helminths were fixed in a 70 % ethanol solution with 5 % glycerol. Cestodes were stained in aceto-alum-carmine solution, mounted in Canada-balsam and identified using the key and description of Tenora *et al*., (1986), Haukisalmi and Henttonen (2003), Haukisalmi and Wickström (2005), Haukisalmi (2009). Nematodes were clarified and mounted in lactophenol, and identified using the keys and descriptions of Erickson (1944), Soulsby (1968), Becklund and Walker (1971), Stringfellow (1972), Gibbons and Khalil (1982), Lichtenfels and Hoberg (1993). For Trichostrongylidae, the number of female belonging to each species was estimated based on the number of males. Some damaged or sexually immature nematodes were classed at family level only.
Prevalence and abundance of each parasite species were computed according to Margolis *et al*. (1982) and Bush *et al*. (1997). Confidence intervals (CI) for prevalences and abundances were calculated using the statistical package QP 3.0 (Reiczigel & Rózsa, 2005).

A sample of the isolated parasites and a copy of the raw working data are available from the Authors.

Results

Main features of the examined animals are summarized in Table 1.
Out of 29 hares, 25 were positive for at least one parasite (86 %). Six parasite species were isolated, 2 cestodes (*Cittotaenia pectinata* and *Paranoplocephala* sp.) and 4 nematodes (*Trichostrongylus retortaeformis*, *Graphidium strigosum*, *Trichuris* sp. and *Teladorsagia circumcincta* - which comprises *Teladorsagia circumcincta circumcincta* and *Teladorsagia circumcincta trifurcata* morphotypes). Trichostrongylidae fourth stage larvae were also collected.
Range, prevalence, abundance, sex ratio and localization of the isolated parasites are summarized in Table 2.
All the 53 specimens of *C. pectinata* collected belonged to a single host, an adult female shot in 1999 in continental Italy. The two specimens of *Paranoplocephala* sp. were recovered in the small intestine of one female shot in Sicily in 2008. The 11 males of *T. c. circumcincta* morphotype were found in the stomach of two animals, an adult female shot in 1997 in Sicily and a sub-adult male shot in 1997 in Sicily, with 9 and 1 intensity, respectively. The 3 male nematodes identified as *T. c. trifurcata* were found in one animal only, the same adult female shot in 1997 in Sicily which had the higher intensity of *T. c. circumcincta*. *Trichuris* sp. was found in continental Italy only, while *T. retortaeformis* and *G. strigosum* were collected both in continental Italy and in Sicily.
Being not *T. circumcincta* and *Paranoplocephala* sp. typical parasites of leporids, a description of the observed specimens will be given in the following sections.

Paranoplocephala sp. (Fig. 1)
Strobila relatively thin with large scolex, 0.6 mm wide, bearing four ovoid protruding suckers, large relative to scolex, crateriform in side view, 0.35 mm in maximum

Table 2. Helminths collected from 29 *Lepus corsicanus* in Italy from 1997 to 2008

		range°	Prevalence % (95 % CI)	Abundance (95 % CI)	Sex Ratio (M:F)	localization
Nematodes						
	Trichostrongylus retortaeformis	1 – 171	86 (68 – 96)	14.59 (6.97 – 35.59)	1:1.4	intestine
	Graphidium strigosum	4 – 67	14 (4 – 32)	3.28 (0.52 – 12.38)	1:1.6	stomach
	Teladorsagia circumcincta§	2 – 76	7 (1 – 23)	2.69 (0.00 – 10.62)	1:5.0	stomach
	T. c. circumcincta	2 – 57	7 (1 – 23)	2.03 (0.00 – 8.07)	1:4.9	
	T. c. trifurcata	19 – 19	3 (0.1 – 18)	0.66 (0.00 – 1.97)	1:5.3	
	Trichuris sp.	1 – 5	10 (2 – 27)	0.31 (0.03 – 0.90)	1:0.3	intestine
	L4*	7 – 26	7 (1 – 23)	1.14 (0.00 – 3.83)	/	stomach , intestine
	Trichostrongylidae**	1 – 179	86 (68 – 96)	17.72 (9.10 – 40.34)	1:1.6	
Cestodes						
	Cittotaenia pectinata	53 – 53	3 (0.1 – 18)	1.83 (0.00 – 5.48)	/	intestine
	Paranoplocephala sp.	2 – 2	3 (0.1 – 18)	0.07 (0.00 – 0.21)	/	intestine

° in infected animals
§ include *T. c. circumcincta* and *T. c. trifurcata* morphotypes
*Trichostrongylidae fourth stage larvae
**Total adult Trichostrongylidae burden, including damaged specimens

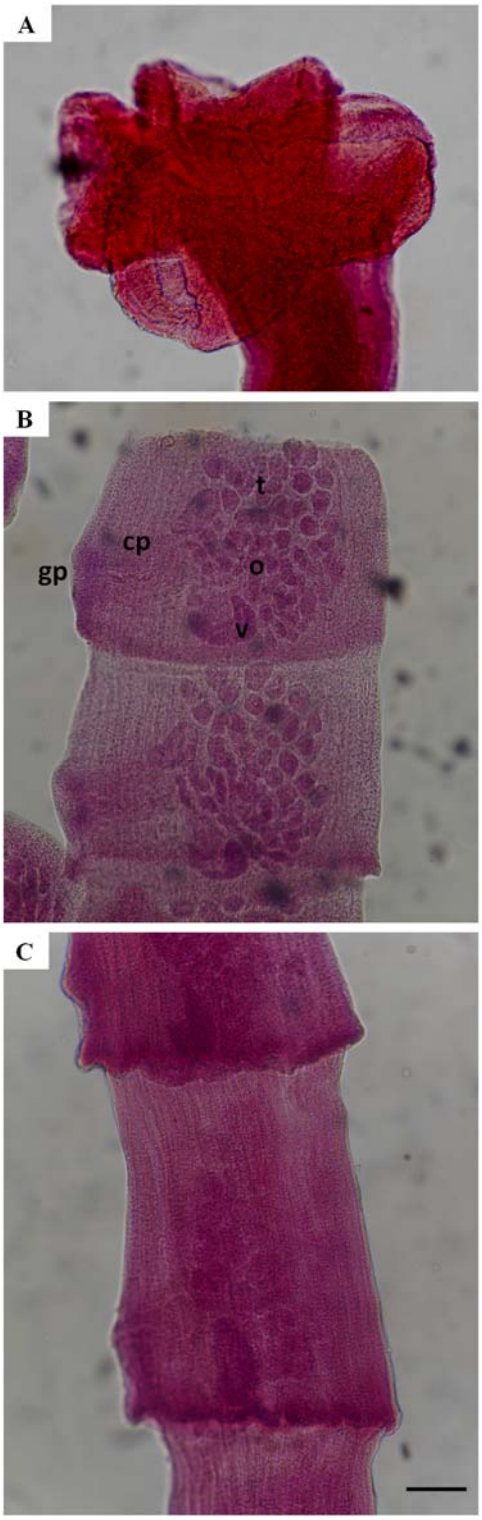

Fig. 1. *Paranoplocephala* sp. A. Scolex; B. Mature proglottids (t: testes, o: ovary, v: vitellarium, cp: cirrus pouch, gp: genital pore; C. Terminal segment strongly elongate. Scale-bar: 100 µm.

diameter, directed antero-laterally. Neck 1.55 mm long, minimum width 0.25 mm.

Craspedote proglottides wider than long (length/width ratio range 0.5 – 0.7); acraspedote proglottides elongated (length/width ratio range 0.7 – 1.3). Absence of eggs in terminal segments (sterility) which are strongly elongate (length/width ratio range 1.3 – 1.9). Genitalia single. Genital pores unilateral, opening in posterior half of segment margin. Testes numerous, distributed antiporally and anterior to ovary. Vitellarium asymmetrically bilobed overlapping posterior margin of ovary. Ovary in median position with margin coarsely lobed. Uterus not visible.

Teladorsagia circumcincta (Fig 2)

Teladorsagia circumcincta circumcincta

Male copulatory bursa with two large lateral lobes and a reduced separate dorsal lobe. Rays of copulatory bursa in 2-2-1 pattern. Ventral rays diverging and coming together at their distal tips; anteroventral ray smaller than posteroventral ray; externodorsal ray arising from the base of the dorsal ray; dorsal ray slender, with one pair of long branches, arising halfway from the main trunk, dividing again at their distal end; dorsal raylets widely spaced, surrounded by an accessory bursal membrane unsclerotized. Spicules rod-like, with an average length of 297.9 µm, branching at their distal fourth. Average body length 7.5 mm.

Teladorsagia circumcincta trifurcata

Copulatory bursa ray pattern as in *T. c. circumcincta*. Accessory bursal membrane sclerotized. Spicules stout, with an average length of 223.3 µm ending with two sharp spurs given off medially just behind the middle and with a large knobbed tip. Average body length 7.2 mm.

Discussion

T. retortaeformis, *G. strigosum*, *Trichuris* sp. and *C. pectinata* are common parasites of lagomorphs and have been reported in Europe both in *L. europeus* (Canestri Trotti *et al*., 1988; Poglayen *et al*., 2002; Bordes *et al*., 2007; Alzaga *et al*., 2009; Dubinský *et al*., 2010) and in *O. cuniculus* (Giannetto *et al*., 1998; Sorgi *et al*., 2004; Eira *et al*., 2007). High frequence and abundance of *T. retortaeformis* and *G. strigosum* are usually reported in *L. europaeus* and the present paper indicates their importance also in *L. corsicanus*. On the contrary, the absence of *Passalurus ambiguus*, usually dominant in European brown hare biocoenosis, is noteworthy. Although the relatively small number of examined Italian hares, the presence of this parasite in *O. cuniculus* in Sicily (Giannetto *et al*., 1998; Sorgi *et al*., 2004) and in *L. europaeus* in continental Italy (Canestri Trotti *et al*., 1988; Poglayen *et al*., 2002) suggests an important role of host specificity in explaining its absence in *L. corsicanus*. However, we would like to point out that parasitological data regarding European hares from Central and Southern Italy are lacking.

The frequent observation of *Trichuris* spp. in *L. europaeus* and the very occasional report in *O. cuniculus* could explain its presence in mainland *L. corsicanus* only, where this species lives in sympatry with *L. europaeus*; the absence of *Trichuris* spp. in Italian hare in Sicily suggests that this host probably does not play the role of reservoir species for this nematode.

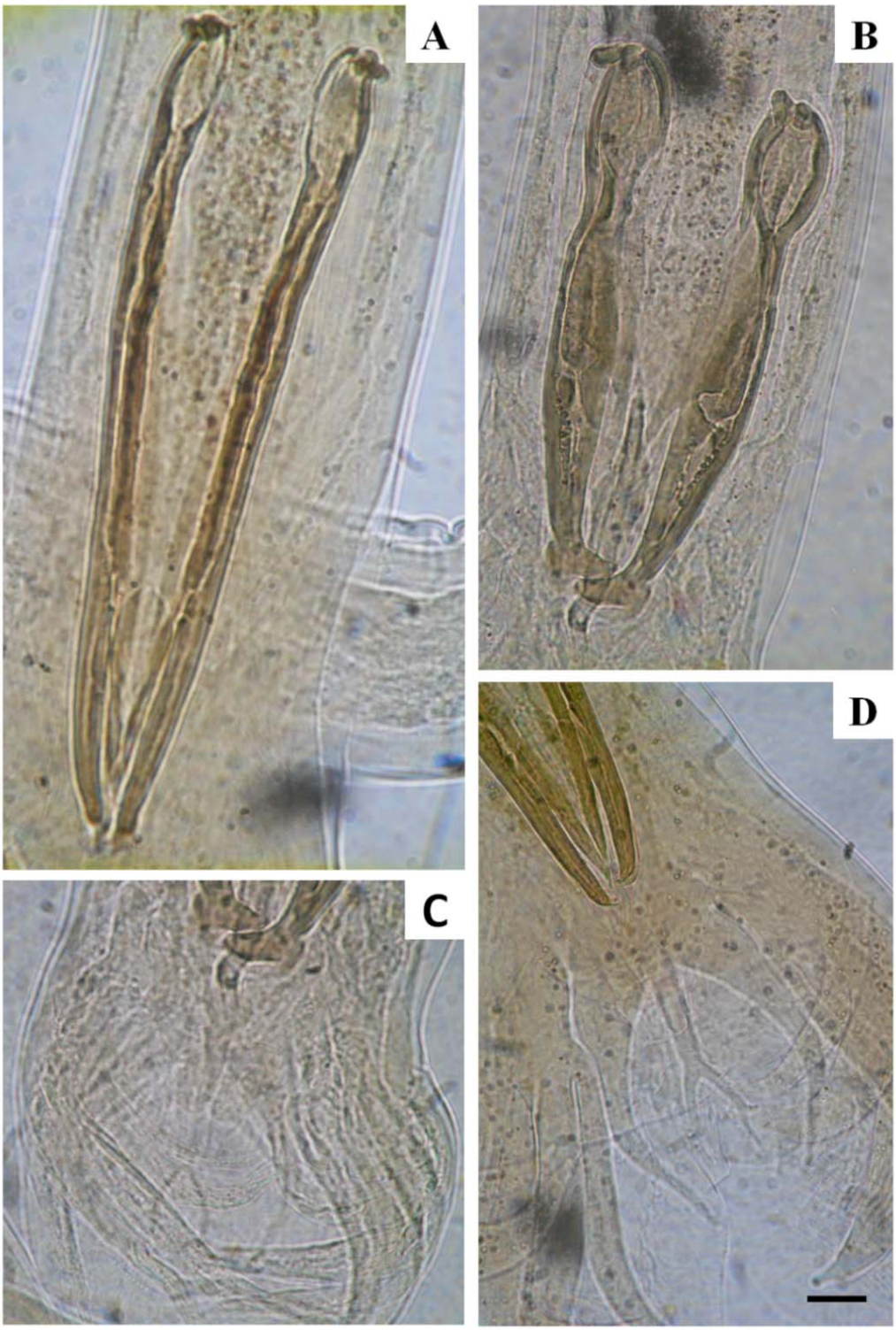

Fig. 2. *Teladorsagia circumcincta*. A spicules and D copulatory bursa of *Teladoragia c. circumcincta*; B spicules and D copulatory bursa of *Teladorsagia c. trifurcata*. Scale-bar: 20 µm.

Atypical parasites

Paranoplocephala (*sensu lato*) is a heterogeneous group of Anoplocephaline cestodes parasitising rodents. The detection of the genus *Paranoplocephala* in lagomorphs should be considered as the first report. In fact, despite *Paranoplocephala wimerosa* had been described from hares and rabbits in Europe, and it was for a long time considered the only *Paranoplocephala* species recorded from lagomorphs (Stunkard, 1941), this species was removed from *Paranoplocephala* genus since 1976 (Haukisalmi, 2009).

Paranoplocephala is morphologically characterized by a partly or completely reticulate (early) uterus and by testes

distributed in the antiporal or antiporal and anterior parts of the segment (Tenora *et al*., 1986; Haukisalmi & Henttonen, 2003). In our specimens it was not possible to appreciate the uterus but, conversely to previous assumption and according to Haukisalmi and Henttonen (2003), the uterus is not to be considered the main determinant for anoplocephaline systematic. Indeed, the following additional criteria, suggested by Haukisalmi and Wickström (2005), have been used to assign the species to the genus *Paranoplcephala* and to differentiate it from the similar genus *Andrya*, typical of leporids: i) the vitellarium of *Andrya* is positioned posterior to the ovary, whereas in *Paranoplocephala* spp. the vitellarium substantially overlaps the posterior part of the ovary, ii) the asymmetrically bilobed vitellarium is also characteristic of *Paranoplocephala* spp. as opposed to *Andrya*'s vitellarium that is more irregular and usually not distinctly bilobed, iii) in addition, the genital pores of *Andrya* are irregularly alternating (Haukisalmi & Wickström, 2005).

Finally, the sterility detected in our specimens is an additional feature described only in *Paranoplocephala* genus, in particular in *P. macrocephala*, *P. kalelai*, *P. omphalodes*, *P. microti* and *P. feodorovi*, which are included in the *Paranoplocephala sensu stricto* group (Haukisalmi & Henttonen, 2003). Consistently with our observations, sterility is typically accompanied by pronounced morphological changes, especially in terminal segments which are strongly elongate (Haukisalmi & Henttonen, 2003). It is not known whether sterility is a permanent or transient feature while, according to Haukisalmi and Henttonen (2003) it is probably related nor to the incompatibility with a particular host *taxon* or to the intensity of infection. Therefore, the sterility of the described specimens does not exclude the adaptation of the parasite to *L. corsicanus* host species.

This is the first record of natural *T. circumcincta* infection in lerporids. The identification was based upon morphologycal characters typical of this species, differing from the other Trichostrongylidae, in particular those reported in natural infections in leporids and specifically from *Obeliscoides cuniculi*. This latter differs from our specimens in the morphology and length of the spicules, in the absence of the *gubernaculum* and in the shape of dorsal ray and dorsal raylets (Graybill, 1923; Erickson, 1944). Moreover, the italian geographical distribution of *O. cuniculi* is restricted to Northern regions, having been reported for the first time in Piedmont in *Sylvilagus floridanus* (whose import has introduced this parasite) by Tizzani *et al*. in 2002 and later in sympatric *L. europaeus* (Meneguz & Tizzani, 2004).

T.c. circumcincta and *T. c. trifurcata* have been definitely recognized as morphotypes of the same species (Leignel *et al*., 2002) that always occurs in host population in more or less fixed proportion with predominant *T. c. circumcincta* (Craig *et al*., 2010). They are typical parasites of ruminants and have never been found in naturally infected lagomorphs. The natural acquisition of ruminant digestive tract nematode species is possible but not frequent in rabbits and hares. For example, Saulai and Cabaret (1998) reported typical ruminant parasites, namely *Trichostrongylus colubriformis* and *Trichostrongylus capricola*, in *O. cuniculus* and *Lepus capensis* respectively. However these infections were sporadic notwithstanding the high density of ruminant species. In Italy, ruminant parasites - *Trichostrongylus vitrinus*, *T. colubriformis*, *Trichostrongylus axei* and *Ostertagia ostertagi* - have been reported in *O. cuniculus* in Sicily (Giannetto *et al.*, 1998) while in Sardinia *T. colubriformis* and *T vitrinus* were formerly reported from wild rabbit (Lai & Arru, 1969). As regards *T. circumcincta*, experimental infections of lagomorphs with cultured larvae have been proved difficult but achievable in *L. europaeus* and not in *O. cuniculus* (Stott *et al.*, 2009). The susceptibility of *L. corsicanus* to *T. circumcincta* is probably peculiar of this host, as this parasite, widespread in both wild and domestic ruminants in Northern Italy (Capelli *et al*., 1998; Zaffaroni *et al.*, 2000; Stancampiano & Battelli, 2002; Citterio *et al.*, 2006) has never been recorded in *L. europaeus* from Northern italian areas (Canestri Trotti *et al*., 1988; Poglayen *et al*., 2002).

Regarding the role of parasites in Italian hare populations, further analysis of available data, with special attention on parasite biocoenosis and host-parasite relationship, are going to be performed.

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