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First report of *Obeliscoides cuniculi* in European brown hare (*Lepus europaeus*)

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Abstract *Obeliscoides cuniculi* is a New World nematode parasite of the *Trichostrongylidae* family infecting the gastric tract of different domestic and wild Lagomorph species. This parasite is reported for the first time from a European brown hare (*Lepus europaeus*). The nematodes isolated from the gastro-intestinal tract were identified using the primary characteristics used to differentiate the species of this genus as adults morphology (length and thickness) and other characteristics of spicules, cervical papillae, cuticular ridges and striations. In the Italian study area, the European brown hare lives in sympatry with the eastern cottontail (*Sylvilagus floridanus*), species in which the nematode was previously isolated; therefore, the possibility of a cross-infestation must be considered.

The expansion of an animal or plant species out of its natural environment due to human intervention represents one of the greatest threats to bio-diversity (Simberloff et al. 2000). In some cases, the impact of the invasion by non-indigenous animal species on the environment is particularly severe for animal health, especially when mediated by the introduction of "new" pathogens (Stebbins 1992; Dobson 1996). A well-known example of this possibility is represented by the squirrel poxvirus, which played a clear role in the severe competition between the introduced invading grey squirrel (*Sciurus carolinensis*) and the native red squirrel (*Sciurus vulgaris*) in Europe (Sainsbury et al. 2008).

In Italy, one of the allochthonous invaders is the eastern cottontail (*Sylvilagus floridanus*) which was introduced illegally as game in France (1953), Italy (1966), Spain (1980) and Switzerland (1982). Northwest Italy is the only region, in Europe, where this rabbit has become naturalised (Spagnesi and Toso 1999) and is still expanding its distribution area (Spagnesi and Toso 1999; Silvano et al. 2000). In the plain and hills of this region, eastern cottontail populations have reached high densities, and in the areas of sympatry, have largely outnumbered the native European brown hare (*Lepus europaeus*) (Bertolino 2003). Presence of *S. floridanus* is an obvious matter of concern for conservation of *L. europaeus* since the potential exists for both ecological (Vidus-Rosin et al. 2010) and sanitary (Tizzani et al. 2002) competition between the two Leporids. Remarkably, four macroparasites so far exotic to the European parasitic fauna have been reported in eastern cottontails naturalised in Northwestern Italy: three are nematodes (*Obeliscoides cuniculi*, *Trichostrongylus calcaratus*, *Passalurus nonannulatus*) and one is a flea (*Euhoplopsyllus glacialis*) (Meneguz and Tizzani 2002).

In December 2009, an adult European brown hare found dead beside a road in the Alessandria Province (44°54' N; 8°37' E) was submitted to our Laboratory. It was a female in poor body condition, weighing only 952 g. The necropsy confirmed the traumatic origin of the death, probably due to collision with a vehicle. Serum was harvested and examined for EBHS (European Brown Hare Syndrome) and tularaemia, with negative results.

The digestive tract was opened longitudinally and all helminths were collected following examination of diluted aliquots of the content under a stereomicroscope (Georgi and Georgi 1990). Nematodes were fixed in 70% alcohol, then clarified in lactophenol for easier identification according to keys in Skrjabin et al. (1954). Measurements were taken of the spicules length and the reproductive tract of females was inspected for the presence of eggs. For comparison, the same measures were taken of 20 *O. cuniculi* specimens (10 males and 10 females) collected from four eastern cottontails of sympatric naturalised northwestern Italian population. These animals were part of a shooting plan made in January 2010 in the same area.

The aforementioned measures were also assumed as fitness parameters, according to Poulin (2007), that speak about life history strategies in nematodes: to a greater size of male reproductive organs and increased fertility in females corresponds a higher fitness of the parasite.

There were 59 specimens from the stomach and were red in color. Males ($N=15$) were 9.6 to 11.7 mm long and 0.2 to 0.5 mm wide, whereas females ($7V=44$) were 13.6 to 20.6 long and 0.3 to 0.5 mm wide. All were identified as adult *O. cuniculi* (Graybill 1924). In particular, body size, the length and shape of spicules, the absence of mouth cavity, the presence of neck papillae and the presence of longitudinal cuticular ridges and a fine transversal line pattern were consistent with the original

description (Graybill 1924). Male specimens had a bursa with two large lateral lobes, a small dorsal lobe and ventro-ventral rays diverging from the lateral ventral ones though reconverging at tips. Large externo-lateral rays extend separately from the other lateral ribs, which are smaller and closely approximated. The externodorsal ray, small and somewhat bent, is dorsally split into two branches, from each of which emerges a bifid tip. Spicules were $567 \pm 29 \mu\text{m}$ long (median $570 \mu\text{m}$, range $520\text{--}620 \mu\text{m}$, $N= 15$) distal end bifurcated, each bifurcation ends in a hook (Fig. 1). All females had eggs in the uterus. The vulva was located distally, in the posterior quarter of the body.

To the best of our knowledge, this is the first report of *O. cuniculi* in *L. europaeus*, and the first report of this helminth species in a native European host. In our samples we observed: (1) all females were pregnant, confirming that the hare is a competent host for the reproduction of *O. cuniculi*; (2) biometric measures of male reproductive organs (spicules) when compared with similar measurements obtained from a sample of *O. cuniculi* parasitizing *S. floridanus*, are significantly higher (Mann-Whitney *U* test $-Z= -4.09$, $p < 0.05$), suggesting that fitness of *O. cuniculi* in *L. europaeus* is not at all compromised.

Other 60 nematodes (15 males and 45 females) were collected in the small intestine. They were identified as adult *Trichostrongylus retortaeformis* (Zeder 1800), which is typical in *L. europaeus* (Canestri-Trotti et al. 1988). No nematode was found in the large intestine.

The Trichostrongylid *O. cuniculi* is known to infect the stomach of a range of tame and wild (native and allochthonous) Lagomorphs in the New World, including the snowshoe hare *Lepus americanus* (MacLulich 1937; Gibbs et al. 1977; Measures and Anderson 1983), the Black-tailed jackrabbit *Lepus californicus* (Ward 1934), the eastern cottontail *S. floridanus* (Alicata 1932; Jacobson et al. 1978; Andrews and Davidson 1980; Wiggins et al. 1980; Measures and Anderson 1983), the Marsh rabbit *Sylvilagus palustris* (Tomkins 1935; Stringer et al. 1969), the Swamp rabbit *Sylvilagus aquaticus* (Ward 1934; Smith 1940) and the tame rabbit *Oryctolagus cuniculus* (Jensen et al. 1980). Occasionally, it has been signalled as a "capture" in rodents and ungulates, namely in *Marmota monax* (Twichell 1939; Rausch and Tiner 1946; Fleming et al. 1979; Measures and Anderson 1983) and *Odocoileus virginianus* (Maples and Jordan 1966; Prestwood et al. 1973).

In Europe, *O. cuniculi* was signalled for the first time at the turn of this century, as a dominant species in naturalised eastern cottontails in Italy (Meneguz and Tizzani 2002), and this invader is the obvious origin of the infection which is object of the present note. Notwithstanding, fitness parameters suggest that *O. cuniculi* found a favourable "niche" in the stomach of the examined *L. europaeus* and apparently became adapted to the new host. More hares from northwestern Italy will need to be surveyed to confirm if the present finding is an occasional "capture" as occur in Saulai and Cabaret (1998) for parasite nematodes of ruminants or the first evidence of the ongoing adaptation of an exotic nematode to a new native host. It is worth signalling that representatives of the genus *Lepus* are amongst the usual hosts of *O. cuniculi* in the New World (see above), and that the nematode has been reported in an African hare (*Lepus capensis*) introduced in the United States in sympatry with *L. americanus* (Measures and Anderson 1983).

The larger size of spicules and body size may even be evidence of increased fitness acquired by the nematode in the change of host and therefore its possible greater evolutionary success (Combes 1997; Poulin 2007).

The consequences of possible adaptation of *O. cuniculi* to the European brown hare are unknown. Tompkins and Begon (1999) reported a reduced survival in naturally infected snowshoe hares (*L. americanus*), and pathogenic effects (haemorrhagic gastritis, reduced weight gain, anaemia and scour) were demonstrated in experimentally infected tame rabbits (Solod et al. 1968). The stomach of *L. europaeus* is, *de facto*, a sort of "empty ecological niche" (Hudson and Greenman 1998), the only exception being the presence of the nematode, *Graphidium strigosum*, in hares sympatric with wild rabbits (*O. cuniculus*) (Boag 1987; Bordes et al. 2007). Though well tolerated by the original rabbit host, *G. strigosum* is associated with severe stomach lesions in hares (Broekhuizen and Kemmers 1976). In this particular multi-host model, apparent parasite-mediated competition (Holt 1977) to the detriment of hares has been postulated (Hudson and Greenman 1998). An interesting hypothesis to be tested is that *O. cuniculi* may play a similar role, favouring the spread of the eastern cottontail in the typical European brown hare biotopes.

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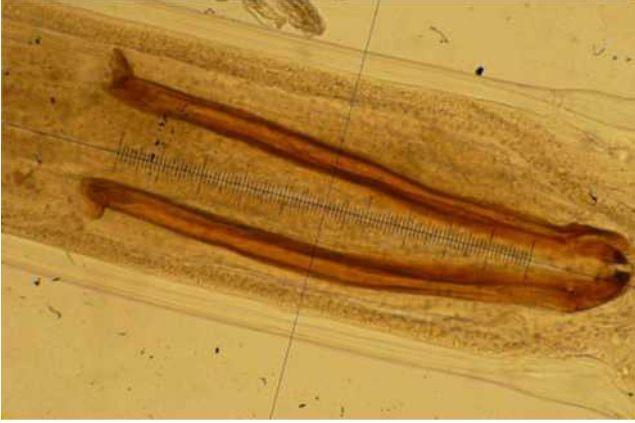


Fig. 1 Male of *Obeliscoides cuniculi*: spicules detail (x250).