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The Pampas fox (*Lycalopex gymnocercus*) as new definitive host for *Spirometra erinacei* (Cestoda: Diphyllobothriidae)

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ABSTRACT

Spirometra erinacei, Faust, Campbell and Kellogg, 1929, is a pseudophyllidean cestode of the family Diphyllobothriidae. The genus *Spirometra* is cosmopolitan and these parasites infect carnivores, specially felids and canids. In Argentina, *S. erinacei* and *S. mansonoides* have been reported sporadically only in domestic definitive hosts. The Pampas fox, *Lycalopex gymnocercus*, is the most abundant native carnivore in southern South America, where it inhabits grasslands and open woodlands and areas highly modified by extensive ranching and agricultural activities. This report describes the first finding of *S. erinacei* infecting Pampas fox, and provides an estimate prevalence of this cestode in rural areas of southern Buenos Aires province, Argentina based on 78 complete Pampas fox intestine samples analysis. This study found a 15.4% of prevalence of *S. erinacei* in small intestine (adult stage) and a 21.8% in fecal samples (egg stage). In the present work, the first case of S. *erinacei* in a wild definitive host from Argentina was reported expanding the list of definitive hosts of *S. erinacei* in South America.

1. Introduction

The genus *Spirometra* (Faust, Campbell and Kellogg, 1929) contains pseudophyllidean cestodes of the family Diphyllobothriidae (Bray et al., 1994). Two major species are recognized, *Spirometra erinacei* Faust et al., 1929 (syn. *Diphyllobothrium mansoni* Cobbold, 1883, *Spirometra erinaceieuropaei* Rudolphi, 1819) and *S. mansonoides* Mueller, 1935 (Wardle and McLeod, 1952; Mueller, 1974). The species of *Spirometra* genus are cosmopolitan and include intestinal parasites of carnivores, especially felids and canids, and requires two intermediate hosts. The first is a copepod (*Cyclops* spp.) and the second intermediate host or paratenic host is a vertebrate tetrapod (i.e., humans and other mammals, amphibians, reptiles and birds) (Miyazaki, 1991). Their life cycle has been studied in detail, mostly because plerocercoids (termed spargana) are agents of the human disease sparganosis and also because they were found to secrete a hormone-like factor stimulating overgrowth in mammals (Denegri and Reisin, 1993; Hirai, 2003).

Spirometra mansonoides is considered a parasite only found in North America, where their main definitive host are dogs and cats (Mueller, 1936; Rep, 1975) and also some wild mammals like *Lynx rufus*, *Procyon lotor* (Harkema and Miller, 1964; Heidt et al., 1988). However, this species extends into several countries of Central and South America, where it has been found in cats and in several wild definitive hosts, namely *Oncifelis guigna*, *Puma concolor*, *Panthera onca*, *Leopardus pardalis*, *Cerdocyon thous*, *Lycalopex gymnocercus*, *Atelocynus microtis* (Schmidt and Martin, 1978; Fernández and Villalba, 1984; Santa Cruz and Lombardero, 1987; Tantaleán and Michaud, 2005; Araújo de Lima, 2009; Aranda et al., 2013). There are also reports of *S. mansonoides* in the Caribbean (Rep, 1975).

On the other hand, *S. erinacei* is widespread in the Old World, exhibiting a focal distributional pattern and there are also records in the Americas, Africa and Australasia (Georgiev et al., 2006). The

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main definitive hosts are dogs and golden jackal (*Canis aureus*) in Asia (Cho et al., 1981; Dalimi and Mobedi, 1992) and in cats and red foxes (*Vulpes vulpes*) in Europe and Australasia (Ryan, 1976; Shimalov and Shimalov, 2003; Ugarte et al., 2005). In Central and South America this species has been found in dogs and cats as well in the wild carnivores *Cerdocyon thous* and *Conepatus chinga* (Cram, 1927; Acholonu, 1977; Mueller, 1974; Venturini, 1980; Torres and Figueroa, 1982; Valerio et al., 2004; Santos et al., 2004; Gómez-Puerta et al., 2009).

In Argentina, *Spirometra* has only rarely been reported, and so far exclusively in domestic definitive hosts. *Spirometra mansonoides* was found in cats (Santa Cruz and Lombardero, 1987) and *S. erinacei* in cats and dogs (Venturini, 1980, 1989; Denegri and Reisin, 1993).

South America has one of the largest wild canid diversity, with 10 species, of which 6 are present in Argentina (Eisenberg and Redford, 1999; Sillero-Zubiri et al., 2004; Diaz and Lucherini, 2006). The Pampas fox, *Lycalopex gymnocercus* Fisher, 1914, is the most abundant of them and inhabits grasslands and open woodlands of the Southern Cone of South America, and it also occur in areas highly modified by extensive ranching and agricultural activities (Lucherini et al., 2004). Regarding its eating habits, it is described as an omnivorous predator showing an opportunistic behavior since consumption of items varies according to seasonal availability and geographic location (Farias and Kittlein, 2008).

There are reports on heminths infecting Pampas fox in Argentina (Martínez, 1985; Martínez et al., 2000, 2005; Fuchs et al., 2006; Scioscia et al., 2013), but none confirmed the presence of adults or eggs of *Spirometra* spp. Here we describe the first finding of *S. erinacei* infecting Pampas fox providing the prevalence of this cestode in rural areas of southern Buenos Aires province, Argentina.

2. Materials and methods

2.1. Study area

The study was conducted in rural areas located in six departments of the south of Buenos Aires province, Argentina, namely Azul, Villarino, Benito Juárez, Tandil, Necochea, Lobería, encompassing the ecoregions El Espinal (southwest) and La Pampa (southcentral and southeast) (Fig. 1). Currently, the study area is dominated by cattle farming and agricultural activities and it is home to high densities of Pampas foxes.

2.2. Source of samples

We used 78 complete intestine samples from Pampas foxes. They were obtained from opportunistically found road-killed foxes and dead animals provided by licensed hunters during the authorized hunting season in the province of Buenos Aires during 2010 and 2012. In addition, we received a permit from the Ministerio de Asuntos Agrarios and Dirección de Flora y Fauna of Buenos Aires Province to collect and transport samples from these wild animals. The intestinal tracts were carefully removed from each carcass and subsequently isolated by ligatures (pylorus and rectum). Each sample was individually packed and labeled with relevant information, including place of origin, sampling date, age, and sex of the animal. All samples were kept at −20 °C prior to processing.

The fecal samples were obtained from each rectum and processed for coproparasitological analyses. Each sample was analyzed by two methods of egg concentration: Ritchie sedimentation (Young et al., 1979) and Sheather flotation (Benbrook and Sloss, 1965). Then, they were examined microscopically (by duplicate) at ×100, ×400, and ×1000 for cestode eggs identification (Miyazaki, 1991).

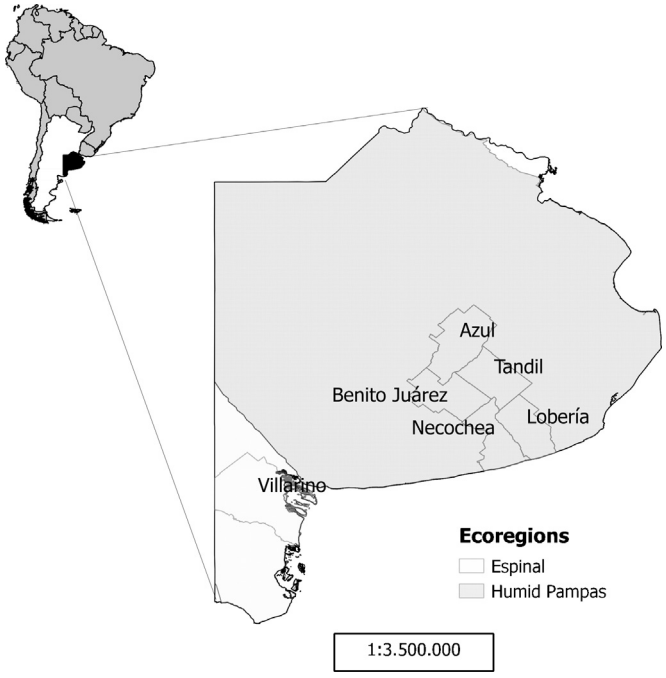

Fig. 1. Sampling sites in Buenos Aires province, Argentina. The map was made with the program Quantum GIS.

Examination of the intestinal content was performed using the sedimentation and counting technique described by Eckert et al. (2001) with modifications described by Scioscia et al. (2013). Obtained sediments were examined in small portions of 5–10 ml round petri dishes with magnifier lens at 65× to identify small helminths, scolexs, and proglottids of cestodes. The cestodes collected were washed in saline solution and then some were preserved in ethanol 70% (for future molecular studies) and the rest were conserved in formol 3% until stained with hydrochloric carmine, dehydrated in a series of alcohols (70°, 85°, 96° and 100°), cleared in beechwood creosote and mounted in Canada balsam. The proglottid was measured under a light microscope using an eyepiece graticule. All measurements of adult cestodes are given in millimeters. The identification of specimens recovered was based on the morphological characteristics of the strobilas and of the eggs, according with descriptions of Mueller (1936, 1974), Wardle and Mcleod (1952) and Miyazaki (1991).

3. Results

A total of 78 Pampas foxes (34 females and 44 males) were necropsied in the present work. The foxes belonged to different departments of the province of Buenos Aires, being 24 from Azul, 49 from Villarino, two from Tandil, one from Benito Juárez, one from Lobería and one from the Necochea (Fig. 1). All foxes were classified as adults according to their size. The postmortem exam revealed that 67 foxes (85.9%) had at least one intestinal helminth species.

Cestodes of *Spirometra* genus were collected in small intentinals of 12 (15.4%) Pampas fox (7 males and 5 females): seven from department Azul (hunted), one from departament Necochea (roadkill), one from departament Benito Juarez (roadkill) and three from departament Villarino (roadkill). In most samples, only short strobila or loose proglotids were found, scolices (5) were found in only one intestine. No full specimen was recovered. In feces of these twelve foxes *Spirometra* sp. eggs were also found through the two coproparasitologic techniques used (flotation and sedimentation).

Table 1
Measurement of gravid, mature and immature proglottids from different adults studied.

	Average measurement (mm)		Range (mm)
Proglottids	Length	Width	
Immature ($n=7$)	0.43	1.97	0.25–0.60 × 0.74–3.25
Mature ($n=9$)	0.86	2.77	0.50–1.60 × 1.60–4.70
Gravid ($n=27$)	2.18	4.46	0.55–7.37 × 2.60–9.0

On the basis of morphological examination the specimens were identified as *Spirometra erinacei.* The cestodes presented strobilas with a length of 27–33 cm. The scolex was flat with two longitudinal bothrias projecting as dorsal and ventral spatulas. The neck was prominent, long and thin and are slightly craspedotes proglottids. The findings of measurements of the proglottids are detailed in Table 1. The main characteristics (based in mature and gravid proglottids) that determined the diagnosis were: presence of anterior and posterior uterine coils in the longitudinal median line of the proglottids. The cirrus is surrounded by the seminal receptacle and opens out separately from vagina and is near to the uterine pore. The uterine pore is in the ventral middle third of the gravid proglottid. The uterus opened by a pore well separated from and posterior to the vagina, and presented a varying number of loops in the terminal heavy walled portion in an "S" shape. The vagina passed traversing from its vestibule in an approximately straight path in the median line, was thrown into lateral undulations of different amplitude. The vesicular testes are numerous and placed in two ventral–dorsal layers (Fig. 2).

The coproparasitological study showed a prevalence of 21.8% (17/78) of *Spirometra* sp. eggs (14/17 with both techniques; 15/17 with Sheather flotation and 16/17 with Ritchie sedimentation).

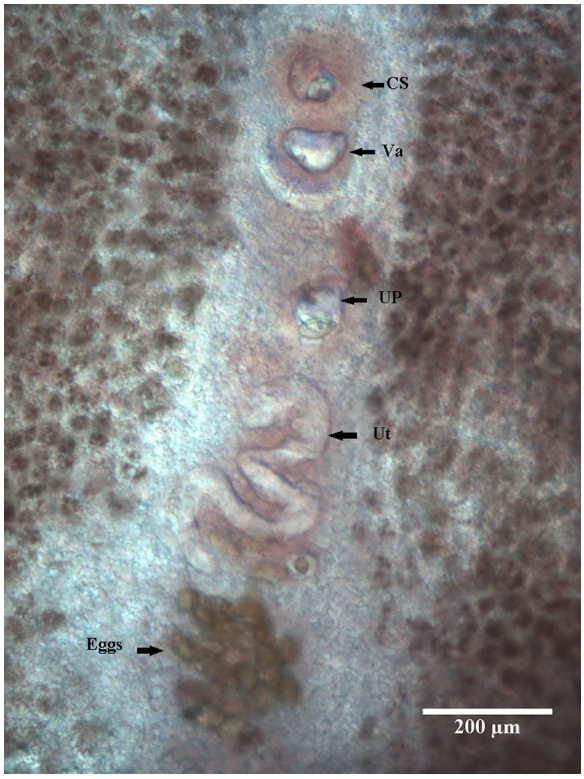

Fig. 2. Gravid proglottid of *Spirometra erinacei*. Scale bar: 200 µm. CS: Cirrus Saco. Va: Vagina. UP: Uterine Pore. Ut: Uterus. Eggs: eggs of *Spirometra erinacei* within uterus.

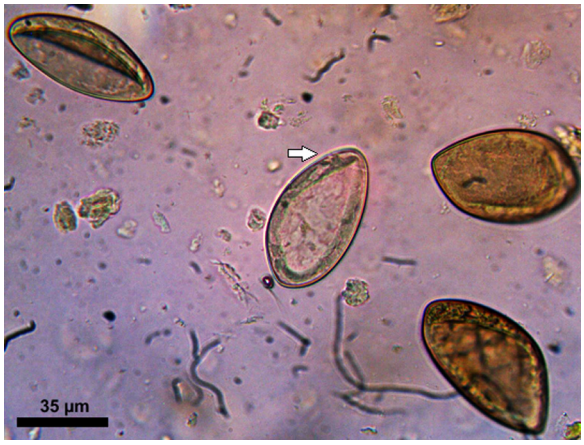

Fig. 3. Eggs of *Spirometra erinacei.* The arrow indicates the operculum of the egg. Scale bar: 35 µm.

These eggs showed the following characteristics: light brown, with pointed ends and the cap quite evident, but widespread pleomorphism eggs was observed within the same preparation (Fig. 3). Measure from 66.64 µm (range = 55.23–78.9) by 37.11 µm (range = 28.93–44.71) ($n=63$).

4. Discussion

The taxonomy of the genus *Spirometra* is confounded by inadequate descriptions. The term *Spirometra* was suggested as Subgenus within *Diphyllobotrium* by Faust, Kellog Campbel in 1929, to include diphillobothrids with coiled uterus and stacked eggs with pointed ends, unlike rosette uterus and eggs with rounded ends typical of the genus *Diphyllobothrium*. Subsequently, *Spirometra* was elevated to the level of genus (Mueller, 1935). At present, there is still discrepancy among researchers about the denomination of species in the genus *Spirometra*, but in general it is accepted that there are two species, *S. erinacei* and *S. mansonoides*. The basic differences between the two species are: the vagina of *S. mansonoides* is located medially and descends in a straight line, whereas the vagina of *S. erinacei* lies besides the midline and descends in waves of different amplitude. The other difference between the two species is the shape of the uterus, which is simple, uniform and always presents two turns anteriorly in a "C" in the case of *S. mansonoides*, as opposed to the uterus of *S. erinacei* which lacks uniformity in the number of turns (between three and seven loops), besides having irregular arrangement and size (Mueller, 1936, 1974; Wardle and McLeod, 1952). Despite these morphological differences, both species have similar biologic, physiological and epidemiological characteristics (Mueller, 1936; Lee et al., 1997). Based on the morphological examination, the species in our sample were identified as *Spirometra erinacei*.

Pampas fox was found parasitized by *S. mansonoides* in Paraguay (Schmidt and Martin, 1978) and in Brazil there are reports of *Spirometra* sp. eggs in fecal samples from this host species (Lucas, 2000; Ruas et al., 2002, 2008). Here we report the first confirmed cases of *S. erinacei* in Pampa fox. The diet of this fox is very varied including small mammals, amphibians, reptiles and birds, among other items (Canepuccia et al., 2008; Vuillermoz, 2001; Medel and Jaksic, 1988), which could be the intermediate hosts for *S. erinacei.* Furthermore, this parasite appears to be particularly suited to the red fox and attains a very large size (Coman, 1973).

The information on the epidemiology of *S. erinacei* is scarce, worldwide. In dogs from Korea, the prevalence reported was of 2% ($n=102$) (Cho et al., 1981), in golden jackal from Iran 7% ($n=100$)

and in red fox from Australia 29% ($n = 930$) (Ryan, 1976; Dalimi and Mobedi, 1992). In Central and South America, there are no reported *S. erinacei* prevalences in definitive hosts. Compared with the results of studies cited above, our study we found a relatively high prevalence of *S. erinacei*, both in small intestine (adults) and in fecal samples (eggs) of Pampas fox in the province of Buenos Aires, Argentina.

In Argentina, there are no published studies confirming the presence of *Spirometra* species in other wild definitive hosts. There are only studies that reported the presence of the family Diphyllobothridae in fecal samples of domestic and wild canids (Milano and Oscherov, 2005; Gonzalez, 2012). The genus *Diphyllobothrium* sp., has been diagnosed in fecal samples in both domestic and wild animals (Martínez et al., 2000, 2005; Zunino et al., 2000).

The combination of the two coproparasitological techniques used in this study (flotation Sheather and sedimentation Ritchie) allow us to obtain eggs of *Spirometra* sp. These eggs were found in fecal samples of every intestine in which adult *Spirometra* were recovered. Moreover we believe the non-recovery of adult forms from the five intestines with positive feces for *Spirometra* sp. was due to the disintegration of the adult, since in different intestines were found proglottids in poor condition, so we suspect that the time and manner of storage of those samples was not adequate.

Regarding the zoonotic importance of *Spirometra*, human cases have been reported for this parasite in Asia (Sim et al., 2002); sporadically in Australia, USA, South Africa and South America (Miyazaki, 1991; Botero and Restrepo, 1998; Beaver et al., 2003). *Spirometra* cestodes do not reach the adult stage in humans except in rare cases, and when this happens, the presence of adult parasite not a serious medical problem (Beaver et al., 2003). The most common episodes are caused by the dissemination of larvae in different tissues, a condition known as sparganosis and whose clinical manifestations vary depending on the location of the parasite (Botero and Restrepo, 1998; Beaver et al., 2003; Beveridge et al., 1998; Orihel and Ash, 1995). In Argentina, three cases of Sparganosis have been reported. Two cases occurred in adults; one with brain localization (Boero et al., 1991) and the other with cutaneous localization (De Roodt et al., 1993). In addition, Jones et al. (2013) have reported one case of Cerebral Sparganosis in a child. But all this cases were individuals of bordering countries. However, the knowledge of prevalence of *Spirometra* sp. in wild animals of Argentina is necessary due to ongoing changes on the environment that affect parasite ecology and its transmission dynamic. *Spirometra* sp. is a good model for ecological interaction studies, allowing us to understand and define trophic levels of the intermediate and definitive hosts, and later to establish the parasite distribution within a host population (Denegri, 2008).

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