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Short communication

Fasciola hepatica infections in livestock flock, guanacos and coypus in two wildlife reserves in Argentina

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ABSTRACT

Between autumn and spring 2006, a coprological survey was performed in two wildlife reserves located in the north of Argentine Patagonia to determine the prevalence of *Fasciola hepatica* and the number of parasite eggs per gram (epg) of feces in wild guanacos (*Lama guanicoe*), coypus (*Myocastor coypus*), and locally born and raised goats and sheep. Snails of the Family Lymnaeidae were collected in freshwater habitats, identified taxonomically and analyzed parasitologically.

Prevalence of patent infection was 100% in sheep ($n=69$) and coypus ($n=9$), 84% in goats ($n=20$) and 0.5% in guanacos ($n=224$). No significant differences in epg were found among animals, but the median epg of coypus (160) and sheep (160) was higher than that of goats (80). For guanacos and goats, a negative binomial model estimating the population egg-count frequency could be fitted, while for coypus and sheep parasite egg-count frequencies trended toward a normal distribution, indicative of a more even, and much less aggregated distribution across sampled hosts. All snails ($n=175$) were *Lymnaea truncatula* and none of them was found infected. This is the first report of fascioliasis in free-ranging guanacos in Argentina. Coypu appears to be a major wildlife reservoir of *F. hepatica*, which was presumably introduced locally by livestock.

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1. Introduction

Fasciola hepatica, the liver fluke, has an indirect life cycle involving domestic and wild herbivorous mammals and humans as definitive hosts, and freshwater gastropods of the Family Lymnaeidae as intermediate hosts (Andrews, 1999). Infections with this parasite give rise to a cosmopolitan zoonotic disease, fascioliasis, which is a food-borne trematodiasis (Torgerson and Claxton, 1999). In farmed livestock the disease causes important economic losses due to liver condemnation, decreased meat, milk and wool production and increased mortality (Dargie, 1987).

In Argentina, fascioliasis is of concern in farmed livestock. Liver condemnation rates at abattoirs can be nearly 80%, with highest values reported for cattle from Patagonia (Kleiman et al., 2007). In this region detailed epidemiological surveys involving both definitive and intermediate hosts have been performed in cattle ranches (Kleiman et al., 2005, 2007; Rubel et al., 2005), but as yet there is no information concerning fascioliasis in natural protected areas where animals may, or may not, become infected and play a local role in transmission.

In South America *F. hepatica* has been reported in two large rodent species: the coypu or false nutria (*Myocaster coypus*) in Argentina, and the capybara (*Hydrochoerus capybara*) in Venezuela (cited in Sutton, 1989). South American camelids, e.g. alpacas (*Lama pacos*) and llamas (*Lama glama*), are known to be highly susceptible to

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fascioliasis but generally observed prevalence is low (~8%) inasmuch as mortality in severe acute infections can be 100% (Leguía, 1991).

In this paper, we report the findings of an epidemiological survey in two wildlife reserves in Argentina and document for the first time active transmission foci of *F. hepatica* and infection in wildlife.

2. Material and methods

The study area comprised the Natural Reserves of La Payunia (36°00'S, 68°34'W) and Laguna de Llanquanelo (35°45'S, 69°08'W), both located in the Department of Malargüe, Province of Mendoza, northern Patagonia, Argentina. The La Payunia Reserve occupies 450,000 Ha and hosts the largest (about 10,000 heads) wild guanaco (*Lama guanicoe*) population in the country (Candiar et al., 1993). This camelid has experienced a dramatic population decline in the last 50 years due to severe hunting pressure (Puig et al., 2003) and competition with livestock for grazing pastures (Baldi et al., 2004). The Llanquanelo Reserve occupies 65,000 Ha and was declared a Ramsar site in 1995 (SADSN, 2008).

The climate in the region is cold and arid, with mean temperatures in winter and summer of 6 and 20 °C respectively; and an annual accumulated precipitation of 255 mm (Puig et al., 2003). Biogeographically, the region is included within the Andean–Patagonian domain, Patagonian province, Payunia district, which is characterized by xerophytic vegetation (Cabrera and Willink, 1980). There are scarce water bodies, which are used as water sources by livestock, wild animals and humans (Issia et al., 2008). The local population is small and houses are scattered over a large area (density less than 1 inhabitant/km²); goat and sheep rearing is the main economic activity and cattle are raised for domestic use (Issia et al., 2008). Livestock are allowed to graze freely and generally anthelmintics are only sporadically applied.

To search for *Fasciola* infection, coprological and snail surveys were performed between June (end of autumn) and December (end of spring) 2006. Fresh fecal samples were taken from 20 sheep, 69 goats, 224 guanacos and 9 coypus. Feces of sheep and goats were collected from the ground, immediately after deposition. Migrating guanacos were followed by sight to identify defecating animals, and fecal samples were taken once they had moved away. The members of a colony of coypus inhabiting a stream were individually identified and their feces were collected on the banks and margins. In all cases feces were kept at about 7 °C until processing. The prevalence (number of infected

individuals/number of examined individuals) and the number of eggs per gram (epg) of feces were estimated for each herbivorous species analyzed. Two grams of each fecal sample were processed following the methodology of Kleiman et al. (2005). Eggs of *F. hepatica* were counted under a binocular microscope at 20×.

Egg-count data were recorded from each individual, and then entered onto an electronic format using Microsoft Excel™. The data thus collated were analyzed using MS Excel™ and R statistical package® v2.8.0 (Ihaka and Gentleman, 1996). For prevalence data, confidence intervals (CI₉₅) were estimated using the exact method as described in Armitage and Berry (1994). Significant differences in the prevalences among mammals were tested using the Chi-square test of proportions from several independent samples (Fleiss, 1981). Significant differences in epg were tested using the Kruskal–Wallis test followed by the Dunns test (Siegel, 1956). The significance level was set at $p < 0.05$. To determine infection frequency distributions for individual hosts, infection data were analyzed using Risk Solver Platform™ (Frontline Systems Inc.) in MS Excel. Risk Solver Platform is a powerful simulation and optimization tool that enables distribution prediction using Chi-Square and Kolmogorov–Smirnov tests of independence for non-normal distributions (Steel and Torrie, 1980).

A total of 175 lymnaeid snails were hand-collected from several sampling sites along two streams, one in La Payunia and the other in Llanquanelo. Snails were transported alive to the laboratory in plastic flasks containing water and vegetation from the collection site. The identification of snails at species level was performed in 64 of the largest specimens collected in the two reserves, so as to increase the probability of sexual maturity. These were relaxed, sacrificed, preserved in Railliet–Henry's fluid (Paraense, 1984), and taxonomically determined by features of the shell and internal organs. Live snails were individually placed in small containers with de-chlorinated tap water and then exposed to light to stimulate emergence of cercariae and their subsequent encystment. Snails from which cercariae did not emerge were dissected to search for developing trematode larvae in the viscera.

3. Results

The prevalences and median epg values for the different herbivores analyzed are shown in the Table 1. The Chi-square test revealed significantly different prevalences among sheep, goats, coypus and guanacos (Chi-square = 270, $p < 0.05$). Further analysis indicated that

Table 1
Prevalence and fecal egg counts (epg) of the animals studied in the reserves La Payunia and Llanquanelo, northern Patagonia, Argentina.

Herbivores	No. analyzed	Prevalence		Eggs per gram			
		%	95% CI	Min–Max	Median (Q1–Q3)	k parameter (95% CI)	
Domesticated	Goat	69	84.1	73.3–91.8	2–920	80 (18.5–250.3)	0.2631(0.1229–0.4031)
	Sheep	20	100.0	83.2–100.0	6–680	160.5 (61.8–277.8)	NA
Wild	Guanaco	224	0.5	0.0–2.5	–	–	<0.01
	Coypu	9	100.0	66.4–100.0	14–254	160 (90–183)	NA

The only infected guanaco had an epg of 19.

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