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# Invasive species and their parasites: eastern cottontail rabbit *Sylvilagus floridanus* and *Trichostrongylus affinis* (Graybill, 1924) from Northwestern Italy.

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**Abstract** The eastern cottontail *Sylvilagus floridanus* is a native American lagomorph. Within the genus *Sylvilagus,* the eastern cottontail is the species with the widest distribution. From 1950s, the species was introduced to several European countries. A rapid territorial expansion of the introduced eastern cottontails has been observed in many areas of Italy. The eastern cottontail has been demonstrated to play a main role as carrier of exotic parasites. To date, three nematode species, exotic in Italian ecosystems, have been reported from introduced S. *floridanus.* However, its parasite fauna biodiversity is richer in native populations of the American continent. The aim of this work was to further investigate the gastrointestinal parasites of S. *floridanus,* to evaluate the potential presence of other exotic species. During 2010, 101 hosts were examined, and three nematodes were collected from their digestive tract. Two parasite species *(Obeliscoides cuniculi, Trichostrongylus calcaratus)* were already reported in ltaly; the isolation of *Trichostrongylus affinis* is instead the first report of this nematode in Italy and in Europe as a whole. This study wants to highlight the great risks related to the introduction of allochthonous species. The impact of the invasion by alien animal species may be particularly severe for public and animal health, due to the potential introduction of new pathogens. The good number of exotic parasites found in introduced eastern cottontails, together with the few sanitary surveys carried out, suggests that an epidemiological survey, with specimens from multiple localities on a wider geographic range, could lead to interesting findings on parasites of native and alien lagomorphs in Europe.

**Introduction** The eastern cottontail rabbit, *Sylvilagus floridanus,* ranges from the northern part of South America (Colombia and Venezuela) to southern Canada (Saskatchewan, Manitoba, Ontario, and Quebec), and the eastern part of the USA (Chapman et al. I980). Since the 1950s, cottontails have been introduced as a game species in Europe, more precisely in France (1953), Italy (1966), Spain (1980), and Switzerland (1982). They became established only in North-western Italy (Spagnesi and Toso 1999; Andreotti et al. 2001). The increasing population trend of S. *floridanus* contrasts with declining sympatric populations of the endemic and threatened European brown hare *(Lepus europaeus)* within the same region. The decline of the brown hares has been attributed to direct ecological competition between the two lagomorphs (Vidus Rosin et al. 2010).

Herein, we postulate that gastrointestinal parasites of the invasive eastern cottontail may impair the competition with the sympatric European hare because of the spill over of these nematodes from an alien to an autochthonous, naive host species. Exotic nematodes typical of cottontail rabbits have already been found to infect local hares (Meneguz and Tizzani 2002; Tizzani et al. 2011). In this paper, we document the occurrence of the nematode *Trichostrongylus affinis* in introduced cottontails; this is a new finding in Europe and another potential threat to the conservation of *L. europaeus.*

**Materials and methods** Within the study area, an agricultural plain close to the village of Rivalta Scrivia (44°50' N, 8°48' E), Piedmont, Italy, S. *floridanus* lives at high densities (> I 00 individuals/km²), in sympatry with the European brown hare. During 2010,51 males (18 juveniles and 33 adults) and 50 females (16 juveniles and 34 adults) were acquired from animals harvested within science-based wildlife management programs for the eradication of invasive species (Resolution of the Provincial Council of Alexandria (Piedmont Region, Italy) no. 84114760, November 24, 2003). This program was managed by the government of the City of Alessandria, and it was independent from the present study. All individuals were necropsied, and each gastrointestinal tract was isolated and dissected. Intestinal contents were washed and sieved in order to remove the finest particulates. Helminths were collected with the aid of a stereoscope by examination of diluted aliquots of the intestinal content, as described by Georgi and Georgi (1990). Isolated worms were fixed in 70 % ethanol and subsequently clarified with lactophenol for morphological identification, in accordance with standard reference keys (Skrjabin et al. 1954).

**Results and discussion** Three nematode species were collected from the digestive tract of the examined cottontail rabbits. Two species *(Obeliscoides cuniculi, Trichostrongylus calcaratus)* were previously reported from S. *floridanus* in North-western Italy (Meneguz and Tizzani 2002), while a second *Trichostrongylus* species, collected from the large intestine, is a new finding for any lagomorph or other mammalian hosts in Europe.

The *Trichostrongylus* species had the following characteristics:

Male The length ranged from 5 to 7.5 mm. The spicules were equal in length, short, curved ventrally, and, at the proximal end, were provided with a thin, rounded appendage, concave on its anterior face. At the distal end, they were provided with two blunt, curved hooks on their ventral aspect. On the left spicule, one of these hooks was larger, and it formed the end of the spicule itself. The other hook was slightly behind the tip of the spicule

and more lateral than the other. Similarly to the left spicule, the right one ended with a hook. In this case, the distal hook was the smaller of the two, whereas the other hook was located considerably further forward. The spicules were 131-156 μm long. The gubcmaculum (length 74 - 86 μm, width 21-38 μm) was approximately ellipsoidal from a dorsal view, while from a lateral perspective, it had a triangular body with a narrow, curved appendage extending anteriorly from the antero-dorsal angle.

Female The maximum width was at the posterior region of the body. The anterior end was rounded; length, 8.7-9.25 mm; maximum width, 106-177 μm; and width of the proximal end, 16 μm. The anus was located 141-164 μm from the posterior end, while the vulva was located 1.6-1.7 mm from the end. Well-developed ovejectors were present.

The nematodes were identified as *T affinis* according to the original species description (Graybi11 1924, Fig. I) and Skrjabin identification key (Skrjabin et al. 1954).

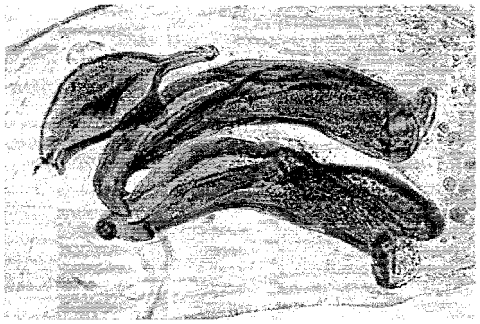

Fig. 1 Gubemaculum and spicules of *Trichostrongv1us affinis*

The prevalence of *T affinis* in our sample *(n*= 101) was 49 %, and its mean intensity was 101.6±147.4. Sex-related differences of these parameters were not found, but juvenile hosts showed a greater risk of infestation (odds ratio=5.06; *p<0.00I).*

Despite common occurrence in native populations (Jacobson et al. 1974, 1978; Andrews ct al. 1980; Lepitzki ct al. 1992), this study is the first report of *T affinis* outside the natural distribution range of its host. Although the pathologic significance of *T affinis* is unknown, the discovery of this parasite in sympatric European hares may be of conservation significance, emphasizing the risk associated with the introduction of allochthonous species and the possible spillover of pathogens to naive endemic hosts (Mack ct al. 2000; Torchin ct al. 2002).

Out of the 18 nematode species parasitizing S. *floridanus* in its natural range (Jacobson ct al. 1974, 1978; Andrews ct al. 1980; Lepitzki et al. 1992), only four have been described in the invasive cottontails in Italy (Meneguz and Tizzani 2002), with a reduction of parasite richness (Torchin ct al. 2003).

This effect can be due to (a) founder effect: introduced populations often derived from a relatively small subset of the native population; (b) host population bottlenecks after introduction may break transmission of the parasites present in the founder population; and (c) many parasites have a complex life cycle requiring more than one host or specific environmental conditions. If suitable hosts (Dobson and May 1986; Torchin et al. 2003) or environmental conditions are

not met in the new habitat, egg or larval development, or transmission cycle, may be impeded (Torchin et al. 2003).

The prevalence and intensity of *T affinis* in our survey are much lower than in the native population (Andrews et al. 1980; Lepitzki et al. 1992). This is also consistent with what has been reported for other parasites of invasive host species (Torchin et al. 2003).

This situation can potentially benefit the allochthonous species with a demographic advantage.

In Northwestem Italy, the overlap between the distribution ranges of the introduced S. *floridanus* and the native *L. europaeus* might create conditions for the transmission of *T affinis,* as of other exotic parasites previously reported (Meneguz and Tizzani 2002; Tizzani et al. 2002) in a new biocenosis. Host switching has been previously demonstrated when the nematode O. *cuniculi* was isolated for the first time from the stomach of a European brown hare (Tizzani et al. 2011). In cottontail rabbits, this nematode is associated with parasitic gastritis; it has the potential to cause similar pathology in a new host such as the European hare (Sollod et al. 1968; Jacobson and Mcginnes 1978). The impact of new parasites on a naive population of European hares is still unknown, but an apparent parasite-mediated competition impacting hare populations has been assumed (Holt 1977; Hudson and Greenman 1998). This effect might be caused not only by a direct pathogenic impact but also by sublethal effects of the alien nematodes, as described for other species (Munay et al. 1997).

This study provides another example of the potentially detrimental effects on biodiversity that may be caused by invasive species when they disturb the delicate equilibrium of a whole ecosystem (Kemledy and Guégan 1994; Mack et al. 2000; Rizzo and Garbelotto 2003). Further research is needed to evaluate if other exotic parasites

were introduced in Italy with S. *floridanus* and if more alien pathogens found a suitable host in the European brown hare. Baseline information on the health and parasitological status of leporid populations in Italy is fundamental to understand if the recent expansion of S. *floridanus* and the contemporary decreasing population trend of *L. europaeus* are the consequence of the introduction of alien parasites in a new ecosystem.

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