

Intestinal parasites of *Leopardus geoffroyi* (Mammalia, Felidae) inhabiting the Paraná River Delta (Argentina): a coprological study

Parásitos intestinales de *Leopardus geoffroyi* (Mammalia, Felidae) en el Delta del Río Paraná (Argentina): un estudio coprológico

Zonta María Lorena^{1*}, Ezquiaga María Cecilia¹, Demergassi Natalia², Pereira Javier Adolfo³, Navone Graciela Teresa¹

ABSTRACT: *Leopardus geoffroyi* (Geoffroy's cat) is distributed from southern Brazil and Bolivia to southern Argentina and Chile in the Patagonia region. The aim was to identify intestinal parasites of *L. geoffroyi* inhabiting the Paraná River Delta (Buenos Aires province, Argentina) and to detect species of zoonotic importance. Thirteen fecal samples were obtained from specimens captured. Feces were processed using Ritchie and Sheather modified techniques. Prevalence and dominance were calculated for each parasite species found. All samples were parasitized. Twelve parasite species were identified. Polyparasitism was observed in all cats. *Aelurostrongylus abstrusus*, *Spirometra* sp., *Ancylostoma* sp. and *Toxocara cati* were the most prevalent and dominant species. *Eimeria* sp., *Rodentolepis nana*, *Platynosomum* sp., *Eucoleus aerophilus*, *Pterygodermatites* sp., *Spirurida*, *Trichuris* sp. and *Trichostrongyloidea* were also detected. This study reports *A. abstrusus*, *Spirometra* sp., *R. nana* and *Platynosomum* sp. for the first time in the Geoffroy's cat for Argentina. Species of zoonotic importance such as *Spirometra* sp., *Ancylostoma* sp., *T. cati*, *E. aerophilus*, *Trichuris* sp. and *R. nana* are recorded. The diversity of endoparasites found in the Geoffroy's cat responds to the generalist habits of this carnivore and to its interaction with domestic animals, which favors the transmission of parasites of zoonotic importance.

Keywords: Geoffroy's cat, enteroparasites, Buenos Aires province, feces, zoonoses.

RESUMEN: *Leopardus geoffroyi* (gato montés) se distribuye desde el sur de Brasil y Bolivia hasta la región patagónica de Argentina y Chile. El objetivo de este trabajo fue identificar los parásitos intestinales de *L. geoffroyi* del Delta del río Paraná (provincia de Buenos Aires, Argentina) y detectar especies de importancia zoonótica. Se obtuvieron 13 muestras fecales de especímenes capturados. Las heces se procesaron utilizando las técnicas de Ritchie y Sheather modificada. Se calcularon la prevalencia y la dominancia para cada especie parasitaria encontrada. Todas las muestras estuvieron parasitadas. Se identificaron 12 especies de parásitos. Se observó poliparasitismo en todos los gatos. *Aelurostrongylus abstrusus*, *Spirometra* sp., *Ancylostoma* sp. y *Toxocara cati* fueron las especies más prevalentes y dominantes. Además se detectó *Eimeria* sp., *Rodentolepis nana*, *Platynosomum* sp., *Eucoleus aerophilus*, *Pterygodermatites* sp., *Spirurida*, *Trichuris* sp. y *Trichostrongyloidea*. Este estudio reporta por primera vez a *A. abstrusus*, *Spirometra* sp., *R. nana* y *Platynosomum* sp. en el gato montés de Argentina. Se registran especies de importancia zoonótica tales como *Spirometra* sp., *Ancylostoma* sp., *T. cati*, *E. aerophilus*, *Trichuris* sp. y *R. nana*. La diversidad de endoparásitos encontrados en el gato montés responde a los hábitos generalistas de este carnívoro y a su interacción con los animales domésticos, la cual favorece la transmisión de parásitos de importancia zoonótica.

Palabras clave: identificación molecular, diagnóstico, nematode.

INTRODUCTION

Parasitological studies are important for monitoring the population health of free-ranging wildlife, especially where wild species are in contact with domestic animals (Fiorello et al., 2006). Therefore, when habitat fragmentation and human activities that affect the landscape increase, encounters of wild felids with domestic fauna and humans become more frequent.

In this way, parasitic infection constitute an important concern not only for wild animal conservation but also for human health (Solórzano-García et al., 2017, and references therein).

Carnivores play very important roles as hosts and reservoirs of numerous intestinal parasites, some of which could infect humans (e.g. *Toxoplasma gondii*, *Spirometra* spp., *Ancylostoma* spp., *Toxocara* sp.)

¹Centro de Estudios Parasitológicos y de Vectores (CEPAVE-CONICET-UNLP). Boulevard 120 S/N entre 60 y 64, La Plata (1900), Argentina; ²Fundación Temaikèn. Ruta 25 Km 0,700, Belén de Escobar (1625), Argentina; ³Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (CONICET). Ángel Gallardo 470, Ciudad de Buenos Aires (C1405DJR), Argentina.

Correspondencia: lorenazonta@cepave.edu.ar

(Soulsby, 1987; Tantaleán and Michaud, 2005). The mechanisms of infection vary according to the species involved; they may be by direct or indirect contact with the secretions or depositions of infected animals, or by ingestion of an intermediate host (Traversa et al., 2014).

Leopardus geoffroyi (d'Orbigny and Gervais, 1844) (Geoffroy's cat) is distributed from southern Brazil and Bolivia to southern Argentina and Chile in the Patagonia region (Cuyckens et al., 2016). This species appears to be a highly adaptive predator occurring in wetlands, dry forests, grasslands and scrublands in both pristine and disturbed areas (Nowell and Jackson, 1996). Small mammals usually constitute the bulk of the diet of this felid within its distribution range, however, in some localities, other prey items (e.g., hares, waterbirds, fish, amphibians) can reach high frequencies in its diet (Johnson and Franklin, 1991; Manfredi et al., 2004; Canepuccia et al., 2007; Bisceglia et al., 2008; Pereira et al., 2012; Migliorini et al., 2018). Its conservation status is Least Concern according to the IUCN (Pereira et al., 2015).

Aspects related to the natural history of this felid species have been studied, some of them focused in to describe its parasitic fauna (Fiorello et al., 2006; Gallas and Fraga da Silveira, 2011; Vieira et al., 2008). Especially in Argentina, Beldomenico et al. (2005) recorded 10 species of gastrointestinal parasites from the Monte Desert (La Pampa province) and Uhart et al. (2012) studied pathogens in two Geoffroy's cat populations detecting antibodies for *T. gondii* and *Dirofilaria immitis*. Moreover, in a study of helminths in carnivores from Argentina, Moleón et al. (2015) found 19 helminth species, 7 of them belonging to the Geoffroy's cat.

The aim of this study was to identify intestinal parasites of *L. geoffroyi* in the Paraná River Delta (Buenos Aires province, Argentina) and to detect species of zoonotic importance.

MATERIALS AND METHODS

This study was carried out in the lower delta of the Paraná River, the final portion of one of the largest wetlands in South America (Kandus and Malvárez, 2004). The delta was originally covered by freshwater marshes and riparian forests, but an intensive forestry activity (i.e., commercial plantations of poplar and willow) and livestock raising have strongly modified its physiognomy. In addition to the Geoffroy's cat, the crab-eating fox (*Cerdocyon thous*), the lesser grison (*Galictis cuja*) and the southern river otter (*Lontra longicaudis*) are typical carnivores of this region. Domestic cats and dogs are widespread animals in the area, usually associated with human residences.

During October 2014 and July 2015, 24 Geoffroy's cats were captured and fitted with radio collars

for a project focused on the spatial ecology and conservation of the species in this wetland (Disposition 149/14 of Dirección de Flora y Fauna, Buenos Aires province). Cats were caught using homemade traps and immobilized with ketamine and dexmedetomidine administered intramuscularly. Cats were weighed and sexed. After anesthesia, every cat was placed in a recovery kennel for approximately 3 hours until they were released. Thirteen feces were collected from the homemade traps or recovery kennels if available, preserved in 3% formaldehyde for coproparasitological studies and sent to the Laboratory of Biodiversity and Parasitic Epidemiology of the Centro de Estudios Parasitológicos y de Vectores (La Plata, Buenos Aires, Argentina). Nineteen of the Geoffroy's cats were categorized as adults (11 males and eight females), three as subadults (one male and two females) and two as juveniles (one male and one female). All specimens appeared to be in good physical condition at the moment of capture (i.e., with no apparent disease).

All stool samples were homogenized, filtered and processed using the Ritchie -formaldehyde-ethyl acetate- and Sheather modified -saturated sucrose solution- concentration techniques. The Ritchie sedimentation technique was employed as a standard procedure: a 10 ml filtered suspension was centrifuged, then both a 7 ml formaldehyde, and a 3 ml ethyl acetate suspensions were added to the resulting pellet. Tubes were vigorously shaken and centrifuged during 5 minutes at 400 g. The plug was carefully removed, and the pellet was examined under a light microscope (Cociancic et al., 2018a). The Sheather modified flotation technique used a sucrose saturated solution ($\delta=1.3$). The filtered suspension (10 ml) was placed in a tube forming a meniscus in which a cover is placed. The coverslip was placed on a slide for observation after 20 min (Becerril Flores and Romero Cabello, 2004).

Every sample was examined by two experts using a Leica DM 500 light microscope of 100× and 400× magnifications. The eggs, cysts and larvae identification was based on their measures, morphological features, and specific bibliography (Thienpont et al., 1979; Mehlhorn et al., 1992; Beldomenico et al., 2005). Prevalence and dominance were calculated following Morales and Pino (1987).

RESULTS

All fecal samples belonging to different host specimens were parasitized. Twelve parasite species were found by the Ritchie technique. Additionally, six of those species were found by the Sheather technique (Table 1). Helminths were more prevalent than protozoa ($P=100\%$ vs 30.7%).

Sporulated and unsporulated oocysts of *Coccidia* were detected among protozoa. Sporulated oocysts

Table 1. Intestinal parasites of *Leopardus geoffroyi* from the Paraná River Delta (Argentina) detected by Ritchie and Sheather modified

	Parasite	Ritchie	Sheather modified	Prevalence (%)	Dominance (%)
Protozoa	<i>Eimeria</i> sp. (Coccidia: Eimeriidae)	P	P	30.8	33.3
Cestoda	<i>Rodentolepis nana</i> (Cyclophyllidae: Hymenolepididae)	P	A	15.4	16.7
	<i>Spirometra</i> sp. (Pseudophyllidae: Diphyllbothriidae)	P	P	76.9	83.3
Trematoda	<i>Platynosomum</i> sp. (Digenea: Dicrocoeliidae)	P	A	7.7	8.3
Nematoda	<i>Aelurostrongylus abstrusus</i> (Strongylida: Angiostrongylidae)	P	P	92.3	100.0
	<i>Eucolus aerophilus</i> (Enoplida: Trichuridae)	P	P	30.8	33.3
	<i>Ancylostoma</i> sp. (Strongylida: Ancylostomatidae)	P	P	76.9	83.3
	<i>Pterygodermatites</i> sp. (Spirurida: Rictulariidae)	P	A	7.7	8.3
	Spirurida	P	A	7.7	8.3
	<i>Toxocara cati</i> (Ascaridida: Ascarididae)	P	P	46.2	50.0
	<i>Trichuris</i> sp. (Enoplida: Trichuridae)	P	A	15.4	16.7
	Trichostrongyloidea (Strongylida)	P	A	7.7	8.3

References: P=Presence; A=absence

with 4 sporocysts were ellipsoidal measuring 23.9 x 17.4 µm mean, with a yellow and smooth wall and were compatible with *Eimeria* sp. (Fig. 1a). Unsporulated oocysts were not identified.

Regarding Platyhelminthes, *Rodentolepis nana* and *Spirometra* sp., (Cestoda) were identified. *Rodentolepis nana* measured 67.0 x 54.8 µm mean, and the oncosphere inner membrane showed polar thickenings bearing several filaments (Fig. 1b). *Spirometra* sp. eggs measured 59.0 x 34.6 µm mean, light brown colored with an evident cap, and pointed ends (Fig. 1c). Eggs attributable to *Platynosomum* sp. were found among Trematoda. They were golden brown colored, oval, thick-shelled, and embryonated. The operculum was situated on one extremity, and they measured 36.0 x 22.0 µm mean (Fig. 1d).

Additionally, several Nematoda species were detected. First stage larvae (L1) were identified as *Aelurostrongylus abstrusus* according to morphological –terminal oral opening and “S” shaped tail– and morphometric (305.5 x 14.5 µm mean) features (Fig. 1e). Eggs of *Eucolus* (=Capillaria) *aerophilus* measuring 65.3 x 27.5 µm mean were found. They showed protruding, transparent polar plugs, and a fine granular shell (Fig. 1f). Eggs resembling *Ancylostoma* sp. were found, measuring 67.3 x 38.4 µm mean. They were ovoid, with similar bluntly rounded poles, barrel-shaped side walls, and a thin shell (Fig. 1g). Eggs resembling *Pterygodermatites* sp. were

detected measuring 38.8 x 22.8 µm mean. They were embryonated and thick-shelled (Fig. 1h). Embryonated eggs of Spirurida –33.0 x 21.8 µm mean– with smooth thick shells were found (Fig. 1i). Eggs of *Toxocara cati* –73.6 x 58.9 µm mean– were almost spherical, with a thick rough, and pitted shell (Fig. 1j). Medium-sized eggs resembling *Trichuris* sp. measuring 63.5 x 31.0 µm mean, with two clearly protruding transparent polar plugs, and a thick shell with smooth surface were found (Fig. 1k). Long, ovoid eggs measuring 79.0 x 36.0 µm mean belonging to Trichostrongyloidea were also found. They possessed parallel side walls, similar poles, and a thin shell with smooth surface (Fig. 1l). They were embryonated and could be an accidental parasite.

Aelurostrongylus abstrusus, *Spirometra* sp., *Ancylostoma* sp. and *T. cati* were the most prevalent and dominant parasites. The remaining species were poorly represented with values under 35% (Table 1).

Polyparasitism was observed in all cats. In this sense, 46.1% of them were parasitized by 3 species and the most frequent co-occurrence was *A. abstrusus*/*Spirometra* sp./*Ancylostoma* sp. In addition, 38.5% were parasitized by 5 species, and 15.4% by 6.

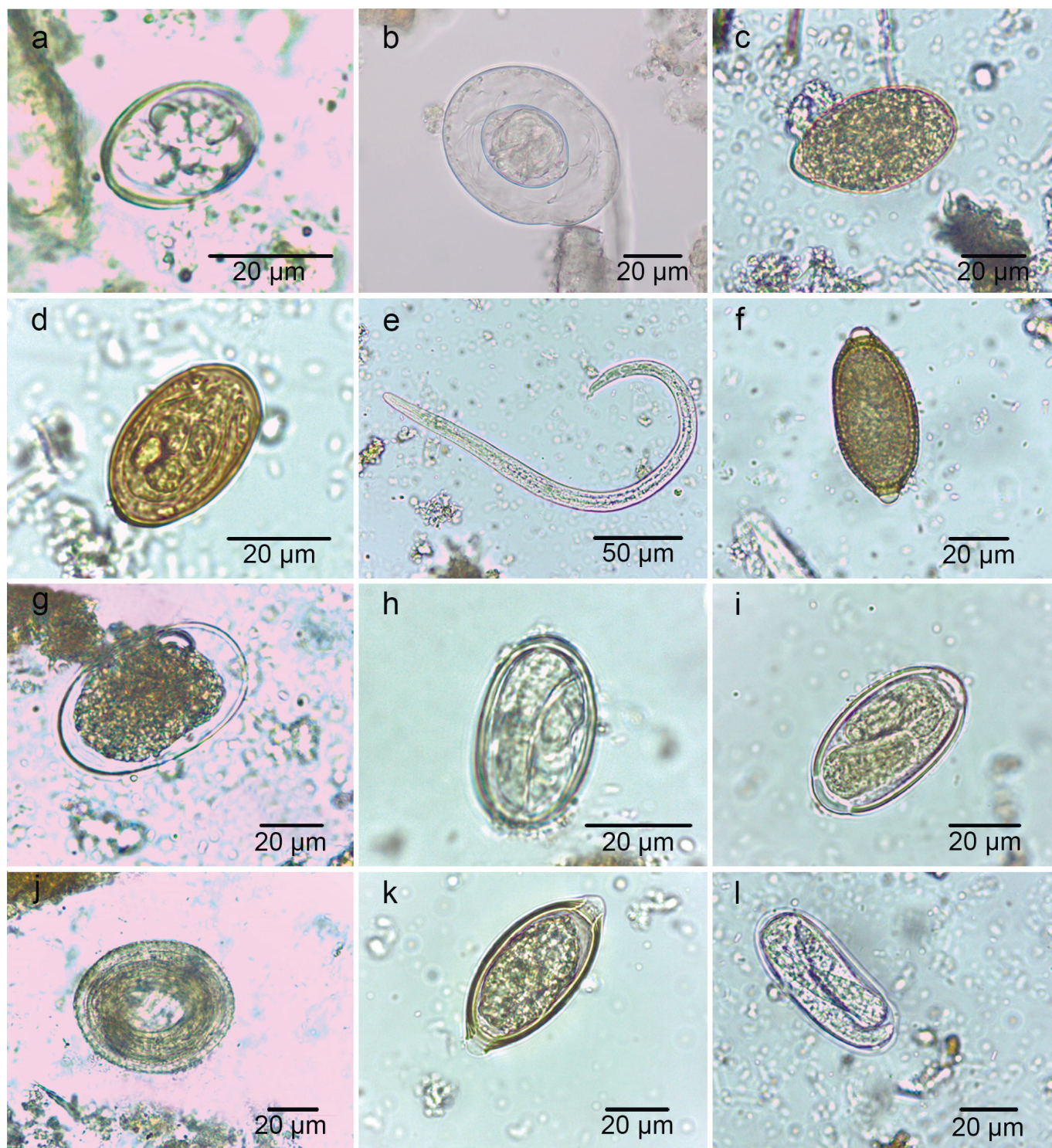


Figure 1. Intestinal parasites identified in *Leopardus geoffroyi* from Paraná River Delta, Argentina. a) *Eimeria* sp. b) *Rodentolepis nana* c) *Spirometra* sp. d) *Platynosomum* sp. e) *Aelurostrongylus abstrusus* f) *Eucoleus aerophilus* g) *Ancylostoma* sp. h) *Pterygodermatites* sp. i) *Spirurida* j) *Toxocara cati* k) *Trichuris* sp. l) *Trichostrongyloidea*.

DISCUSSION

In this study, we contribute to the knowledge of intestinal parasites present in Geoffroy's cats inhabiting the Paraná River Delta (Argentina) through the use of coproparasitological methods. When comparing both Ritchie and Sheather methods we conclude that the Ritchie's was the technique that showed a better detection of parasitic forms in fecal samples.

The intestinal parasites prevalence in our study was high and 12 parasite species were found. The most prevalent parasites were *A. abstrusus*, *Spirometra* sp. and *Ancylostoma* sp. Moreover, zoonotic species such as *Spirometra* sp., *Ancylostoma* sp., *T. cati*, *E. aerophilus*, *Trichuris* sp. and *R. nana* were found.

Among the protozoa, sporulated oocysts of *Eimeria* sp. were detected in this study. Dib et al. (2018) identified *Eimeria* sp. in other felid species in Rio de Janeiro (Brazil) and Beldomenico et al. (2005) found immature coccidian oocysts in Geoffroy's cats from central Argentina. The infection of felids with these protozoa occurs by the ingestion of sporulated oocysts especially in water or by pseudoparasitism (Dib et al., 2018).

The parasites of the genus *Spirometra* belong to one of the twelve genera of the family Diphylobothriidae, with several species of zoonotic importance whose definitive hosts are carnivorous mammals (Petrigh et al., 2015). Several studies in Latin America reported the presence of this cestode in wild carnivores (e.g. jaguars, pumas, ocelots, Geoffroy's cats, jaguarundis, crab-eating foxes) (Tantaleán and Michaud, 2005; Fiorello et al., 2006; Solórzano-García et al., 2017) and in domestic cats (Valerio et al., 2004). In Argentina, this parasite has been recorded in pampas foxes and in domestic cats and dogs (Venturini, 1980; Santa Cruz and Lombardero, 1987; Scioscia et al., 2014; Petrigh et al., 2015). The finding of *Spirometra* sp. becomes relevant since humans can be infected with this species by accidentally drinking water contaminated with copepods (first intermediate host) infected with the proceroid larva or by consuming raw or undercooked meat of any of the second intermediate or paratenic hosts (vertebrates) infected with the larva plerocercoid. This larva (spargana) causes a serious parasitic zoonosis named sparganosis (Scioscia et al., 2014; Petrigh et al., 2015). On the other hand, *R. nana* is a cestode that parasitizes rodents and humans. Its life cycle is direct and infection in humans can occur through contaminated food or soil (Thompson, 2015; Fitte et al., 2017). The presence of this cestode in the Geoffroy's cat may be related to the ingestion of the habitual host (e.g. rodents) and indicates that *R. nana* is circulating in the environment and carnivores carry out the role of post-cyclic host, disseminating eggs with the feces. To date, two cestode species were recorded in the Geoffroy's cat from Argentina: *Taenia* sp. and

Echinococcus oligarthrus (Schantz and Colli, 1973; Beldomenico et al., 2005). In this work, we add two more species for *L. geoffroyi* in Argentina: *Spirometra* sp. and *R. nana*.

Platynosomiasis is a liver disease caused by digeneans of the genus *Platynosomum* that affects birds and mammals in tropical and subtropical areas, including Latin America (De Castro and Albuquerque, 2008; Lenis et al., 2009; Vieira et al., 2009). The species of this genus are hepatic trematodes which cause 'lizard poisoning' in cats. The life cycle involves terrestrial snails and isopods as intermediate hosts, and birds, felids, marsupials and canids as definitive hosts (De Castro and Albuquerque, 2008; Lenis et al., 2009; Basu and Charles, 2014). Lizards, geckos, frogs and toads can act as paratenic hosts (Pinto et al., 2014). In this study, *Platynosomum* sp. is recorded for the first time in the Geoffroy's cat, expanding the host and geographical distribution of this parasite species.

Aelurostrongylus abstrusus is a nematode that parasitizes domestic cats and has a wide geographical distribution. In Argentina, this parasite causes an emerging disease that has been described in domestic cats in several cities of the province of Buenos Aires (Cardillo et al., 2014). However, previous studies recorded the presence of this species in wild felids of South Africa (Di Cesare et al., 2016) and in Geoffroy's cats and ocelots (*Leopardus pardalis*) of Bolivia (Fiorello et al., 2006). In this study, *A. abstrusus* is reported for the first time in Geoffroy's cats in Argentina. The high prevalence of *A. abstrusus* observed seems to be directly related to its proximity to rivers since its life cycle involves intermediate hosts such as snails or slugs that inhabit this environment. Additionally, frogs, birds and lizards can act as paratenic hosts by eating the infected mollusks. Definitive hosts become infected by ingesting the intermediate or paratenic hosts. Adult nematodes live in the terminal respiratory bronchioles and alveolar ducts of the felid definitive host and L1 are eliminated in host's feces to the environment (Cardillo et al., 2014; Valente et al., 2017).

Eucoleus aerophilus and several species of *Trichuris* are very common parasites of domestic cats and dogs. In *L. geoffroyi*, *E. aerophilus* was mentioned previously by Moleón et al. (2015) for Argentina, and Fiorello et al. (2006) for Bolivia. Beldomenico et al. (2005) detected adults of *T. campanula* in this felid in central Argentina regarding *Trichuris* sp.

Ancylostoma sp. and *Toxocara* sp. are the most common intestinal parasites detected in dogs and cats in temperate, tropical and subtropical climate areas (Szwabe and Błaszowska, 2017). Humans can become accidental hosts by being in contact with infective eggs from contaminated soil, unwashed raw vegetables or by skin penetration. However, contact with contaminated soil is the most important route

of transmission for human infections. Children are frequently infected by being exposed to contaminated soil while playing in sandboxes or playgrounds (Szwabe and Blaszkowska, 2017; Cociancic et al., 2018b). There are two species parasitizing domestic cats and dogs, *Ancylostoma caninum* and *A. tubaeforme*. However, in the present study the species were not identified due to eggs are morphologically indistinguishable. Previous studies have recorded *A. tubaeforme* and *T. cati* in the Geoffroy's cat in central Argentina and Bolivian Chaco (Beldomenico et al., 2005; Fiorello et al., 2006; Moleón et al., 2015). This is the first record of *T. cati* in the Geoffroy's cat in Paraná River Delta.

In this study, the genus *Pterygodermatites* might be recognized by egg morphometric features although we could not identify the species. However, previous studies reported the presence of *P. cahirensis* in the Geoffroy's cat (Beldomenico et al., 2005).

There are 3 species of Spirurida recorded in Argentina parasitizing the Geoffroy's cat: *Vigisospirura potekhina*, *Didelphonema longispiculata* and *Physoleptera praeputialis* (Beldomenico et al., 2005; Moleón et al., 2015). In our research we could not recognize the species due to the morphometric features did not match our findings.

Unfortunately the samples sent to the laboratory were fixed in 3% formaldehyde. Therefore, no molecular analysis could be performed.

The coproparasitological examination performed is of considerable relevance to advance on the parasitological knowledge of the Geoffroy's cat population. Several studies have indicated that a low to moderate parasitic load in wild animals is normal and that such load is not necessarily related to a clinical manifestation. However, co-occurrence of infection and intensity are more severe in individuals with poor physical condition (Beldomenico and Begon, 2009; Ezquiaga et al., 2014). Although the cats analyzed had polyparasitism, they appeared in good physical condition at the time of capture.

The endoparasites richness found in the Geoffroy's cat responds to the generalist habits of this carnivore (both in terms of habitat use and diet) and to its interaction with domestic animals of the area and with a landscape that has been modified by forestry and livestock. This situation could favor the transmission and risk for infection of parasites of zoonotic importance. Several factors such as agricultural practices, wildlife-livestock contact, urbanization, changes in lifestyle, globalization of food supply and deforestation alter ecosystems, thereby increasing the risk of human parasitoses (Odeniran and Ademola, 2016). Studies in areas with different perturbation degrees could help reveal the effects of human-wildlife contact on the host-parasite ecology (Solórzano-García et al., 2017).

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