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VERSITA

Natural infection of *Oryctolagus cuniculus* (Lagomorpha, Leporidae) by *Gongylonema neoplasticum* (Nematoda, Gongylonematidae) in Portugal

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Abstract

Gongylonema neoplasticum was identified in the oesophagus of 14 wild rabbits (*Oryctolagus cuniculus*) from Portugal. This is the first record of *Gongylonema neoplasticum* in a naturally infected lagomorph species in Europe. This paper presents the most relevant measurements of adult worms and some of their surface features seen by scanning electron microscopy. Epidemiological aspects of *G. neoplasticum* such as geographical distribution, host spectrum and biological features are discussed.

Key words

Gongylonema neoplasticum, Nematoda, *Oryctolagus cuniculus*, Lagomorpha, Portugal

Introduction

Although the helminth fauna of the wild rabbit (*Oryctolagus cuniculus* L., 1758) (Lagomorpha, Leporidae) and of representatives of the genus *Lepus* L., 1758 (Leporidae) have been extensively studied in the Iberian Peninsula, no species of the genus *Gongylonema* Molin, 1857 were ever detected in this group of mammals (Moreno-Montáñez *et al*. 1979, Maia *et al*. 1994, Blasco *et al*. 1996, Molina *et al*. 1999, García-Romero *et al*. 2001). However, *Gongylonema neoplasticum* (Fibiger et Ditlevsen, 1914) was recently found in the oesophagus of several wild rabbits from Portugal. This is the first time that *G. neoplasticum* has been recorded in a naturally infected lagomorph species in Europe.

Materials and methods

The study was based on the examination of 122 wild rabbits shot by hunters during game seasons between 2000 and 2004 in Dunas de Mira (Coimbra District, Portugal). Dead wild rabbits were frozen (within a maximum of 2 hr after death) until their routine helminthological analysis, which occurred directly after thawing. During analysis, *G. neoplasticum* adult specimens were collected from the oesophagus of several *O. cuniculus* and were preserved in 70% ethanol. After being mounted on slides with Amann lactophenol, 10 males and 10 gravid females were measured (all values are given in micrometres). Other specimens were processed for scanning electron microscopy (SEM) as described by Miquel *et al*. (1995) and observed using a Zeiss DSM 940 A electron microscope at 10 to 15 kV.

Results

Forty adult *G. neoplasticum* specimens were found embedded in the oesophageal mucosa and submucosa of 14 wild rabbits (12%), with a mean intensity of 2.86 (1–10) specimens. Some morphoanatomical characters of *G. neoplasticum* are shown in Figures 1 to 4.

Members of the genus *Gongylonema* are easily recognized because their cuticle is covered by large verruciform thickenings, especially prominent on the anterior part of the body

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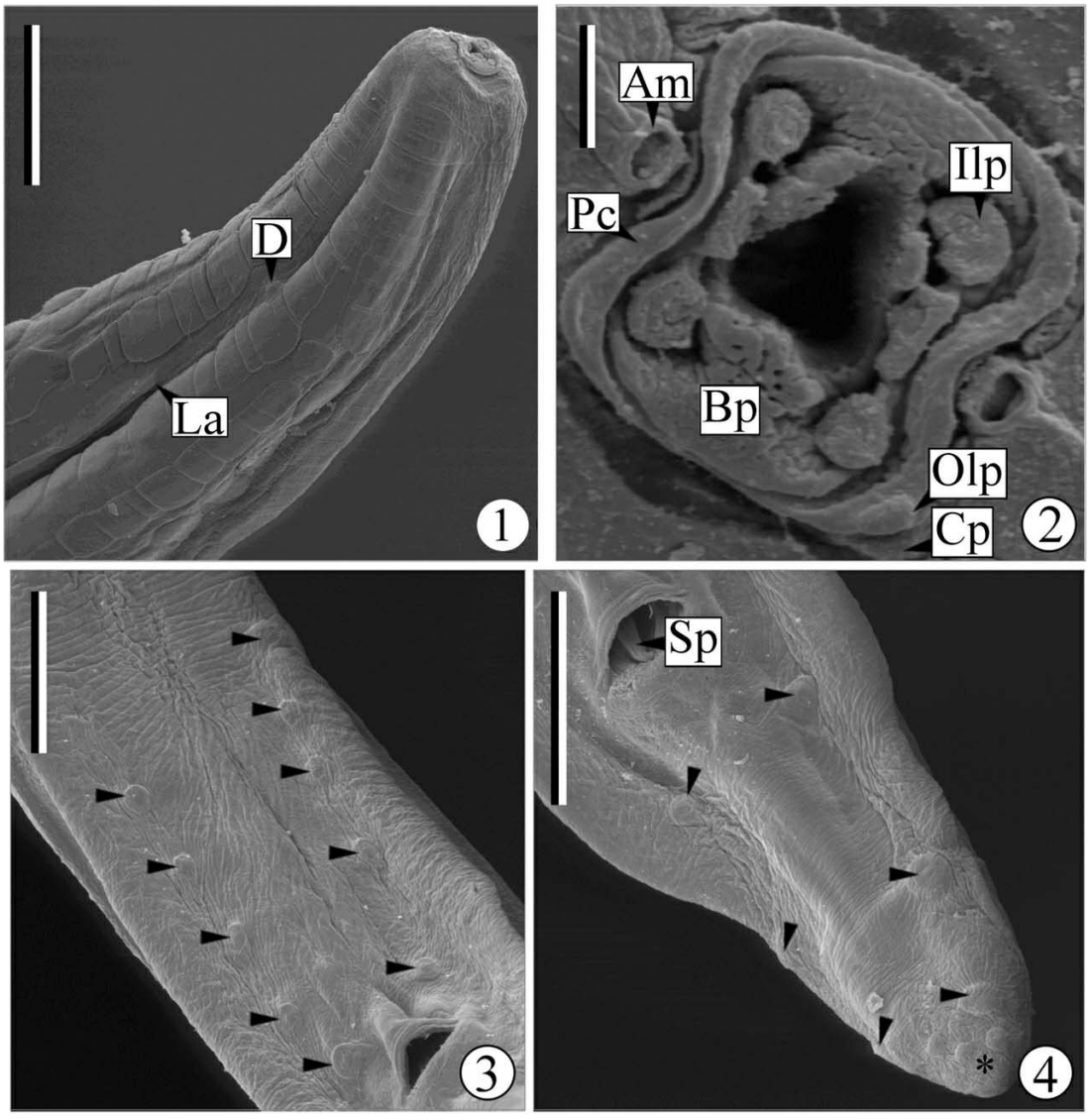
(Fig. 1). A thick peribuccal collar surrounds the triangular mouth opening (Fig. 2). The peribuccal collar presents in its internal face (1) the inner circle of labial papillae constituted by six well-developed labial papillae arranged in two groups of three papillae located laterally, and (2) two large buccal plates located in a dorsal and ventral face (Fig. 2). In the external face of the peribuccal collar there is an outer circle of labial papillae formed by four small papillae (Fig. 2). Finally, more externally, there is a circle of cephalic papillae constituted by four large papillae (Fig. 2). The outer labial papillae and the cephalic papillae are arranged in pairs. In a lateral face between these pairs of external labial and cephalic papillae there are two prominent amphids (Fig. 2). Two digitiform cervical papillae or deirids are also present (Fig. 1). Two lateral alae, each arising just behind the cervical papillae, extend throughout the body length (Fig. 1).

Males present chitinized spicules unequal in form and size, and two subsymmetrical caudal alae which are supported by 8–9 pairs of lateral papillae arranged asymmetrically, 5–6 precloacal (Fig. 3) and 3 postcloacal (Fig. 4). All lateral papillae are pedunculate except for the last pair which is composed by sessile papillae. There is one pair of phasmids and



Figs 1–4. SEM micrographs of *Gongylonema neoplasticum*: **1.** Anterior extremity showing verruciform thickenings, deirid and initial portion of lateral ala. **2.** Apical view of anterior extremity showing the oral opening. **3.** Detail of posterior extremity of a male specimen showing lateral precloacal papillae. **4.** Detail of posterior extremity of a male specimen showing lateral postcloacal papillae and posterior tip with a pair of papillae and one pair of phasmids. **Abbreviations to all figures:** Am – amphid, Bp – buccal plate, Cp – cephalic papilla, D – deirids, Ilp – inner lateral papilla, La – lateral alae, Olp – outer lateral papilla, Pc – peribuccal collar, Sp – spicule; arrows – lateral cloacal papillae, *posterior tip. Scale bars = 50 µm (Figs 1, 3 and 4); 5 µm (Fig. 2)

another pair of sessile papillae very near to the posterior end (Fig. 4).

In females, the tail is blunt and the vulva is located in the last fifth of the body. Eggs are thick-shelled and embryonated on deposition.

The most relevant measurements of adult *G. neoplasticum* specimens found in naturally infected wild rabbits from Dunas de Mira are:

Male: Body length 13,252 (11,568–16,017). Body width 29 (26–33) at cephalic extremity, 58 (54–64) at posterior end of pharynx, 181 (139–216) at posterior end of oesophagus, and 141 (126–177) at cloacal level. Deirids, nerve ring and excretory pore situated at 111 (90–141), 226 (208–255) and 366 (303–434), respectively, from anterior extremity. Pharynx length 34 (28–39). Length of muscular and glandular portions of oesophagus 453 (411–494) and 2,877 (2,673–2,993), respectively. Left spicule 556 (447–656) in length and 11 (8–13) in maximum width. Right spicule 117 (108–126) and 16 (13–18). Gubernaculum 62 (46–82) in length.

Female: Body length 43,259 (34,171–58,644). Body width 48 (39–64) at cephalic extremity, 86 (75–100) at posterior end of pharynx, 317 (298–355) at posterior end of oesophagus, and 187 (167–244) at anal level. Deirids, nerve ring and excretory pore situated at 140 (113–172), 298 (257–342) and 568 (499–687), respectively, from anterior extremity. Pharynx length 39 (28–49). Length of muscular and glandular portions of oesophagus 634 (514–733) and 4,911 (4,169–5,707), respectively. Tail length 276 (198–330). Vulva located at 7,588 (5,191–9,607) from posterior extremity corresponding to 16.9% (13.3–19.5) of body length. Eggs 53 (46–59) long and 34 (28–36) wide.

Discussion

A comparison of the measurements obtained in the present study with those from other species (see Ashour and Lewis 1986) allowed us to identify the specimens as *Gongylonema neoplasticum*. Only a small difference was detected in the vulva location from the posterior extremity (≈ 17% in our specimens vs ≈ 10% of body length).

Gongylonema species occur embedded in the mucosa and submucosa of the anterior region of the gut of birds and mammals including man. Human infections by these nematodes are, however, rare and since the first report by Leidy (1850) only around fifty cases have been reported throughout the world (Cappucci *et al*. 1982, Illescas-Gómez *et al*. 1988, Wilson *et al*. 2001, Haruki *et al*. 2005) mainly involving *G. pulchrum* Molin, 1857.

Gongylonema neoplasticum is a common parasite of wild rats (Hasegawa *et al*. 1994, Pham *et al*. 2001, among others) and has also been reported in laboratory rats (Hsu 1979). This nematode had been previously associated with stomach cancer in wormy rats, but later it was assumed that the lesions were nonmalignant and primarily caused by a vitamin A deficiency, although they were exacerbated by the worms (see Campbell 1997). This species also occurs much more sporadically in other rodents such as representatives of *Apodemus*, *Mus*, *Meriones* and *Dryomys* (see Oliveira Rodrigues and Lopes dos Santos 1984, Berdyev *et al*. 1987, Asakawa *et al*. 1998, Shimalov and Shimalov 2000).

In Spain, *G. neoplasticum* has been reported in *Rattus norvegicus*, *Apodemus sylvaticus* and *Mus musculus* (Muridae) (see Cordero del Campillo *et al*. 1994). However, infections were previously unknown in Portugal. So, the finding of *G. neoplasticum* in wild rabbits from Portugal, as mentioned above, enlarges its wild host spectrum and constitutes the first record of the nematode in Portugal. Surprisingly, this first finding has occurred in a host (*O. cuniculus*) belonging to a group of mammals (order Lagomorpha) where the only previous reports, to our knowledge, refer to *O. cuniculus* in Brazil (Vicente *et al*. 1997) and experimentally infected rabbits (Skrjabin *et al*. 1971). Therefore, although other lagomorph species are known to be naturally infected by *Gongylonema* species, such as the cottontail rabbit (*Sylvilagus floridanus*), which is host to *G. pulchrum* (see Andrews *et al*. 1980), the present finding constitutes the first report of *G. neoplasticum* in a naturally infected wild lagomorph species in Europe and suggests that *O. cuniculus* can act as a reservoir for this nematode.

Gongylonema species follow a terrestrial two-host life cycle with coprophagous insects (beetles and cockroaches) as intermediate hosts and birds and mammals as definitive hosts, which feeding on these insects acquire the infection. Larvae of *Gongylonema* species develop initially in the haemocoel of the insect but eventually most third-stage larvae become encapsulated in muscles (Anderson 2000). To date, no precise information about the life cycle of *G. neoplasticum* in the Iberian Peninsula is available and the role of the involved insects is probably different according to region. In Peninsular Malaysia, Jeffery *et al*. (2003) identified the American cockroach (*Periplaneta americana*) as an intermediate host for *G. neoplasticum*. In the Iberian Peninsula, insects were never reported as an item included in the wild rabbit diet (Gomes 2000, Martins *et al*. 2002, Alves *et al*. 2006). The infection of *O. cuniculus* by *Gongylonema* should result from the unintentional ingestion of coprophagous insects harbouring the third-stage larvae. Taking into account the relatively high prevalence of this nematode in the present study, one must emphasise that the widely reported coprophagous behaviour of the rabbit (Hirakawa 2001, among others) surely enhances the possibilities of ingestion of the intermediate host, especially considering the high number of species included in dung beetle communities associated with rabbit dung heaps (Verdú and Galante 2004).

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