



Natural infection in Pampas fox (*Lycalopex gymnocercus*) by *Lagochilascaris major* Leiper, 1910 (Nematoda: Ascarididae) in Buenos Aires, Argentina

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Abstract

Lagochilascariosis is an emerging parasitic disease limited to the American continent, caused by nematodes of the genus *Lagochilascaris*. Its life cycle is heteroxenous, involving natural definitive hosts (wild carnivores), accidental hosts (domestic carnivores and humans), and intermediate hosts (rodents). Here we report, for the first time, the occurrence of *Lagochilascaris major* in female of a Pampas fox (*Lycalopex gymnocercus*) which was found in the Mar Chiquita basin, Buenos Aires province, Argentina. At necropsy, multiple *Lagochilascaris* larvae and several adults (1 female and 5 male) were observed inserted in the mucosa of the trachea and a male was also collected in the stomach. The diagnosis was made by observing the different stages with optical microscopy, by scanning electron microscopy of adult males and coproparasitological analysis. Although cases of lagochilascariosis have been reported in wildlife in different countries of the American continent, in Argentina, cases are really rare and were only found in domestic cats.

Keywords Nematoda · *Lagochilascaris major* · Wild mammals · South America

Highlights

- The Lagochilascariosis is an emerging parasitic disease in the Americas caused by the nematode *Lagochilascaris* sp.
- There are five species and the most important ones are *L. minor* and *L. major*, described in felines and canines; *L. minor* also affects man.
- In Argentina, sporadic cases of Lagochilascariosis were reported only in domestic cats.
- Here we report the first record in a wild carnivore (*Lycalopex gymnocercus*) naturally infected with *Lagochilascaris major*.

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Introduction

Lagochilascariosis is an emerging parasitic disease in the Americas caused by the nematode *Lagochilascaris* spp. This genus belongs to the Order Ascaridida, to the Family Ascarididae and is distributed from Mexico to Argentina and the Caribbean Islands. Five species have been recognized in this genus: *Lagochilascaris minor* Leiper, 1909, *L. major* Leiper, 1910, *L. turgida* (Stossich, 1902) Travassos, 1924, *L. buckleyi* Sprent, 1971 (Sprent 1971a, b), and *L. sprenti* Bowman et al., 1983. *Lagochilascaris minor* and *L. major* are the two most commonly reported species and both are biologically similar and produce similar injuries. However *L. minor* is more invasive and pathogenic than *L. major* (Pena et al., 2002) and is the etiological agent of human lagochilascariasis in South America (Campos et al. 1992; Pa ô et al. 1999). For instance, more than 80% of human cases were found in Brazil mainly in the Amazon region near forests and rural areas (Vieira et al. 2000; Leão et al. 2005).

The life cycle of *Lagochilascaris* is heteroxenous, involving natural definitive hosts (wild carnivores), accidental hosts (domestic carnivores and humans), and intermediate hosts

(rodents). The adult forms of this nematode inhabit the first portions of the digestive and respiratory tracts of carnivores. The eggs eliminated in feces contaminate the soil and wild rodents become infested by ingesting these embryonated eggs that plays an important role in the transmission of this parasite (Campos et al. 1992; Campos and Barbosa 2016). Man and carnivores are infected by consuming *Lagochilascaris* larvae (L3 infective) that are encysted in the muscle and other tissues of wild rodents (uncooked or partially cooked meat). An auto-infecting cycle has also been reported in both humans and cats (Campos et al. 2017).

Clinical cases caused by *L. major* have been described in domestic cats in Argentina (Led et al. 1968; Sprent 1971a; Romero and Led 1985; Ribicich et al. 2009; Rubio et al. 2015), Brazil (Dell'Porto et al. 1988; Amato et al. 1990), and Uruguay (Castro et al. 2009) and in a raccoon (*Procyon lotor*) and a dog from North America, and in a Pampas fox (*Lycalopex gymnocercus*) from Uruguay (Craig et al. 1980, 1982; Capellino et al. 2003).

The Pampas fox is the most common carnivore in southern South America (Lucherini et al. 2004). It prefers grasslands, open woodlands, and areas highly modified by extensive ranching and agricultural activities (Redford and Eisenberg 1992). The Pampas fox is a generalist carnivore and its diet includes domestic and wild vertebrates, especially birds and rodents, fruits, insects, carrion, and garbage (Canel et al. 2016).

The aim of this work is to report, for the first time in Argentina, a naturally infected wild carnivore (*Lycalopex gymnocercus*) with the nematode *Lagochilascaris major*.

Materials and methods

Necropsy technique, samples collection, light, and scanning electron microscopy

A death adult female of Pampas fox of 1 year was collected (2016) during nocturnal road surveys in the Mar Chiquita Basin (37.36°–28.60° S and 57.22°–30.01° O), Buenos Aires province, Argentina. It was transported to the laboratory of Animal Health at INTA-EEA Balcarce, where the necropsy was performed and parasitological samples were taken.

The Pampas fox was examined externally, where Bruises were observed due to polytrauma caused by the road accident suffered. Subsequently, the necropsy and parasitological inspection of the internal organs were performed, where several endoparasites were found. The pharynx and trachea were opened out and the parasites observed macroscopically were collected and placed in a saline solution. Examination of the stomach and intestinal content was performed using the sedimentation and counting technique described by Scioscia et al. (2013). Obtained sediments were examined in small portions of 5–10 ml in Petri dishes with magnifier lens at $\times 65$. The

nematodes were washed in a saline solution and then fixed and stored in 4% formaldehyde until morphological examination. Two males and one female worms were cleared in lactophenol, examined microscopically, and identified following Sprent (1971a, b). For scanning electron microscopy (SEM), three males were dehydrated using a graded series of ethanols, dried by evaporation with hexamethyldisilazane, sputter-coated with gold palladium, and examined using a JEOL JSM 6460LV SEM (JEOL, Tokyo, Japan). The morphometric data are expressed in millimeters unless noted otherwise. In addition, a fecal sample of the rectum was obtained and analyzed by the Sheather technique (Benbrook and Sloss 1965).

Results

Lagochilascaris nematodes were found in the interior of the trachea up to the bifurcation (four males, one female, and numerous larvae). Also, a male adult *Lagochilascaris* was found in the stomach.

Based on morphological examination, the specimens were identified as *L. major*. There were three protruding lips, which were wider than longer (0.079–0.105 width \times 0.067–0.084 length, $n = 2$). Lips were distinctly separated from the body by a continuous postlabial groove. Behind the lips, there was a conspicuous collar from which the three interlabia projected forwards between the lips (0.056 long, $n = 1$). Denticles were absent. The dorsal lip had two double subdorsal papillae, each one of the subventral lips had one double ventrolateral papilla, one lateral papilla, and an amphidial pore. The interlabia were triangular and had a relatively wide base and attenuated rounded tips. The alae extended from the nerve ring for the entire length of the body. In the male ($n = 2$), the total body length was 17.29 mm (range, 17.07–17.5) and the maximum width was 0.49 mm (0.47–0.50). The length of the esophagus was 2.39 mm (2.30–2.47) and spicules were 0.85 mm. The spicules were alate with rounded tips and with funnel-shaped anterior end. The ejaculatory duct was longer than the spicules and tail, measuring 1.05 mm (1.00–1.10) and 0.17 mm, respectively. There were numerous precloacal and postcloacal papillae. In the female ($n = 1$), the total body length was 20.50 mm and the width 0.42 mm. The length of the esophagus was 2.02 mm, the vulva was located 9.75 mm from the anterior end (47.56% body length from anterior end), and the tail had a small conical tip of 0.39 mm. We found eggs in the uterus ($n = 6$, length 54.67–61.35 μm , range 45–65.75 \times 50–71.01).

In the scanning electron microscope, three lips were separated from the body by a deep notch in the adult males. The anterior edge of each lip was divided into two prolongations that closed in a central hole. We observed a double ventrolateral papilla on one of the ventral lips and a lateral papilla next to an amphid. Projecting between the lips, three elongated

interlabia were present. Behind the notch, the cuticle formed a flange and continued striated throughout the body. Anterior to the cloaca were present a median double papilla and up to 40 pairs of precloacal papillae arranged in two rows. There was also a double papilla on each side behind the cloaca, and four postcloacal papillae: two subdorsals (fused) and two subventrals with the phasmid between (Fig. 1a–d).

In the coprological analysis, spherical or oval eggs were observed of 70.35×74.95 (range, $65.75\text{--}76.27 \times 68.38\text{--}84.16$; $n = 4$) with a prominent reticulate pattern of ridges forming pits on the surface ($n = 35\text{--}40$ pits); inside was a blastomere surrounded by a clear space (Fig. 2).

Discussion

Lagochilascaris minor and *L. major* are very similar species, however in view of their widely different hosts and distribution, specific differentiation between these species is possible (Sprent 1971a, b). There are slight morphological differences between these two species and the most distinct ones are the shape of the interlabia and the size and number of pits on the eggs (Sprent 1971b). The identification of *L. major* found in trachea and stomach of *L. gymnocercus* has been made based on the general morphology of adults and in the number of pits on the eggs (Sprent 1971a). The measurements of the males and female are within the range recorded from naturally infected cats (Sprent 1971a; Romero and Led 1985; Ribicich et al. 2009) and also from experimental infected cats (Pena et al. 2002).

In Argentina, clinical cases of lagochilascariasis have been reported only in domestic cats (e.g., Spampinato et al. 2004; Cosentino 2017) and species identification (*L. major*) was only achieved in some cases (Led et al. 1968; Sprent 1971b; Romero and Led 1985; Ribicich et al. 2009; Rubio et al. 2015).

Fig. 1 Adult male of *Lagochilascaris major* with scanning electron microscope. **a** Anterior body (side view). **b–d** Posterior body (**b** view latero ventral; **c** latero ventral view; **d** ventral view). L lip, il interlabia, ao anal opening, Prc.p precloacal papillae, Poc.p postcloacal papillae, dpp double postcloacal papilla, Svp subventral papillae

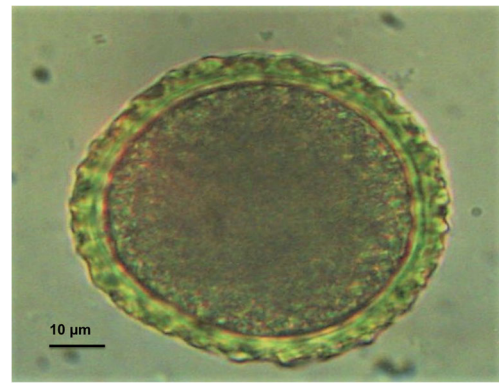
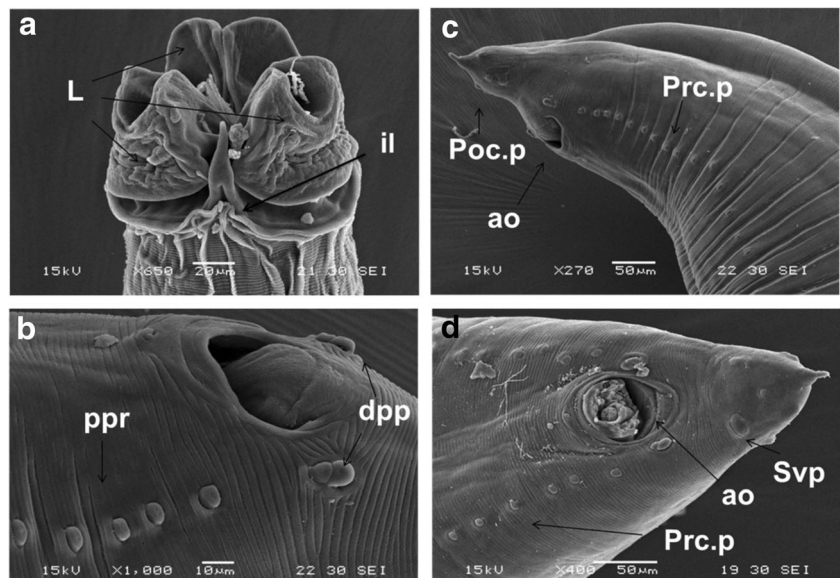


Fig. 2 *Lagochilascaris major* egg obtained by flotation technique of fecal sample from *Lycalopex gymnocercus*. $\times 40$

Recently, Vizcaychipi et al. (2016) found *Lagochilascaris* spp. eggs in bush dog feces in the province of Misiones. Our data confirmed that *L. gymnocercus* could be considered a definitive host for *L. major* in southern of Buenos Aires province, Argentina. Therefore, this is the first report of *L. major* in a wild carnivore from Argentina. The source of infection to the Pampas fox is unknown. However, since species of rodents are included in their feeding habits (Canel et al. 2016); it is probable that some of their prey were infected with this nematode. The results presented in this article matches with other studies of lagochilascariasis, which reported that the main source of infection on humans and animals is generally through consumption of wild rodents (Campos et al. 1992; Pa ô et al. 1999; Faccio et al. 2013).

So far, no human infection by *L. major* has been reported, but this cannot be excluded because of the apparently close relationship between the other species of this genus (Craig et al. 1980). Lagochilascariasis is mostly a chronic human disease that persists for several years, in which the parasite burrows into the subcutaneous tissues of the neck, paranasal

sinuses, and mastoid. Other localizations of the parasite are the central nervous system, lungs, sacral region, eyeballs, and dental alveoli (Campos et al. 2017). It is important to highlight the report of *Lagochilascaris* eggs in a public park in southern Brazil (Moura et al. 2012). Therefore, it should be included in the differential diagnosis of granulomatous diseases in both veterinary and human medicine in the Neotropical region of Latin America (Rubio et al. 2015). Furthermore, an adequate identification of the *Lagochilascaris* species is necessary, whether in domestic and wild animals as in humans. This would allow us to know more about the hosts involved, the geographic range, and the ecologic niche of each species of *Lagochilascaris*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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