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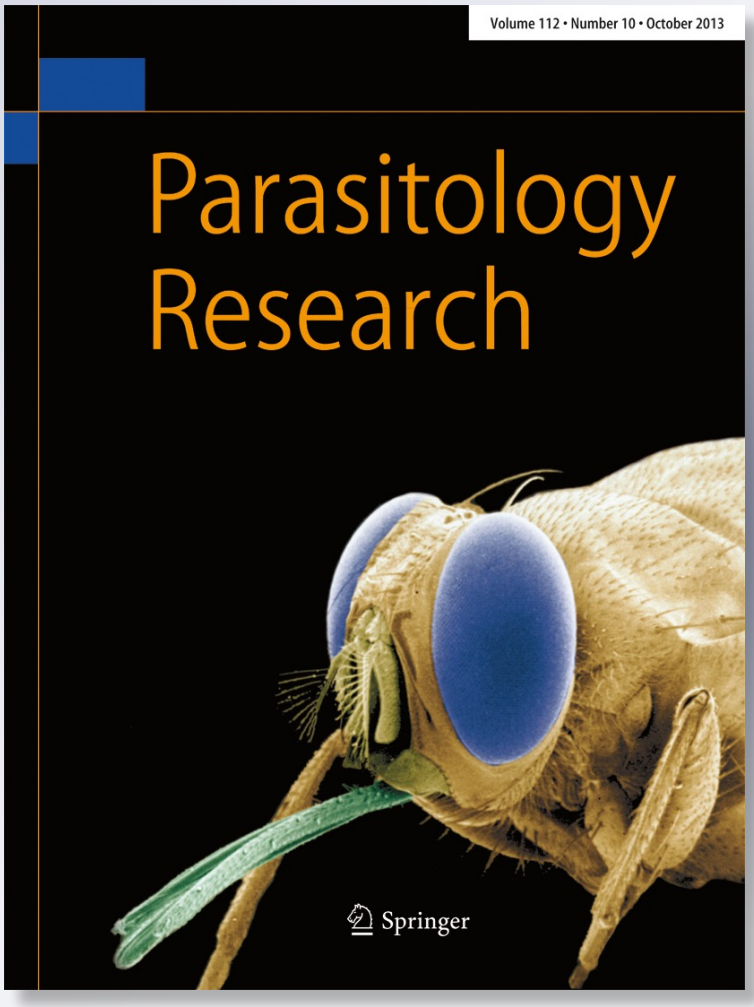

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ORIGINAL PAPER

Epidemiological studies on *Echinococcus* in Pampas fox (*Lycalopex gymnocercus*) and European hare (*Lepus europaeus*) in Buenos Aires province, Argentina

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Abstract In Argentina, hydatid disease caused by *Echinococcus granulosus* is widespread. The south of Buenos Aires province, Argentina, is one of the three regions where hydatidosis is endemic. Although domestic dogs and sheep are considered to be the main hosts for *E. granulosus*, the potential role of wildlife in the local transmission of *E. granulosus* has not been investigated. The aim of this study was to estimate the hydatidosis/echinococcosis prevalence in European hare (*Lepus europaeus*) and Pampas fox (*Lycalopex gymnocercus*), two abundant species with a strong predator–prey relationship in rural areas of Buenos Aires province using different diagnostic tests. A total of 61 fox intestines were examined, finding that 52 (85.2 %) harbored at least one helminth species. However, no adult or immature form of *Echinococcus* sp. was found in the intestinal contents. Coproparasitological analysis and Copro–ELISA followed by Copro–PCR were used as supplementary diagnostic tests. Only one (1.7 %) of 59 fecal samples was positive to Taeniidae eggs by coproparasitological analysis, but this same sample was negative by the Copro–ELISA test. The analysis by Copro–ELISA showed 6 of 57 (10.6 %) positive samples, but the Copro–PCR tests carried out on these samples were negative to *E. granulosus*. A total of 6,808 lungs, 3,576 livers, and 3,542 hearts of hunted hares were examined and palpated, but no structure resembling hydatid cysts were detected. Our results suggest that hares and Pampas foxes are not currently important wild reservoirs of *E. granulosus* in the studied area.

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Introduction

Hydatidosis is a zoonotic disease caused by larval stages of cestodes of the genus *Echinococcus* (Rudolphi 1801) (Cestoda: Taeniidae) that may cause severe illness and even death in the intermediate host. The life cycles of these parasites necessarily involve two mammalian hosts that have an obligatory predator–prey relationship. The adult cestode inhabits the small intestine of carnivores (definitive host), and metacestodes (hydatid cysts) develop in the internal organs of intermediate hosts (herbivorous and omnivorous mammals, including humans) as a result of ingestion of *Echinococcus* sp. eggs shed into the environment with feces of carnivores (Jenkins et al. 2005).

Five *Echinococcus* species are currently recognized as valid *Echinococcus granulosus* sensu lato Batsch 1786, *Echinococcus multilocularis* Leuckart 1863, *Echinococcus*

oligarthrus Diesing 1863, *Echinococcus vogeli* Rausch and Berstein 1972, and *Echinococcus shiquicus* Xiao et al. 2005. *E. granulosus* s. l. is the most widely distributed species with high prevalence rates reported in Eurasia, North and East of Africa, Australia, and South America (Eckert et al. 2001; Jenkins et al. 2005; Zhenghuan et al. 2008). The transmission of *E. granulosus* s. l., particularly within the domestic context, is influenced by human habits and activities, public policies, and the presence of wildlife reservoirs (Jenkins et al. 2005).

In Argentina, there are three regions where hydatid disease has a high rate of transmission: the Patagonia in the south, the province of Buenos Aires in the center, and the Northeastern region. These three regions are associated with small ruminant production (OPS 2004).

From the extensive list of mammals that were documented to be naturally infected with *E. granulosus* s. l., just a few are really relevant for the epidemiology of the disease in humans (D'Alessandro 2002). In Australia, *E. granulosus* persists in two transmission cycles, domestic and wild. The first includes sheep as intermediate host and the second, marsupials (kangaroos and wallabies) (Durie and Riek 1952; Kumaratilake and Thompson 1982). These two cycles are linked through different carnivores (domestic dogs, wild dogs, dingoes, and red foxes) that act as definitive hosts (Thompson and McManus 2002). In the Iberian Peninsula, the wolf (*Canis lupus*) and the deer (*Cervus elaphus*) appear to be successful wild hosts for *E. granulosus* (D'Alessandro and Rausch 2008). In North America, *E. granulosus* occurs in at least four wild systems: wolf–elk (*Cervus canadensis*) (Riley 1933; Rausch 1952), coyote (*Canis latrans*)–deer *(Odocoileus* spp.) (Brunetti and Rosen 1970; Liu et al. 1970), jackal (*Canis mesomelas*)–sambhur (*Rusa unicolor*) (Paramananthan and Dissanaike 1961; Dissanaike and Paramananthan 1962), and wolf–moose (*Alces alces*) (Jenkins et al. 2005). Massive presence of cysts of *E. granulosus* was documented in wild boar in Spain (Martín Hernando et al. 2008). Recently, Himsworth et al. (2010), reported a sylvatic cycle of *E. granulosus* s. l. between wild cervids and domestic dogs in an indigenous community from Canada.

In contrast, there are few reports about the role of wildlife in the maintenance of infection by *E. granulosus* s. l. in South America. The first studies in Argentina on the existence of wild reservoirs of *Echinococcus* were made by Szidat (1963) who reported a prevalence of 12 % (6/50) in culpeo fox (*Lycalopex culpaeus*) captured in the pre-Andean cordillera. Blood and Lelijveld (1969) reported 3.6 and 15.5 % infection rates in Pampas fox (*Lycalopex gymnocercus*) and the gray fox (*Lycalopex griseus*), respectively, but no evidence of infection was found in 31 specimens of culpeo fox captured in southern Argentina. An independent sylvatic cycle involving culpeo fox and European hare (*Lepus europaeus*) was described in Neuquén province, Argentina, with a prevalence of 25 % (4/16) in the fox and 5.6 % (4/71) in the hare (Schantz et al. 1972). In Chile, Alvarez (1961) failed to find the parasite in 534 culpeo fox samples but Aguilera (2001) reported natural infection with *E. granulosus* s. l. in 2.2 % of 45 of gray fox examined. A recent study carried out in Tierra del Fuego province reported the presence of a single adult of *E. granulosus* s. l. in one of 81 (1.2 %) gray foxes examined (Zanini et al. 2006).

Experimental infection by feeding cysts of *E. granulosus* to culpeo fox, gray fox, and Pampas fox showed that all three species are susceptible to infection. The susceptibility between the culpeo fox and dogs was similar, but most gray foxes and Pampas foxes were less susceptible than dogs, although gravid strobilae were recovered from some individuals of both species (Schantz et al. 1976). To our knowledge, no experimental infection using hares has been carried out, but there has been work in another Lagomorph, the European rabbit, where inoculation with protoscoleces of *E. granulosus* resulted in the development of hydatid cysts (Heath 1970; Sahin et al. 1997; Yetim et al. 2005; Ahmadnia et al. 2009).

The Pampas fox inhabits grasslands and open woodlands of the Southern Cone of South America, where it is one of the most common and widespread carnivore species, and it also occur in areas highly modified by extensive ranching and agricultural activities (Lucherini et al. 2004). The current Pampas fox population in Buenos Aires province is estimated at 260,000 individuals (Porini and Ramadori 2007). 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). In Buenos Aires province, frequent food items include rodents, European hares, birds, insects, fruits, carrion, and garbage (Crespo 1971; García and Kittlein 2005; Farias and Kittlein 2008). Other studies found significant contribution of domestic livestock (Drittanti et al. 1998).

The European hare, *L. europaeus*, (Pallas 1778) (Lagomorpha: Leporidae) was introduced into Argentina in 1888 from Germany to promote sport hunting (Grigera and Rapoport 1983). Its own characteristics and the prevailing land use change facilitated its proliferation. It became a resource of economical importance in the country as its meat is exported, and it is considered a pest for forestry and agriculture (Bonino 2006).

Although the preventive measures implemented by the hydatidosis/echinococcosis control program had a significant effect on the domestic dog–sheep cycle, the continuing infection of wild canids is possible due to some prevailing eco-epidemiological conditions (Zanini et al. 2006).

The present work was aimed at studying the occurrence of *Echinococcus* sp. in Pampas fox and European hare in Buenos Aires, and thus to obtain information about their possible role in a sylvatic life cycle. These two species are of relevance because of their abundance and strong predator–prey relationship in the study area.

Materials and methods

Study area

The study was conducted in rural areas located in seventeen departments of the south of Buenos Aires province, Argentina, 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 and European hares.

Source of samples

Potential wild definitive host of E. granulosus

We used 61 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 2011 (Fig. 1). 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 for at least 1 month prior to processing in order to inactivate *Echinococcus* spp. eggs.

Examination of the intestinal content was performed using the sedimentation and counting technique described by Eckert et al. (2001) with some modifications. The small intestine was separated from the large intestine, and then each section of intestine was placed in different trays and cut lengthwise. Coarse material and large parasites of the small intestine were removed. A fecal sample was obtained from the rectum for coproparasitological and molecular analyses (see below). Then, each intestine section was immersed in 9 % saline solution at 37 °C for 30 min. Intestinal walls were scraped with a microscope slide, and all the intestinal content of each section were poured into individual glass bottles and left to stand for 20 min. The supernatant was discarded and physiological saline solution was added to dilute the sediment. This procedure was repeated several times until the supernatant was almost translucent. Obtained sediments were examined in small portions of 5 to 10 ml round petri dishes with magnifier lens at ×65 to identify small helminths.

The helminths found were cleaned with saline solution and deposited in recipients with either 4 % formalin or 70 % ethanol for further taxonomic and molecular examination, respectively.

Small fecal aliquots were separated for Copro–ELISA test (subsample a) and Copro–PCR test (subsample b). These were stored frozen until processed. Only the positive samples

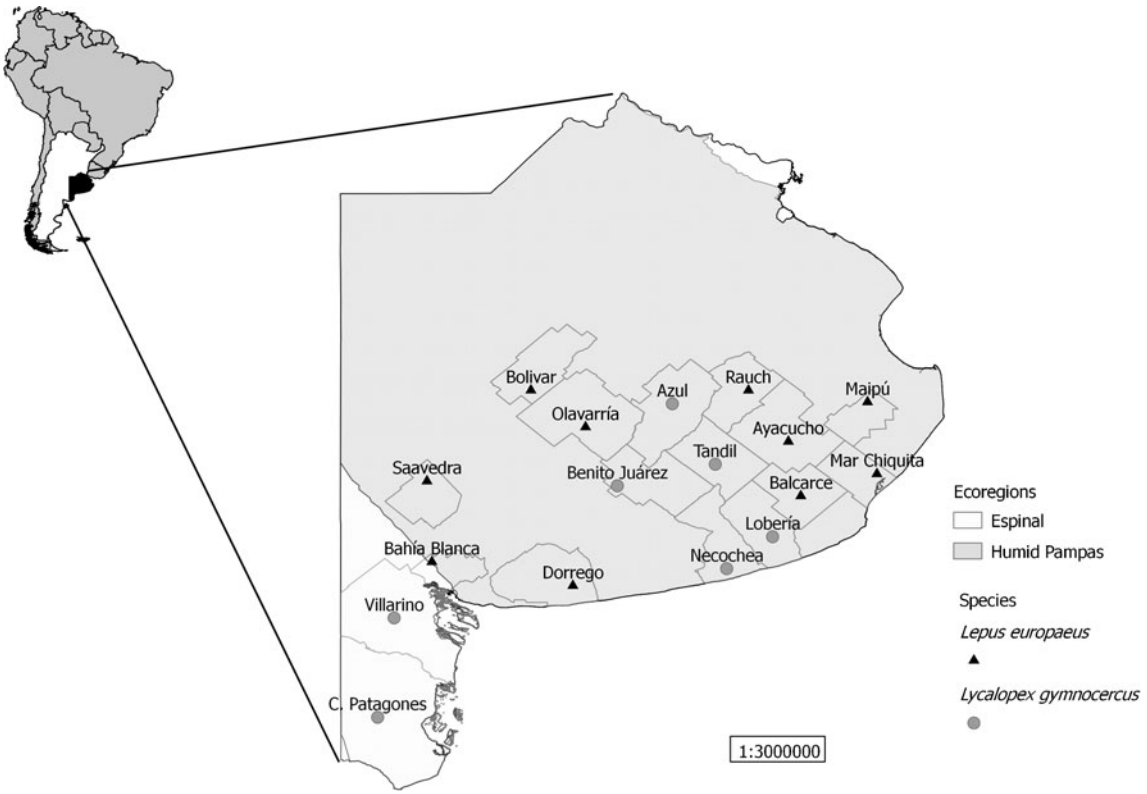

Fig. 1 Sampling sites in Buenos Aires province, Argentina. The map was made with the program Quantum GIS

determined by Copro–ELISA tests were processed through Copro–PCR to confirm the presence of *E. granulosus* DNA. The remainder of each sample was concentrated by Ritchie sedimentation (Young et al. 1979) and Sheather flotation (Benbrook and Sloss 1965) methods. Then, they were examined microscopically (by duplicate) at ×100, ×400, and ×1,000 for taeniid egg identification (Soulsby 1987).

(a) Copro–ELISA: One part of phosphate buffered saline (PBS) 0.15 M with 0.3 % of Tween 20 was added to fox fecal supernatant. Resulting mix were shaken vigorously with a vortex, and it was centrifuged at 3,500 rpm for 30 min at room temperature (Pierangeli et al. 2010). The Coproantigen test was performed using a sandwich ELISA protocol described by Pierangeli et al. (2010). The cutoff value was calculated as described below. In all plates, blank (PBS instead of fecal sample) positive controls (fecal sample with 5 μg/ml of *E. granulosus* somatic antigen) and negative controls (fecal sample from helminth-free dogs) were tested. All fecal samples and controls were assayed by duplicate. The cutoff was performed with receiver operating characteristic curves, and the value was determined by the mean plus three standard deviations of the optical densities of 30 negative fecal samples (Pierangeli et al. 2010).

(b) Copro–PCR: DNA from 150 mg of fecal samples was extracted using *AccuPrep*® Stool DNA Extraction Kit (BIONEER, Daejeon, Republic of Korea) following the manufacturer's instructions, but incubating 2 h at 56 °C with 800 μg/ml of proteinase K (Biobasic, New York, USA). Finally, DNA was eluted with 100 μl of elution buffer. For *E. granulosus* DNA detection, a region of 125 bp of the mitochondrial cytochrome C oxidase subunit 1 (*cox1*, AN: HF947597.1) was amplified by PCR using the following primers: Ech1F 5′CTATAGTGTGTTTGG GTAGCAGGG 3′ and Ech1R 5′CACCTTTATACCAGT AGGAACCC 3′. PCR was performed in a final volume of 12.5 μl containing 200 μM of each dNTP (Finnzymes, USA), 0.4 μM of each of the primers (IDT, Iowa, USA), 2 mM of $MgCl_2$ (Fermentas, USA), 5 % *v*/*v* of DMSO (Finnzymes, USA), and 0.65 units of *Taq* DNA polymerase (Fermentas, USA) in 10× *Taq* Buffer with $(NH_4)_2SO_4$. In all samples, we used 2 μl of DNA and 1/10 DNA dilution to PCR inhibition control. A positive control (*fkbp* gene from *E. granulosus*) was used to check the reaction. PCR conditions were as follows: an initial denaturing step (94 °C for 3 min) followed by 40 cycles at 94 °C for 45 s (denaturation), 50 °C for 45 s (annealing), 72 °C for 45 s (extension), and a final extension step (72 °C for 10 min). A negative control was included in all PCR assays. The specificity and size of amplification products were assessed by electrophoresis using a 100-bp DNA ladder in 2 % (*w*/*v*) Tris–borate/EDTA agarose gels and stained with SYBR® Gold Nucleic Acid Gel Stain (Invitrogen, Buenos Aires, Argentina).

Potential wild intermediate hosts of E. granulosus

It is noteworthy that the hare is an exotic species in Argentina, and its hunting is permitted in several provinces of this country, including Buenos Aires. The inspection of the organs was done in authorized registered slaughterhouses located in Azul, Tapalqué, and Coronel Vidal cities of the Buenos Aires province. The red viscera (lung, liver, and heart) of about 7,000 hunted European hares from several departments of the province of Buenos Aires were analyzed between May and July for 3years (2010 to 2012) (Fig. 1). Each organ was carefully examined and palpated in search of hydatid cysts.

Results

A total of 61 Pampas foxes (30 females and 31 males) were necropsied in the present work. The foxes belonged to different departments of the province of Buenos Aires, being 13 from Azul, 42 from Villarino, 2 from Tandil, 1 from Benito Juárez, 1 from Lobería, 1 from the Necochea, and 1 from Carmen de Patagones (Fig. 1). All foxes were classified as adults according to their size. The postmortem exam revealed that 52 foxes (85.2 %) had at least one intestinal helminth species. However, no adult or immature form of *Echinococcus* sp. was found in the intestinal contents. In the small intestine, the overall prevalences to nematodes, cestodes, and trematodes were 52, 43, and 31 %, respectively. In addition, 8 % of the large intestines contained nematodes. Only one of 59 fecal samples analyzed was positive to Taeniidae eggs by both concentration techniques used, but this sample was negative to Copro–ELISA.

The analysis by Copro–ELISA of 57 fox fecal samples showed six (10.6 %) positive samples, corresponding to three individuals from Azul and three from Villarino. All samples were run in duplicate. The cutoff point was 0.230, and the average optical densities of positive samples was 0.248. However, the Copro–PCR tests carried out on these samples was negative to *E. granulosus*.

A total of 13,926 organs from hunted hares from several departments of the province of Buenos Aires were thoroughly examined and palpated, corresponding to 6,808 lungs, 3,576 livers, and 3,542 hearts (Table 1). No structure resembling to hydatid cysts was detected.

Discussion

In Argentina, hydatid disease is widespread, being *E. granulosus* s. l. species involved. The southeast of Buenos Aires province is

Table 1 Organs analyzed of European hare (*L. europaeus)* from rural areas of Buenos Aires different departments during the commercial hunting season

Departments	Heart (*n*=3,542)	Liver (*n*=3,576)	Lung (*n*=6,808)	Total
Ayacucho	1,325	611	1,325	3,261
Azul	385	188	449	1,022
Bahía Blanca	114	158	348	620
Balcarce	180	399	507	1,086
Benito Juárez	405	211	565	1,181
Bolivar	43	23	43	109
Dorrego	158	67	224	449
Maipú	–	31	47	78
Olavarría	–	36	137	173
Saavedra	623	707	1,417	2,747
Rauch	282	449	797	1528
Tandil	27	500	651	1,178
Mar Chiquita	–	196	298	494

a region where hydatid disease is a real public health problem because it is endemic, and it occurs at high prevalences in humans, livestock, and dogs (Dopchiz 2006; Lavallén et al. 2011; Dopchiz et al. 2013). No previous *E. granulosus* surveys in either Pampas foxes or hares have been conducted in Buenos Aires province. This is the first epidemiological work that investigated the existence of an independent *E. granulosus* wild cycle involving Pampas fox and European hare in this province using different diagnostic tests.

In this work, inspection of the small intestine was performed exhaustively, but despite the high prevalence of helminths found in the intestine of Pampas foxes (85.2 %), no adult *Echinococcus* sp. was detected in the samples analyzed. Other studies conducted in Pampas fox elsewhere in Argentina and Brazil also failed to detect *Echinococcus* sp. adults, even when they found large burdens of nematodes, trematodes, and cestodes (Ruas et al. 2008; Fuchs et al. 2006). However, many years ago, infection by *Echinococcus* sp. were reported from necropsied foxes from different Argentine localities, although in all cases the prevalences reported were low (Szidat 1963; Blood and Lelijveld 1969; Schantz et al. 1972; Zanini et al. 2006).

In this epidemiological study, we decided to also use other non-invasive diagnostic techniques to detect *E. granulosus* in foxes, such as coproparasitologic analysis and Copro–ELISA followed by confirmation by Copro–PCR to increase the sensitivity of *E. granulosus* detection.

The fox fecal samples were analyzed by two egg concentration techniques to increase the sensitivity of the coprologic exam. Taenidae eggs were observed only in one sample by both concentration techniques. However, this sample was negative to Copro–ELISA, showing that the eggs were not of *E. granulosus*. In fact, this fox was harboring adults of *Taenia* sp. (eggs of all *Echinococcus* and *Taenia* species are morphologically indistinguishable from one another).

Of 57 samples analyzed in this study, only six were Copro–ELISA positive. However, all six samples were negative by Copro–PCR. The Copro–ELISA method used in this work has a sensitivity and specificity of 93.6 and 88.5 %, respectively. It has been validated using domestic dog samples finding a low cross-reactivity with antigens of *Taenia* sp. and nematodes (Pierangeli et al. 2010). This ELISA test has not been validated in wild carnivores. To rule out the possibility of a false positive Copro–ELISA result (Malgor et al. 1997), confirmation by detecting *E. granulosus* DNA in feces using PCR is highly desirable (Abbasi et al. 2003). In this work, the incongruity in the results may be because these techniques detect different targets. In the Copro–ELISA, the target is a protein, while in the Copro–PCR, the target is DNA from the parasite. There is possibility of a Copro–PCR false negative, as DNA amplification could be affected by the presence of PCR inhibitors commonly found in stool samples, which may not be eliminated by the purification kit. However, our negative results by the other techniques employed suggest that false positive Copro–ELISA results should not be ruled out. In any case, even if the Copro–ELISA positive individuals were indeed true positives, there was no indication that the foxes in our sample were a significant source of *Echinococcus* sp. propagules for the ecosystem.

All foxes examined were adults. According to Schantz et al. (1976), the suitability of foxes as definitive hosts of *E. granulosus* varies according to fox species and other factors like age at infection. Experimental studies suggest that they are most susceptible to infection when young (8–12 week old), becoming progressively more refractary as they grow older (Gemmell 1959; Thompson 1983). Therefore, the probability of infection in our sample may be lower that of the population, as it did not include young animals.

Over the last three seasons of hare commercial hunting in Buenos Aires province (2010, 2011, and 2012), we inspected 13,926 organs of hares hunting without detection of hydatid cysts. These negative results coincide with those of Alvarez (1961), who did not find any hydatid cyst in 95 hares examined in Chile. However, in Argentina, Schantz et al. (1972) found fertile hydatid cysts in 5.6 % (4/71) hares examined and Almudevar et al. (1986) found infertile hydatid cysts in 0.4 % (16/3,646) hare viscera. Also, surveys in North America and Europe revealed the presence of hydatid cysts in hare organs (Erickson 1944; Bouvier et al. 1954). We agree with Almudevar et al. (1986), who suggested that the slow larval development of *E. granulosus* (compared to that of *E. multilocularis*) combined with the high hunting pressure makes hares in the field little likely to harbor a mature cyst.

Given the results reported in this work, we conclude that hares and Pampas foxes are not important wild reservoirs of *Echinococcus* sp. in Buenos Aires, confirming that the sheep–

dog cycle is the main cycle in this region of high endemicity. However, the continuous changes that the ecosystems are suffering in this province may result in a change of the current eco-epidemiological conditions, contributing to the development of a wild cycle of *E. granulosus*, as studies already cited demonstrated that some wild species in Argentina are suitable hosts for this parasite. Therefore, the potential role of wildlife must continue to be probed.

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References

Abbasi I, Branzburg A, Campos-Ponce M, Abdel Hafez SK, Raoul F, Craig PS et al (2003) Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified DNA sequence. AmJTrop Med Hyg 69:324–330

Aguilera FJC (2001) Estudio preliminar de equinococosis y helmintiasis gastrointestinal en zorro gris (*Pseudalopex griseus*) silvestre de Tierra del Fuego, Chile. Memoria de Titulo, Med. Vet. Universidad de Concepción, Fac. Med. Vet. Chillán, Chile pp 56

Ahmadnia H, Kalantari MR, Yarmohammadi AA, Nekooee S, Ghanbarizadehe SR (2009) An experimental model of hydatidosis in rabbit testis. Q Horiz Med Sci 14:26–30

Almudevar M, Lamberti R, Sasso W, Tome M (1986) Hidatidosis en libre europea en La Pampa. Vet Arg 27:42–43

Alvarez V (1961) Investigaciones sobre equinococosis silvestre en Chile. Biológica 31:89–94

Benbrook EA, Sloss MW (1965) Parasitología Clínica Veterinaria. Compañía Editorial Continental, México

Blood BD, Lelijveld JL (1969) Studies on sylvatic echinococcosis in southern South America. Z Tropenmed Parasitol 20:475–482

Bonino N (2006) Estado actual del conocimiento sobre la liebre europea y el conejo europeo introducidos en la Argentina. INTA EEA Bariloche Argent Comun Técnica Fauna 61:1–29

Bouvier G, Burgisser H, Schneider PA (1954) Monograph on diseases of hares in Switzerland. Service Vétérinaire Cantonal et Institut Galli Valerio, France

Brunetti OA, Rosen MN (1970) Prevalence of *Echinonoccus granulosus* hydatids in California deer. J Parasitol 56:1138–1140

Crespo JA (1971) Ecología del zorro gris *Dusicyon gymnocecus antiquus* (Ameghino) en la Provincia de La Pampa. Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". Ecología 1:147–205

D'Alessandro A, Rausch RL (2008) New Aspects of neotropical polycystic (*Echinococcus vogeli*) and unicystic (*Echinococcus oligarthrus*) echinococcosis. Clin Microb Rev 41:380–401

D'Alessandro A (2002) Descripción morfológica, ciclo biológico y distribución geográfica de las especies del género *Echinococcus*. In: Denegri GM, Elissondo MC, Dopchiz MC (eds) Situación de la hidatidosis-echinococcosis en la República Argentina. Martín, Mar del Plata, pp 19–30

Dissanaike AS, Paramananthan DC (1962) On the Occurrence and Significance of Hydatid Cysts in the Ceylon Sambhur *Rusa unicolor unicolor*. Ceylon J. Med. Sci (D)11:1–7 http://dl.nsf.ac.lk/bitstream/1/7004/2/CJMS-11(1)-1.pdf. Accessed 10 Apr 2013

Dopchiz MC (2006) Aspectos epidemiológicos de la hidatidosis-echinococcosis en el sudeste de la provincia de Buenos Aires. PhD Thesis, Universidad Nacional de Mar del Plata. Martín, Mar del Plata, p 201

Dopchiz MC, Lavallén CM, Bongiovanni R, Gonzalez PV, Elissondo C, Yannarella F, Denegri GM (2013) Endoparasitic infections in dogs from rural areas in the Lobos District, Buenos Aires province, Argentina. Revista Brasileira de Parasitologia Veterinária. doi:10.1590/s1984-29612013005000008

Drittanti M, Lucherini M, Casanave E (1998) La dieta serrana del zorro, *Pseudalopex gymnocercus*. Dissertation: XIII Jornadas Argentinas de Mastozoología (SAREM) Puerto Iguazú, Misiones. p 129

Durie PH, Riek RF (1952) The role of the dingo and wallaby in the infestation of cattle with hydatids (*Echinococcus granulosus* (Batsch, 1786) Rudolphi, 1805) in Queensland. Aust Vet J 28:249–254

Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS (2001) WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. World Organization for Animal Health (OIE), Paris

Erickson AB (1944) Helminth infections in relation to population fluctuations in snowshoe hares. J Wildl Manag 8:134–153

Farias AA, Kittlein MJ (2008) Small-scale spatial variability in the diet of pampas foxes (*Pseudalopex gymnocercus*) and human-induced changes in prey base. Ecol Res 23:543–550

Fuchs L, Baldone V, Rojas M, Fort M, Gimenez H, Kin M (2006) Endoparásitos Hallados en el Zorro gris Pampeano (*Pseudalopex gymnocercus*) en la Provincia de La Pampa, Argentina. Boletín de divulgación técnica. INTA EEA Anguil. 90: 190–194. http://www.produccion-animal.com.ar/fauna/105-zorro.pdf

García VB, Kittlein MJ (2005) Diet, habitat use, and relative abundance of pampas fox (*Pseudalopex gymnocercus*) in northern Patagonia, Argentina. Mamm Biol 70:218–226

Gemmell MA (1959) Hydatid disease in Australia.VI. Observations on the carnivora of New South Wales as definitive hosts of *Echinococcus granulosus* (Batsch, 17E6) (Rudolphi, 1801), and their role in the spread of hydatidiasís in domestic animals. AustVet J 450–455

Grigera DE, Rapoport EH (1983) Status and distribution of the European hare in South America. J Mammal 64:163–166

Heath DD (1970) The development of Echinococcus granulosus larvae in laboratory animals. Parasitol 60:449–456

Himsworth CG, Jenkins E, Hill JE, Nsungu M, Ndao M, Thompson RCA, Covacin C, Ash A, Wagner BA, McConnell A, Leighton FA, Skinner S (2010) Emergence of sylvatic *Echinococcus granulosus* as a parasitic zoonosis of public health concern in an indigenous community in Canada. Am J Trop Med Hyg 82:643–645

Jenkins DJ, Romig T, Thompson RCA (2005) Emergence/re-emergence of *Echinococcus* spp.—a global update. Int J Parasitol 35:1205–1219

Kumaratilake L, Thompson R (1982) A review of the taxonomy and speciation of the genus *Echinococcus Rudolphi* 1801. Z Parasitenkd 68:121–146

Lavallén CM, Dopchiz MC, Lobianco E, Hollmann P, Denegri GM (2011) Intestinal parasites of zoonotic importance in dogs from the District of General Pueyrredón (Buenos Aires, Argentina). Rev Vet 22:19–24, Available from: http://vet.unne.edu.ar/revista/22-1%202011/RevVet_vol_22_nro_1_2011-04_Lavallen.pdf

Liu IKM, Schwabe CW, Schantz PM, Allison MN (1970) The occurrence of *Echinococcus granulosus* in coyotes (*Canis latrans*) in the central valley of California. J Parasitol 56:1135–1137

Lucherini M, Pessino M, Farias A (2004) Pampas fox. In: Sillero-Zubiri C, Hoffmann M, Macdonald DW (eds) Canids: foxes, wolves, jackals and dogs. Status survey and conservation action plan. IUCN/SSC Canid Specialist Group, Gland, pp 63–68

Malgor R, Nonaka N, Basmadjian I, Sakai H, Carámbula B, Oku Y et al (1997) Coproantigen detection indogs experimentally and naturally infectedwith *Echinococcus granulosus* by amonoclonal antibody-based enzyme linked immunosorbent assay. Int J Parasitol 27:1605–1612

Martín Hernando MP, González LM, Ruiz-Fons F, Garate T, Gortazar C (2008) Messive presence of *Echinococcus granulosus* (Cestoda, Taeniidae) cysts in a wild boar (*Sus scrofa*) from Spain. Parasitol Res 103:705–707

OPS (Organización Panamericana de la Salud) (2004) Informe del Proyecto Subregional Cono Sur de control y Vigilancia de la Hidatidosis: Argentina, Brasil, Chile y Uruguay. Montevideo. ISBN: 9974-7700-5-X. http://www.bvsops.org.uy/pdf/equinoc.pdf. Accessed 10 Mar 2013

Paramananthan DC, Dissanaike AS (1961) Sylvatic hydatid infection in Ceylon. Trans R Soc Trop Med Hyg 55

Pierangeli N, Soriano SV, Roccia I, Bergagna HFJ, Lazzarini LE, Celescinco A, Kossman AV, Saiz MS, Basualdo JA (2010) Usefulness and validation of a coproantigen test for dog echinococcosis screening in the consolidation phase of hydatid control in Neuquén, Argentina. Parasitol Int 59:394–399

Porini G, Ramadori D (2007) Estado de conocimiento sobre el manejo de zorros de interés económico en Argentina. Dirección de Fauna Silvestre, Secretaría de Ambiente y Desarrollo Sustentable 11p. Available from: http://www.ambiente.gov.ar/archivos/web/Pzorros/File/INFORME_ZORROS_2007.pdf

Rausch RL (1952) Hydatid disease in boreal regions. Arctic 5:157

Riley WA (1933) Reservoris of *Echinococcus* in Minnesota. Minn Med 16:744–745

Ruas JL, Muller G, Farias NAR, Gallina T, Andreia SL, Felipe GP, Linkoc L, Brum JGW (2008) Helmintos do Cachorro do Campo, *Pseudalopex gymnocercus* (Fischer, 1814) e do Cachorro do Mato *Cerdocyon thous* (Linnaeus, 1766) no Sul do Estado do Rio Grande do Sul, Brasil. Rev Bras Parasitol Vet 17:87–92

Sahin M, Aydin A, Bülbüloğlu E, Ciralik H (1997) Experimental hydatid disease of the liver. Eur J Clin Investig 27:537–538

Schantz PM, Lord RD, de Zavaleta O (1972) *Echinococcus* in the South American red fox (*Dusicyon culpaeus*) and the European hare (*Lepus europaeus*) in the Province of Neuquén, Argentina. Ann Trop Med Parasitol 66:479–485

Schantz PM, Colli C, Cruz-Reyes A, Prezioso U (1976) Sylvatic echinococcosis in Argentina. II. Susceptibility of wild carnivores to *Echinococcus granulosus* (Batsch, 1786) and host-induced morphological variation. Tropenmed Parasitol 27(1):70–78

Soulsby EJL (1987) Parasitología y enfermedades parasitarias en los animales domésticos. Helmintos, artrópodos y protozoos de los animales domésticos. Nueva Editorial Interamericana, México

Szidat L (1963) Studien uber den Erreger der alveolaren Echinococcen krankheit des Menschen in Sudamerika. Z Parasitenkd 23:80–91. doi:10.1007/BF0026033910.1007/BF00260339. (In German)

Thompson RCA (1983) The susceptibility of the European red fox (*Vulpes vulpes*) to infection with *Echinococcus granulosus* of Australian sheep origin. Ann Trop Med Parasitol 77:75–82

Thompson R, McManus DP (2002) Towards a taxonomic revision of the genus *Echinococcus*. Trends Parasitol 18:452–457

Yetim I, Erzurumlu K et al (2005) Results of alcohol and albendazole injections in hepatic hydatidosis: experimental study. J Gastroenterol Hepatol 20:1442–1447. doi:10.1111/j.1440-1746.2005.03843.x

Young KH, Bullock SL, Melvin DL, Spruill CL (1979) Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. J Clin Microbiol 10:852–853

Zanini F, Laferrara M, Bitsch M, Pérez H, Elissondo ME (2006) Epidemiological studies on intestinal helminth parasites of the patagonian grey fox (*Pseudalopex griseus*) in Tierra del Fuego, Patagonia Argentina. Vet Parasitol 136:329–334

Zhenghuan W, Xiaoming W, Xiaoqing L (2008) Echinococcosis in China, a review of the epidemiology of *Echinococcus* spp. EcoHealth 5:115–126