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Advances in Knowledge of Wild Toxocariasis in Patagonia (Argentina): *Toxocara canis*

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ABSTRACT: Seventeen specimens of carnivores of the families Felidae (*Puma concolor* and *Leopardus geoffroyi*) and Canidae (*Lycalopex culpaeus*) were collected in different localities of Nahuel Huapi National Park (Argentina) from August 2005 to May 2018. The specimens were processed by necropsy, and ascaridid parasites were obtained only from *L. culpaeus*. Morphological analysis indicates all the specimens belong to *Toxocara canis*, and specific identification was confirmed by PCR procedure. This study represents the first molecular identification of *T. canis* from Andean foxes in Argentina, and provides information on the spatial distribution of *T. canis* in wild environments in Patagonia.

KEY WORDS: carnivores, Andean fox, *Lycalopex culpaeus*, Canidae, Patagonia, ITS, PCR, *Toxocara canis*, toxocariasis, wildlife, zoonoses.

Toxocara canis (Werner 1782) is one of the most common parasites of dogs, and has also been reported in different wild mammals in countries all over the world (Wapenaar et al., 2013; Duscher et al., 2015; Fang et al., 2015; Mackenstedt et al., 2015) including Arctic latitudes (Myšková et al., 2019). This ascaridid species is characterized by its high fecundity, the resistance of its eggs to soil environmental conditions, and the survival of its larvae in tissues of paratenic and definitive hosts. It has 4 transmission routes: horizontally, through accidental ingestion of embryonated eggs found in soils, vegetables, fruits, and dog fur, or by ingestion of paratenic hosts that harbor larvae in their tissues; and vertically, by a transplacental or transmammary route (Okulewicz et al., 2012; Antolová et al., 2013; Holland, 2015; Sierra et al., 2015).

In South America, *T. canis* has been cited for *Procyon cancrivorus* (crab-eating raccoon), *Nasua nasua* (South American coati), *Leopardus pardalis* (ocelot), *Cerdocyon thous* (crab-eating fox), and *Puma concolor* (cougar) in Brazil (Vieira et al., 2008); and *Leopardus jacobita* (Andean mountain cat), *Lycalopex culpaeus* (Andean fox), *Lycalopex griseus* (Chilla fox), and *Lycalopex fulvipes* (Darwin fox) in Chile (Jiménez et al., 2012; Oyarzún-Ruiz et al., 2020). In Argentina, *T. canis* was identified morphologically for *L. gymnocercus*, *L. culpaeus*, *P. cancrivorus*, *Chrysocyon brachyurus* (aguará guazú), *Speothos venaticus* (bush dog), and *Oncifelis geoffroyi* (Geoffroy's cat) (Martínez, 1987; Suárez et al., 1990; Rodríguez Camon et al., 2012; Moleón et al., 2015; Vizcaychipi et al., 2016).

Considering the public health importance of toxocariasis and the current gaps in knowledge of its epidemiology, the aim of this work was to identify the presence of *T. canis* in wild carnivores from northwestern Argentinean Patagonia, through morphological and molecular studies.

MATERIALS AND METHODS

All wild hosts were found dead in different localities of Nahuel Huapi National Park, within Neuquén and Río Negro Provinces (Fig. 1). This is a protected area with numerous glacial lakes bordered by deciduous and perennial forests to the west, and steppe to the east.

Seventeen specimens belonging to the families Felidae and Canidae were collected between August 2005 and May 2018 (Table 1) and kept at −20°C until they were processed and necropsies were performed. Digestive tubes were removed from the carcasses of 1 *P. concolor*, 7 *L. geoffroyi*, and 9 *L. culpaeus*; ascaridid specimens obtained from the stomachs and large intestines were counted and classified by developmental stage and sex (Table 2). The ascaridid specimens were fixed in 5% formalin and cleared with lactophenol

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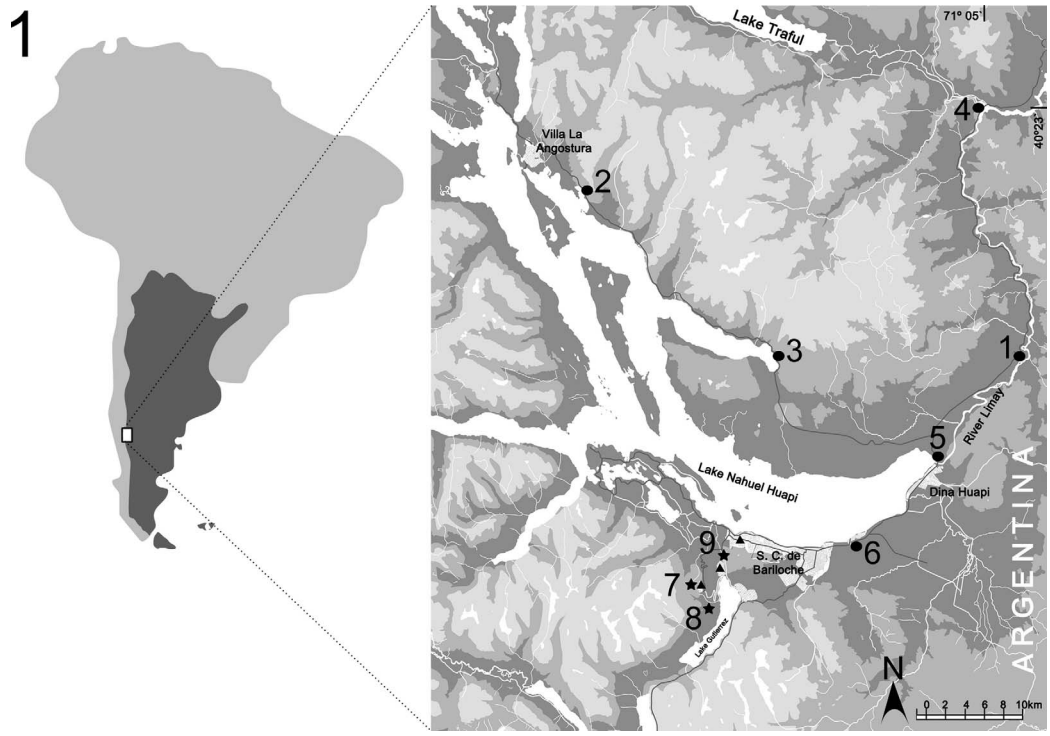

Figure 1. Collection sites for *Lycalopex culpaeus* in Patagonia. Star points: positive specimens for *Toxocara canis.* Triangle points: villages near the collection points of *L. culpaeus* testing positive for *T. canis*. Locality numbers indicated on map: 1. Anfiteatro, Route 237, Dpt. Los Lagos. 2. Route 40, North Dpt., Los Lagos. 3. Quintriqueo. 4. Confluencia, Traful. 5. Secc. Limay, Route 40 (Neuquén province). 6. Las Chacras. 7. Cerro Catedral. 8. Cerro Frey mountain track. 9. Route 82 (Río Negro province).

Table 1. Collection data of Carnivora specimens collected in Nahuel Huapi National Park.*

Family	Specific name	Vernacular name	Date	Sex	Locality and province	Coordinates
Canidae	*Lycalopex culpaeus*	Andean fox	Aug 2012	nr	Anfiteatro Route 237 Dpt. Los Lagos (N)	40°59′S–70°05′W
			Jan 2014	F	Route 40 North Dpt. Los Lagos (N)	40°48′S–71°35′W
			Oct 2015	M	Quintriqueo (N)	40°57′S–71°19′W
			Feb 2016	M	Confluencia Traful (N)	40°43′S–71°05′W
			Jan 2018	F	Secc. Limay, Route 40 (N)	41°03′S–71°09′W
			Mar 2018	M	Las Chacras (RN)	41°06′S–71°12′W
			Apr 2018	F	Catedral Mountain (RN)	41°10′S–71°26′W
			May 2018	Juvenile	Cerro Frey Mountain Path (RN)	41°10′S–71°26′W
			May 2018	M	Route 82 (RN)	41°07′S–71°24′W
Felidae	*Puma concolor*	Cougar	Sep 2010	F	Lake Falkner (N)	40°26′S–71°32′W
	Leopardus geoffroyi	Geoffroy's cat	Aug 2005	M	Las Chacras (RN)	41°06′S–71°12′W
			Oct 2009	M	Mascardi Village (RN)	41°20′S–71°30′W
			Oct 2011	M	Villegas Ranch (RN)	41°31′S–71°27′W
			Aug 2012	M	Bariloche Airport (RN)	41°08′S–71°10′W
			Sep 2012	F	Lake Mascardi (RN)	41°19′S–71°29′W
			May 2013	M	Villegas River (RN)	41°35′S–71°29′W
			May 2013	F	Lakes Mascardi-Gutiérrez Watershed (RN)	41°15′S–71°28′W

*(N) Neuquén province; (RN) Río Negro province; F, female; M, male; nr, not recorded.

Table 2. Content of the digestive tracts and presence of *Toxocara canis* in Carnivora species from Argentinean Patagonia.*

Collection number	Specimens of Carnivora	Date	Contents of digestive tract	*Toxocara canis*			GenBank number
				Number of worms	Stage	Site of infection	
1	*Lycalopex culpaeus*	Aug 2012	nr	Negative			
2		Jan 2014	Fishes, lizard, bones, hair, Nematoceran larvae, leaves	Negative			
3		Oct 2015	nr	Negative			
4		Feb 2016	nr	Negative			
5		Jan 2018	Mice, hair, bones, remains of arthropods	Negative			
6		Mar 2018	Stomach empty	Negative			
7		Apr 2018	Hair, human food	1F 1M	Adults	Large intestine	MK071703
8		May 2018	Stomach empty	6F 4M	Adults	Stomach—large intestine	MK071704
9		May 2018	Bird remains, Muscidae larvae	2F	Adults	Large intestine	MK071702
1	*Puma concolor*	Sep 2010	Hair	Negative			
2	*Leopardus geoffroyi*	Aug 2005	Vegetal remains, chicken feathers, hair	Negative			
3		Oct 2009	Nails, skin, and bones of small sigmodotin, insects, and scales and leg of *Liolaemus pictus*	Negative			
4		Oct 2011	Hairs, mice leg, bucal bones, bones	Negative			
5		Aug 2012	Hairs, little bones, feathers	Negative			
6		Sep 2012	Hairs	Negative			
7		May 2013	Hairs and bones of mice	Negative			
8		May 2013	Feathers, little bones, hairs	Negative			

*F, female; M, male; nr, not recorded.

for taxonomic identification (Anderson et al., 2009), whereas other worms were fixed in 96% alcohol for molecular identification.

A 3.0-mm-long piece from the midbody region of 3 specimens (1 from each host) was removed for DNA extraction, using the "Rapid isolation of Mammalian DNA" protocol (Sambrook and Russell, 2001).

A segment of 320 base pairs (bp) of the nuclear ribosomal DNA internal transcribed spacer 1 (ITS1) of *Toxocara* was amplified by polymerase chain reaction (PCR) using the primer pair 5′-ACGTATGC GTGAGCCG-3′ and 5′-GTGTTTTTGGTTTTTGG CG-3′ (Cardillo et al., 2016).

The PCR mix was made up to a volume of 25 μl, containing 0.5 U of *Taq* polymerase (Invitrogen), 1X Taq buffer, 1.5 mM $MgCl_2$, 0.8 μM of each dNTP, 0.25 μM of each primer, and 5 ng of parasite DNA. The cycling conditions were as follows: 94°C for 3 min, 40 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 10 min. Amplification was carried out in a Px2 Thermal Cycler/ Electron Corporation. Double-distilled water was used as a negative control. The amplified fragments were separated by electrophoresis on a 1.5% agarose gel, stained with GelRed® (Biotium) and compared to a 100-bp DNA ladder molecular weight marker (Fermentas). PCR amplification fragments of the expected size were purified from the agarose gel using an AccuPrep Gel Purification Kit (Bioneer). Sequences were determined using an ABI 3500 Genetic Analyzer (Applied Biosystems). Chromatograms were viewed with Chromas Lite 2.01 and sequences were compared with those in the GenBank database using BLASTn program (https://blast.ncbi.nlm.nih.gov). The ITS1 DNA sequences obtained were deposited in the GenBank database.

RESULTS

All nematode worms removed from the intestine of *L. culpaeus* corresponded to *T. canis* (Table 2), and the infected foxes were collected in the vicinity of Bariloche city, around the villages of Catedral, and Los Coihues (Fig. 1). A total of 14 adults were removed from 3 out of the 9 Andean foxes (Table 2).

Adult worms are cylindrical and white, anterior end curved ventrally, with cervical alae that give a spear-shape appearance. Mouth surrounded by 3 lips. Females are larger in size than males. The vulva is situated ventrally about a quarter of the body length from the anterior end. The posterior end of male has 2 rows of 25–26 pairs of precloacal papillae on each side of the tail, arranged on the ventro-lateral surface. A single precloacal median papilla is situated anterior to the cloaca. On each side of the tail there are 3 pairs of ventro-lateral papillae and 2 pairs of lateral papillae. Based on the morphological characteristics observed, we assigned the worms to *T. canis*. This diagnosis was supported by the ITS1 DNA sequences obtained from 3 of the worms, 1 from each fox. The sequences were deposited in the GenBank database under accession numbers MK071702, MK071703, and MK071704. All three ITS1 sequences were identical, and when compared to the *Toxocara* ITS1 sequences from the GenBank using BLASTn program, they showed the highest homology with *T. canis* (98%).

The remains of fish, lizards, birds, mice, arthropodan larvae, hair, and bones were found in the digestive tube contents of Carnivora specimens, along with plant tissue and human food (Table 2).

DISCUSSION

Although toxocariasis is not a notifiable disease, and in many cases is asymptomatic, it is of major importance to public health, considering that epidemiological studies show *T. canis* to be the most prevalent and ubiquitous zoonotic parasite species from the subpolar region to the tropics, with lower prevalence in industrialized countries (Macpherson, 2016). However, major knowledge gaps exist in its epidemiology, particularly related to its global distribution and prevalence; in particular, the circulation of *T. canis* in wild vertebrates (Ma et al., 2017) constitutes a challenging situation. Molecular and ecological studies are therefore required in order to generate tools for control and surveillance programs, and to contribute to wildlife preservation (Vizcaychipi et al., 2015). Our study presents the first molecular determination of *T. canis* in wild carnivores in Argentina and supports *L. culpaeus* as a definitive host for this species. This specific identification is a prerequisite for epidemiological and population studies because toxocariasis is a worldwide helminthiasis of medical and veterinary significance.

In South America, 5 species of *Lycalopex* are cited: *L. fulvipes*, *L. sechurae*, *Lycalopex vetulus* (Hoary fox), *L. gimnocercus*, and *L. culpaeus*, the last 2 of this list being distributed in Argentina (Jiménez and Novaro, 2004). In particular, *L. culpaeus* is the largest South American fox, distributed along the Andes of South America from the south of Colombia, and present also in Chile and Bolivia (Lucherini, 2016). The Andean fox is found in Argentina on the eastern slopes of the Andes from Jujuy Province in the north, reaching the Atlantic shoreline from Río Negro province and continuing southwards to Tierra del Fuego province (Jiménez and Novaro, 2004). Its nocturnal behavior may be related to prey activity patterns, considering the main prey items are cricetine rodents such as the Southern pericote (*Loxodontomys micropus*), European hare (*Lepus europeus*), and fossorial tuco-tuco (*Ctenomys* sp.); reptiles, birds, and arthropods are also registered as prey (Guzmán-Sandoval et al., 2007; Monteverde and Piudo, 2011).

In wild environments of Argentina, *T. canis* has been identified morphologically in the 2 fox species, *L. culpaeus* and *L. gimnocercus* (Suárez et al., 1990; Moleón et al., 2015). There is a strong probability that infection is present throughout the entire distribution range of both these foxes, considering the transmission routes of ascaridid species and the partially sympatric distributional range of these 2 host species.

The presence of *T. canis* has been recorded in rural dogs in Neuquén (Soriano et al., 2010) and in urban environments of all Patagonian provinces; that is, Neuquén, Río Negro, Chubut, Santa Cruz, and Tierra del Fuego (Sánchez Thevenet et al., 2003; Zanini et al., 2005; Soriano et al., 2010; Flores et al., 2017; Winter et al., 2018; Cociancic et al., 2020). We suspect continuity of transmission between urban, suburban, rural, and wild environments in this region. The current spatial continuity of this zoonosis is probably a consequence of its presence since ancient times, and its eggs have been found in coprolites of *L. culpaeus* in this region (Fugassa et al., 2018). The absence of *T. canis* in felids could be associated with the fact that they do not come close to populated areas, unlike the more daring foxes. It is not so likely, therefore, that

wild felids would be infected with this parasite, *T. cati* infection being more probable for them, which they could acquire through vertical transmission (Otranto et al., 2015).

Although the main prey items found in the dietary remains are the same as those cited by previous authors (rodents, lizards, arthropods, birds), only foxes presented remains of human food, which is another clue that the circulation of *T. canis* between wild and domestic canids would occur. Given that the majority of the foxes analyzed, even the parasitized ones, were registered in the vicinity of inhabited areas, interaction between foxes and dogs is likely to be common (Fig. 1). This situation has previously been registered in our region for another *Toxocara* species, *T. cati* (Vega et al., 2018), and also for other nematode species, *Toxascaris leonina* and *Eucoleus bohemi*, in the northeast of our province (Winter et al., 2018). This steady increase in wild animal presence in urbanized areas has also been observed in other countries, strengthening the link between parasites in wild and domestic canid populations (Otranto et al., 2015).

The multifactorial dynamics of this zoonosis is shown by the complexity of the environments (urban, suburban, rural, and wild) where it developed in Patagonian provinces of Argentina, the increase in human population density and garbage generation in urban areas, the presence of free-roaming dogs, and the urbanization of wild canids that consume the remains of human food. Integrated research including soil and dog feces samples, immunological studies in humans, and analyses of wild carnivores are necessary for clarification of the circulation of this zoonosis in Argentina.

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