Published by Associazione Teriologica Italiana
Hystrix, the Italian Journal of Mammalogy
Volume 33 (1): 98–100, 2022
OPEN ACCESS
Available online at:
http://www.italian-journal-of-mammalogy.it
doi:10.4404/hystrix-00507-2021

Short Note

Intestinal helminths of the endemic Italian hare, *Lepus corsicanus* (De Winton, 1898), in Sicily (Italy)

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Keywords:
intestinal parasites
Sicily
Lepus corsicanus
Trichostrongylus retortaeformis
Anoplocephalidae

Article history:
Received: 23 November 2021
Accepted: 25 March 2022

Acknowledgements
We are very grateful to Dr. Valeria Sergi and Dr. Erica Romi.

Abstract

The Italian hare (*Lepus corsicanus* De Winton, 1898) is endemic to central and southern Italy, where it lives in sympatry with *Lepus europaeus* in the mainland, while in Sicily *L. europaeus* is absent. The only report of endoparasites in *L. corsicanus* in Italy dates back to 2012. After a period of protection, the population density of the Italian hare in Sicily increased. This enabled new parasitological data to be collected on 27 hares which were compared with the data collected on 15 Sicilian hares from a previous study. *Trichostrongylus retortaeformis*, *Paranoplocephala* sp. and *Cittotaenia* (*Mosgovoyia*) sp. were isolated from the intestine. *T. retortaeformis* was the most prevalent and abundant parasite. Its abundance was significantly higher in males than in females. No age differences emerged. *T. retortaeformis* abundance was not related to low body mass and was significantly higher than that calculated from the data of the previous survey. At the same time aggregation, known as a regulatory factor of host-parasite relationship, was also higher in the present study. The possible biases introduced by sampling with different methods prevent any definitive conclusions. However, the relationship between *L. corsicanus* and its intestinal parasite biocoenosis appears to be stable and has possibly improved, in line with the improved status of the host population.

The Italian hare, also known as the Apennine hare (*Lepus corsicanus* De Winton, 1898), was formerly considered a subspecies of the European brown hare (*Lepus europaeus* Pallas, 1778), but it is actually a well-diverged species, endemic to central and southern Italy (Amori and Castiglia, 2018; Pierpaoli et al., 1999). While on the mainland the habitat of the Italian hare is fragmented, in Sicily, where there are no European hares, the Italian hare is quite widespread Lo Valvo (2007); Trocchi and Riga (2007, 2001) and its population density has been steadily growing from 1997 (2.09 hare\ha) to 2021 (5.54 hare\ha) (Trocchi et al., 2021; Trocchi and Riga, 2005). The only report of endoparasites in *L. corsicanus* in Italy dates back to Usai et al. (2012), who described gastro-intestinal helminths from Italian hares collected in 1997–2008 by the Italian National Institute for Wildlife. After a period of protection of the species, hunting has been allowed in some areas on an experimental basis (Anonymous, 2017) which enabled the parasitological data to be updated between 2017–2018. The aim of the present paper is to describe intestinal helminth parasites of *L. corsicanus* collected in Sicily in 2017–2018 and to compare them with the relevant raw data also collected in Sicily by Usai et al. (2012) in 1997–2008. The intestinal tracts of 27 legally hunted *Lepus corsicanus* (18 females and 9 males) were collected in Sicily. The age class was assigned by observing the Stroh sign (Stroh, 1931). The intestinal content was processed with the sedimentation and counting technique according to Hofer et al. (2000). The helminths were identified according to taxonomic keys and specific literature (Haukisalmi, 2009; Haukisalmi and Wickström, 2005; Haukisalmi and Henttonen, 2003; Audebert et al., 2000; Tenora et al., 1986; Durette-Desset and Chabaud, 1977; Levine, 1968; Yamaguti, 1961), and the same procedure as Usai et al. (2012).

Table 1 – Main features (sex, age and weight) of the Italian hares in the present survey (2017–2018) compared with previous data from Usai et al. (2012). Sex was known for 35/42 hares; age was known for 38/42 hares.

	n	Male% (n=35)	Adult% (n=38)	Mean weight (kg) (n=20)
2017–2018	27	34.8	75.0	2.81
Usai et al. (2012)	15	53.3	86.7	-
Total	42	42.1	80.0	2.81

For the comparison, the raw data collected by Usai et al. (2012) on the intestinal helminth community of 15 hares from Sicily (7 females and 8 males) were considered. Stata 12.1 was used for statistical analysis.

Table 1 reports the main features of the examined Italian hares. The sex and age of the hares did not differ from those of Usai et al. (2012) (Fisher's exact test: p 0.324 and 0.672, respectively). A comparison of the data was thus carried out despite the limits due to different sampling procedures, due to the protection of the species during the study by Usai et al. (2012) which only allowed convenience sampling.

Table 2 reports the prevalence of intestinal helminths detected and the comparison with Usai et al. (2012). No prevalence significantly differed between the two sets of data. The presence of the atypical cestodes *Paranoplocephala* spp., usually parasitizing rodents, was confirmed, suggesting a stable relationship between *L. corsicanus* and this parasite taxon (Usai et al., 2012).

The abundances and 95% confidence intervals (CIs) of isolated helminths were 0.07 (CI: 0–22.4) for *Cittotaenia* (*Mosgovoyia*) sp., 0.26 (CI: 0–65.7) for *Paranoplocephala* sp., 42.0 (CI: 18.0–66.0) for Trichostrongylidae and 17.1 (CI: 6.92–27.3) for *Trichostrongylus re-*

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Table 2 – Prevalence (%) and 95% confidence interval (in brackets) of intestinal helminths isolated from Italian hares in the present survey (2017–2018) compared with previous data from Usai et al. (2012). The *p*-values refer to the comparison between the two surveys using Fisher's exact test; n.c.= not computable.

	2017–2018 (n=27)	Usai et al. (2012) (n=15)	*p*-value
Cittotaenia (Mosgovoyia) sp.	3.7 (0–11.2)	0 (n.c.)	1
Paranoplocephala sp.	7.4 (0–17.8)	6.7 (0–20.1)	1
Trichostrongylidae (total)	66.7 (48.0–85.3)	93 (79.9–100)	0.068
T. retortaeformis (Males)	66.7 (48.0–85.3)	66.7 (41.2–92.1)	1

Table 3 – Results of the negative binomial regression evaluating the relationship between abundance of *T. retortaeformis* males and weight, age and sex of the Italian hares (n=17) examined in the present survey (2017–2018).

T. retortaeformis males		Coefficient	95% CI	*p*-value
Constant		-8.09	-17.92–1.75	0.107
Sex	Female	Ref		
	Male	1.99	0.49–3.49	0.009
Age	Young	Ref		
	Adult	0.89	-0.62–2.41	0.245
Weight		3.20	-0.05–6.45	0.054

Table 4 – Results of the negative binomial regression comparing the abundance of *T. retortaeformis* males recovered from Italian hares examined in the present survey (2017–2018) with the data from Usai et al. (2012) (variable "period"), taking into account both the age and the sex of the hosts (n=33).

T. retortaeformis males		Coefficient	95% CI	*p*-value
Constant		-0.39	-1.78–1.00	0.583
Sex	Female	Ref		
	Male	1.090	0.09–2.09	0.033
Age	Young	Ref		
	Adult	0.668	-0.68–2.02	0.332
Period	Usai et al. (2012)	Ref		
	2017–2018	2.257	1.25–3.26	0.000

tortaeformis males. The abundances from Usai et al. (2012) were 0.0 (CI: not computable), 0.13 (CI: 0–0.40), 8.2 (CI: 1.56–14.84), 2.4 (CI: 0.68–4.12), respectively. *Trichostrongylus retortaeformis* was the only parasite species whose abundance was sufficient for statistical analyses. The analyses were performed considering only *T. retortaeformis* males, as females are impossible to differentiate from other species of the family Trichostrongylidae. *T. retortaeformis* abundance was not related to hare age (Tab. 3), while previous studies on *Lepus europaeus* reported that the chances of infection were higher for adults than juveniles (Sergi et al., 2018; Stancampiano et al., 2016). However, in our sample there were very few young hares, and it is therefore not possible to exclude undetected age-related differences.

The abundance was significantly higher in males than females (Tab. 3). In several mammalian hosts, a higher parasite burden has been observed in males, and it is usually attributed to ecological or physiological differences (Poulin, 1996; Zuk and McKean, 1996). In Italian hares, ecological differences that may explain a sex-biased parasite load are related to different spatial behaviour. In fact, given that Appennine hare males have a greater home range overlap than females (Lovari et al., 2020), they have potentially more opportunities to contract the infection. Although the relationship between *T. retortaeformis* abundance and hare weight was not statistically significant (p=0.054; Tab. 3), there was a tendency for a positive association. Despite the common assumption regarding the negative consequences of parasite infection for the host condition, null associations or counterintuitive relationships are often recorded, especially in wildlife hosts (Sánchez et al., 2018). Nematode abundance did not seem to affect the general conditions of the Italian hare, similarly to findings by Sergi et al. (2018) in *Lepus europaeus* in Italy, and Ball et al. (2021) in Irish hares. This assumption appears to be confirmed by Stancampiano et al. (2016), who found a positive relationship between the abundance of *T. retortaeformis* and the weight of *Lepus europaeus*. In addition, Allan et al. (1999) found a positive correlation between increasing wild rabbit weight and *Graphidium strigosum* burden. Table 4 compares our data with the data collected by Usai et al. (2012) in Sicily and highlights that the abundance of *T. retortaeformis* males was significantly greater in 2017–2018, consistently with the confidence intervals previously reported.

A negative binomial regression model with constant terms only was built to evaluate whether the parasite distribution was consistent with an aggregated distribution. The k parameter, negatively related to parasite aggregation, was estimated to be 0.31 (95% CI: 0.17–0.55) and the distribution was significantly different from a Poisson distribution (p<0.001). The k parameter estimated using raw data from Usai et al. (2012) also showed aggregation, but at a slightly lower level (0.69, 95% CI: 0.24–1.96). Aggregation is known as a regulatory factor of host-parasite relationship, balancing the increase in parasite abundance and therefore helping to stabilize host-parasite interaction (Anderson and May, 1978). Some authors have observed a decrease in aggregation of *T. retortaeformis* in the mountain hare *Lepus timidus* with an apparent destabilizing effect on the dynamics of the host population (Newey et al., 2005). In contrast, we observed an increased aggregation, which possibly mitigates the increase in parasite abundance stabilizing the host-parasite interaction. In conclusion, the relationship between *L. corsicanus* and its intestinal parasite biocoenosis appears stable and possibly improved, in line with the improved status of the host population. However, the limited sample size in the data from Usai et al. (2012) and the possible biases introduced by sampling with different methods, and in different periods and locations, mean that no definitive conclusions can be drawn. Therefore, we hope that it will be possible to carry out further investigations in order to support these results and improve our knowledge about the parasites of this precious and elusive host species.

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Associate Editor: C. Romeo