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Toxocariasis in Carnivora from Argentinean Patagonia: Species molecular identification, hosts, and geographical distribution

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ABSTRACT

Twenty four specimens of seven species belonging to the families Felidae, Mustelidae, and Canidae were obtained in Lanín and Nahuel Huapi National Parks from March 1996 to April 2016. Specimens were processed by necropsy in order to contribute to the knowledge of toxocariasis in wild carnivores of Argentinean Patagonia. The only *Puma concolor* and the seven *Leopardus geoffroyi* were positive for *Toxocara cati.* Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the ITS-1 region from larval and adult DNA was carried out to confirm parasite species identification. This is the first molecular determination of *T. cati* from wild felids in Argentina and the study also fill gaps about the spatial distribution and hosts for *Toxocara cati.*

1. Introduction

Toxocara cati is distributed worldwide, especially due to human affection for domestic animals, like cats and dogs (Ma et al., 2017), however the epidemiological role of the dogs in the cycle is discussed (Fahrion et al., 2011). But there still is a relative lack of knowledge of the basic biology and public health significance of the presence of *T. cati,* so gaps and enigmas about its epidemiology remain (Holland, 2015; Ma et al., 2017).

Definitive hosts of *T. cati* are mainly felids and include: cat (*Felis catus*), wild cat (*Felis silvestris*), serval (*Felis serval*), lynx (*Lynx lynx*), Iberian lynx (*Lynx pardinus*), cheetah (*Actinomyx jubatus*), cougar (*Puma concolor*), lion (*Panthera leo*), American leopard (*Panthera onca*), tiger (*Panthera tigris*), jaguarundi (*Herpailurus yagouaroundi*), ocelot (*Leopardus pardalis*), spoted cat (*Leopardus tigrinus*), Geoffroy's cat (*Leopardus geoffroyi*), Chilean pampa cat (*Leopardus colocolo*), tiger cat (*Leopardus tigrinus*), kodkod (*Leopardus guigna*), and Pallas cat (*Otocolobus manul)*, among others (Rodríguez and Carbonell, 1998; Beldoménico et al., 2005; González-Acuña et al., 2010; Okulewicz et al., 2012; Gallas and Fraga da Silveira, 2013; Cruz Hurtado and Muñoz Huamaní, 2016; Heddergott et al., 2016). Nevertheless, there are a few reports of *T. cati* in mustelids (*Martens martes*, European pine marten and *Martens foina*, beech marten) and canids (*Vulpes vulpes*, red fox and *Vulpes macrotis*, kit fox) (Rodríguez and Carbonell, 1998; Górski et al., 2006; Ubelaker et al., 2014).

Considering the gap in epidemiology of *T. cati*, it would be useful to know the relative importance of this ascarid species in paratenic hosts. This information would provide knowledge about the routes of transmission and its dissemination to the definitive host, especially considering its presence in wild, rural, and urban environments. Unfortunately few data exist on the species' identity of wild paratenic hosts like small mammals, such as mice of the families Cricetidae and Muridae, and rats with larvae found in tissues or with seropositive prevalence (Antolová et al., 2013; Strube et al., 2013; Holland, 2015). Other potential routes of transmission should be considered, given that experimental and natural infections were positive in invertebrates, such as oligochaetes (Hadi and Al Amery, 2010), land snails (Cardillo et al., 2016), and different species of Muscidae (Bunchu et al., 2014).

In Americas, records of *T. cati* have been reported from wild carnivores include Chile (González-Acuña et al., 2010), Bolivia (Fiorello et al., 2006), Perú (Aranda et al., 2013), Brazil (Gallas and Fraga da Silveira, 2013), Belize (Patton et al., 1986), México (Solórzano-García et al., 2017), United States (Ubelaker et al., 2014), and Canada (Dare and Watkins, 2012). In Argentina, the findings of *T. cati* larvae and adults were recorded from digestive tracts of *Leopardus geoffroyi* D'Orbigny and Gervais 1844, *Puma concolor* Linneus 1771, and *Leopardus guigna* (Molina, 1782) (Beldoménico et al., 2005; Moleón et al., 2015) from different localities of the country (Table 1).

The aim of this work was to detect the presence of *Toxocara cati* through morphological and molecular identification in wild carnivores of Argentinean Patagonia.

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Table 1
Previous data of *Toxocara cati* in Felidae from Argentinean Patagonia.

Host	Common name	National Park	Province	Site of infection	Source
Puma concolor	cougar	Los Glaciares	Santa Cruz	gastrointestinal tracts	Moleón et al., 2015
Leopardus geoffroyi	Geoffroy's cat	Lihué Calel	La Pampa	stomach and small intestine; gastrointestinal tracts	Beldoménico et al., 2005; Moleón et al., 2015
Leopardus guigna	kodkod	Lanín	Neuquén	gastrointestinal tracts	Moleón et al., 2015
		Los Alerces	Chubut	gastrointestinal tracts	Moleón et al., 2015

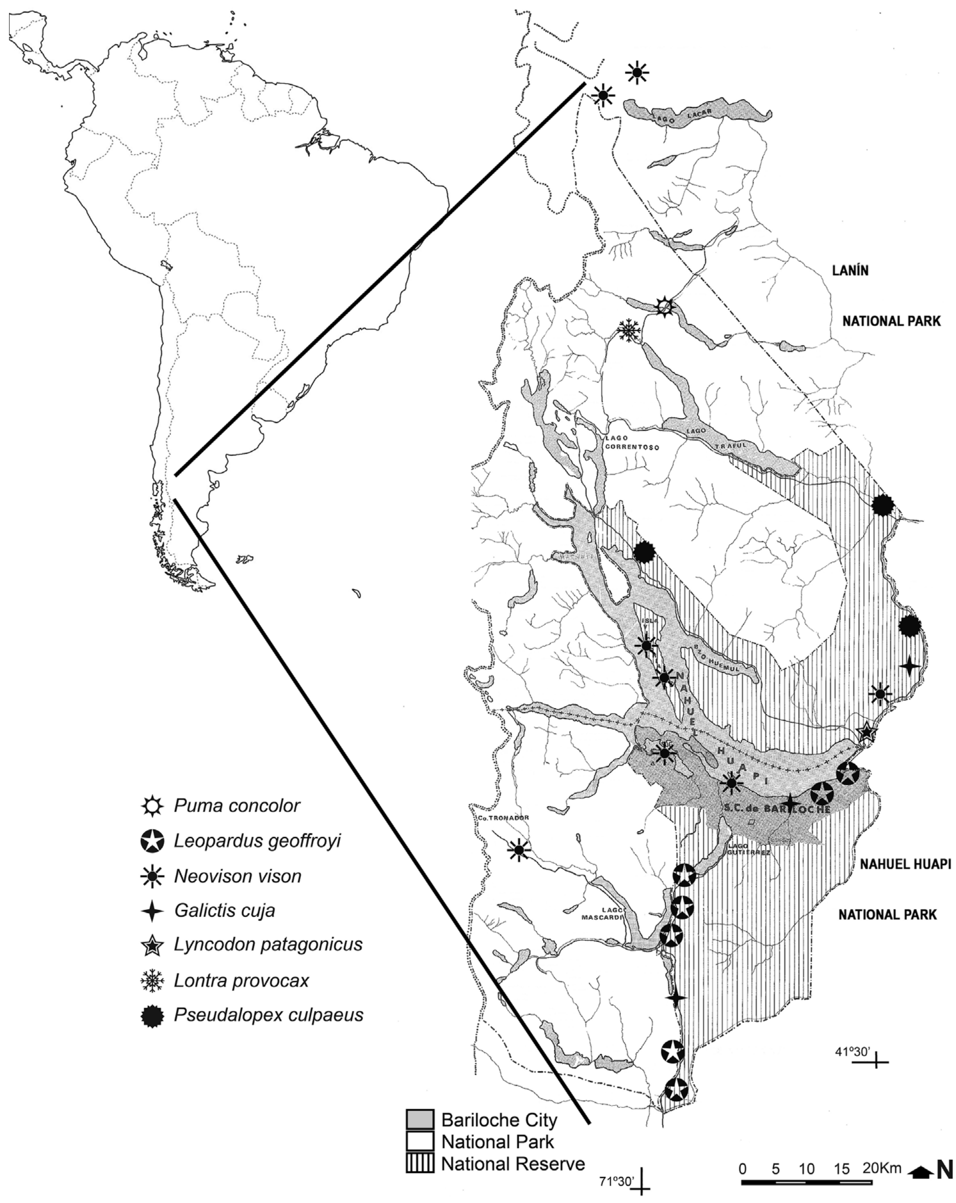

Fig. 1. Collection sites of specimens of Felidae, Mustelidae, and Canidae in Lanín and Nahuel Huapi National Parks from Argentinean Patagonia.

2. Materials and methods

All specimens of Carnivora were road killed or found dead at different localities in Lanín and Nahuel Huapi National Parks, within Neuquén and Río Negro Provinces (Fig. 1). These are protected areas, characterized by a huge hydrographic network with numerous glacial lakes bordered by deciduous and perennial forests on the West, and steppe on the East.

Twenty four specimens of wild carnivores, belonging to the families Felidae, Mustelidae, and Canidae were obtained from March 1996 to April 2016 (Table 2). All specimens were kept at −20 °C. The digestive tracts were examined from: one juvenile of *P. concolor*, seven *L. geoffroyi* (two juveniles and five adults), eigth adults of *Neovison vison* (Schreber, 1777) (American mink), three adults of *Galictis cuja* (Molina, 1782) (lesser grison), one adult of *Lyncodon patagonicus* (de Blainville, 1842) (Patagonian weasel), one adult of *Lontra provocax* (Thomas, 1908) (Southern river otter), and three adults of *Pseudalopex culpeus* (Molina, 1782) (Andean fox) (Table 2). Ascariid specimens were removed from stomach, and small and large intestine, counted, and if it was possible divided in larvae and adults (males and females). *Toxocara cati* specimens were fixed in 5% formalin and cleared with lactophenol for observation and taxonomic identification (Anderson et al., 2009). Others

Table 2
Data of specimens of families Felidae, Canidae, and Mustelidae sampled in Argentinean Patagonia.

Host	Common name	Family	Date	Sex	Locality (National park and province)	Coordinates
Puma concolor	cougar	Felidae	Sep-10	F	Lake Falkner (NHNP, N)	40°26′S-71°32′W
Leopardus geoffroyi	Geoffroy's cat	Felidae	Aug-05	M	Las Chacras (NHNP, RN)	41°06′S-71°12′W
			Oct-09	M	Mascardi Village (NHNP, RN)	41°20′S-71°30′W
			Oct-11	M	Villegas Ranch (NHNP, RN)	41°31′S-71°27′W
			Aug-12	M	Bariloche Airport (NHNP, RN)	41°08′S-71°10′W
			Sep-12	F	Lake Mascardi (NHNP, RN)	41°19′S-71°29′W
			May-13	M	Villegas River (NHNP, RN)	41°35′S-71°29′W
			May-13	F	Lakes Mascardi-Gutiérrez Watershed (NNHP, RN)	41°15′S-71°28′W
Neovison vison	American mink	Mustelidae	Mar-96	NR	Cerro Tronador Route (NHNP, RN)	41°15′S-71°44′W
			Mar-00	M	Hua Hum River (LNP, N)	40°07′S-71°39′W
			Mar-00	M	Hua Hum River (LNP, N)	40°07′S-71°39′W
			Sep-10	M	Fortin Chacabuco (NHNP, N)	40°50′S-71°05′W
			Aug-10	M	Pond Larga, Victoria Island (NHNP, N)	40°53′S-71°32′W
			Aug-10	M	Pond Larga, Victoria Island (NHNP), N	40°53′S-71°32′W
			May-12	F	Lake Nahuel Huapi, Bonita Beach (NHNP, RN)	41°07′S-71°23′W
			Apr-16	NR	Lake Moreno Pisciculture (NHNP, RN)	41°05′S-71°30′W
Galictis cuja	lesser grison	Mustelidae	Aug-05	F	Las Chacras (NHNP, RN)	41°05′S-71°11′W
			Mar-08	M	Rincón Chico (NHNP, N)	40°59′S-71°06′W
			Feb-14	NR	R40, km 1992 (NHNP, RN)	41°26′S-71°29′W
Lyncodon patagonicus	Patagonian weasel	Mustelidae	Oct-10	M	R40N-R231 Crossroad (NHNP, N)	41°02′S-71°11′W
Lontra provocax	Southern river otter	Mustelidae	Apr-10	M	Pichi Traful (NHNP, N)	40°29′S-71°35′W
Pseudalopex culpeus	Andean fox	Canidae	Aug-12	NR	Dpt. Los Lagos (NHNP, N)	40°48′S-71°35′W
			Jan-14	F	Dpt. Los Lagos (NHNP, N)	40°48′S-71°35′W
			Mar-16	M	Confluencia Traful (NHNP, N)	40°43′S-71°05′W

References: (LNP) Lanin National Park; (NHNP) Nahuel Huapi National Park; (N) Neuquen province; (RN) Rio Negro province; NR not recorded.

were fixed in 96° ethanol for later molecular identification. Molecular identification at species level was carry out by PCR-RFLP of ITS-1 region, using enzymes *Tru* I and *Taq* I to generate restriction patterns that allowed to discriminate species, sensu González Prous et al. (2009).

3. Results and discussion

Toxocara cati specimens were obtained from the only specimen of *P. concolor* and from all the specimens of *L. geoffroyi* examined. The other species of Mustelidae and Canidae were negative (Table 3). A total of 11 specimens of *T. cati* were found in the large intestine of *P. concolor* and a total of 382 specimens (larvae and adults) were found in the stomachs and the small and large intestines of *L. geoffroyi* (Table 3). The molecular identity of ascarid specimens from *P. concolor* and from one *L. geoffroyi* were confirmed by PCR-RFLP procedure (Table 3).

Up to now, six species of Felidae are recognized in Patagonia: *P. concolor, L. geoffroyi, L. guigna, L. colocolo*, *Leopardus jacobita*, and *H. yagouaroundi* (Chebez et al., 2014). The first three are hosts for *T. cati,* but for the other species no parasite information is available. The previous known distribution of *T. cati* infections in cougar were recorded in Córdoba and Santa Cruz provinces, and in Geoffroy's cat in La Pampa, Buenos Aires, Entre Ríos, and Santa Fe provinces (Beldoménico et al., 2005; Moleón et al., 2015). For the last host is the southernmost record of *T. cati* in South America. This study fill gaps in the spatial distribution, including the presence of this ascarid zoonotic species in Río Negro province, showing the continuity of the distribution of this parasite species in wild environments in provinces of Patagonia. Presence of *T. cati* in other provinces of Argentina is likely, considering the wide distribution of these two host feline species.

One of the most challenging aspects of preventing exposure to *Toxocara* infection is its complexity of the routes of transmission and sources of infection (Holland, 2015). Four key epidemiological

Table 3
Presence of *Toxocara cati* in *Puma concolor* and *Leopardus geoffroyi* from argentinean patagonia.

Host	Age	Digestive tube content	Locality (National park and province)	*Toxocara cati*		
				N	Stage	Site of infection
Puma concolor	juvenil *	hairs	Lake Falkner (NHNP, N)	11	nr	large intestine
Leopardus geoffroyi	adult	vegetal debris, feathers, hairs	Las Chacras (NHNP, RN)	2	1 male, 1 larvae	large intestine
	adult *	nails, skin, and bones of small sigmodotine rodent, insects, and scales and leg of *Liolaemus pictus*	Mascardi Village (NHNP, RN)	228	nr	large intestine
	adult	Hairs, mice leg, bucal bones, bones	Villegas Ranch (NHNP, RN)	2	1 male, 1 larvae	small intestine
	juvenil	Hairs, bones, feathers	Bariloche Airport (NHNP, RN)	15	larvae	small intestine
	juvenil	Hairs	Lake Mascardi (NHNP, RN)	96	males and females	stomach, small and large intestine
	adult	hairs and bones of mice	Villegas River (NHNP, RN)	10	nr	small and large intestine
	adult	feathers, little bones, hairs	Lakes Mascardi-Gutiérrez Watershed (NNHP, RN)	29	nr	small and large intestine

References: (LNP) Lanín National Park; (NHNP) Nahuel Huapi National Park; (N) Neuquén province; (RN) Río Negro province; nr not recorded; * PCR positive.

reservoirs can be recognized: intestinal infections in definitive hosts, eggs in the environment, larvae in paratenic hosts, and somatic larvae in the definitive host (Overgaauw and van Knapen, 2013). *Toxocara cati* have a faeco-oral transmission cycle, and one of the main routes of transmission to humans is the presence of infective eggs in the environment (Holland, 2015). Humans can be infected through accidental ingestion of infective eggs from contaminated soil (gardens, sandpits, and playgrounds), water, raw vegetables or fruits. Although few embryonated eggs can be found in the hairs of dogs and cats, and the ingestion of larvae in organs or infected muscle tissues that are undercooked, could be other occasional routes of transmission (Deplazes et al., 2011; Holland, 2015; Sierra et al., 2016; Ma et al., 2017). Data from diet remains in definitive hosts would provide clues of other potential hosts in the transmission route. Studies of the diet of the Geoffroy's cat in different ecosystems in Argentina (Monte Central, Mar Chiquita lagoon, Pampasic agricultural lands) showed this small-sized feline is an opportunistic predator capturing vertebrates, with small rodents as the most important prey, while birds and reptiles were the main items in spring and summer (Canepuccia et al., 2007; Bisceglia et al., 2008; Guidobono et al., 2016). Remains of the prey items identified from our specimens of Geoffroy's cat are similar to those of the previous studies. This combined information supports the hypothesis that small rodents may play a paratenic role in the cycle. Unfortunately diet remains in the cougar were too poor to do any comparisons.

In Patagonia, *Toxocara* eggs has been recorded in different environments of Neuquén, Río Negro, and Chubut provinces, and the ranges of prevalence of *Toxocara* spp. in Patagonian cities with arid climate (steppe environments) doesn't differ from that of high moisture localities (temperate forests) in the Andes (12,7% to 33,3% versus 11%–33%) (Zunino et al., 2000; Sánchez et al., 2003; Fillaux et al., 2007; Soriano et al., 2010; Semenas et al., 2014). Fillaux et al. (2007) performed the only study in Patagonia comparing human seroprevalence (31,6%) with soil contamination degree (35,1%) in steppe environments in these three provinces. They showed that despite environmental prevalence, values of human seroprevalence were low compared with those reported from other areas in Argentina, where the seroprevalence values were always more than 3 times higher than those of contaminated soils (Chiodo et al., 2006; Radman et al., 2010). Temperature and moisture are important factors for the embryonation, development, and viability of eggs, although permanent drought could lower transmission. The last factor might explain the peculiar epidemiologic situation in Patagonian steppe (Fillaux et al., 2007). Seroprevalence studies suggest that exposure to the parasite is extremely common, especially in children (Macpherson, 2013), so it's necessary to perform other human immunological studies in Patagonia, especially in humid localities near the Andes, to develop a better understanding of the epidemiologic situation of this zoonoses in the South of Argentina.

Toxocariasis is not an official notifiable disease but it is of major importance as a public health problem, because it is especially prevalent among socioeconomically disadvantaged children in both developing and developed nations (Chen et al., 2012; Macpherson, 2013; McGuinness and Leder, 2014). In the last decades, research trends for this zoonoses showed a notable increase in published papers in the world, including in Argentina (Zyoud, 2017). This is coincident with the discovery of the high seroprevalences in humans and prevalences in dogs (Fillaux et al., 2007; Vizcaychypi et al., 2013). However, in those works, toxocariasis in wild animals as another source of soil contamination in other environments is not mentioned. The positive animals in the areas surrounding the populated center of Bariloche city, could contribute to the circulation of toxocariasis among wild, rural, and urban environments. In addition, this study represents the first molecular evidence of *T. cati* in wild felids in Argentina. These genetic information could be another helpful tool, especially given the complexity of the environments in which these zoonoses dwells (urban, rural, and wild areas) (Otranto et al., 2015). Likewise, given that the early stages of development are difficult to identify among soil nematodes, molecular studies allow us to fill gaps in the knowledge of this zoonoses.

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