Coproparasitological evaluation of nematodes and coccidia in a wild vicuña (*Vicugna vicugna*) population in the Argentinean Andean Altiplano

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Abstract

A parasite screening evaluation was conducted to determine the presence of gastrointestinal nematodes and coccidia in wild vicuñas (*Vicugna vicugna vicugna*) from Jujuy, in Argentina´s Andean Altiplano. Faecal samples were collected during the capture of wild vicuñas. Of the 150 vicuñas that were sampled, 40.66 % (61/150) tested positive to gastrointestinal nematodes eggs, 7.33% (11/150) to coccidia oocysts, 4.66% (7/150) to *Nematodirus* sp. eggs and 1 animal tested positive to cestodes eggs shedding. The absence of clinical signs of parasite infestation, combined with the low egg and oocysts counts observed in this survey, suggest low levels of parasitism in wild vicuña (*Vicugna vicugna vicugna)* living in their free-ranging natural (the Santa Catalina region of the Laguna de Pozuelos Basin). This would not represent a threat to the wild vicuñas or to domestic livestock.

Keywords: Argentinean Altiplano, coccidian, gastrointestinal parasites, nematodes, wild vicuñas

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Introduction

Three of the four species of South American camelids (SACs), llamas (*Lama glama*), guanacos (*Lama guanicoe*) and vicuñas (*Vicugna vicugna*) are found in Argentina. The first of these is a domestic species, together with the alpaca (*Vicugna pacos*), while the other two are wild species. Although camelids have a wide distribution in the Andes, vicuñas are restricted to the high altitude Puna or Altiplano (between 3200 to 4700 metres above sea level) of Perú, Bolivia, Chile and Argentina. These vicuñas also have one of the finest fibres in the world (Wheeler and Hoces, 1997). Twenty years after the introduction of international protection laws, some of the Argentinean populations are now classified in appendix II of the Convention on International Trade in Endangered Species (CITES). This has allowed sustainable use of the species through the practice of capture, shearing and release back to nature (chakus) schemes. The Argentinian vicuña is the southern subspecies, *Vicugna vicugna vicugna,* and its distribution includes the Northwest (NW) of the country (Figure 1).
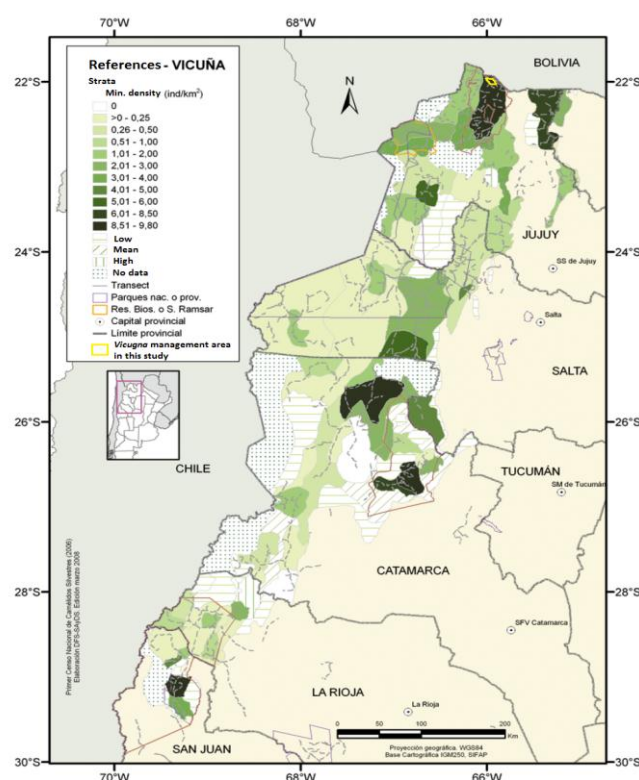

Figure 1. Minimum densities (Indi/km2) of *Vicugna vicugna* in Argentina using the distant transect method and location of vicugna management area for the "Local Management Plan: Conservation and sustainable use of wild vicuñas (*V. vicugna vicugna*) in fields of the Cooperative of Agronomics Producers of Santa Catalina", in this study. (Modified map after Baigún et al., 2008).

Etiological agents of parasitic gastroenteritis in cattle and sheep, such as *Ostertagia* spp*., Haemonchus contortus, Trichostrongylus* spp*., Cooperia* spp. and *Oesophagostomum venulosum,* are also causes of clinical disease in domestic SACs (Fowler, 1998; Cafrune *et al.*, 2001; Beldomenico *et al.*, 2003; Marcoppido *et al.*, 2013). Likewise, host-specific nematodes (*Graphinema aucheniae, Camelostrongylus mentulatus, Nematodirus lamae, Lamanema chavezi, Trichuris tenuis*), protozoa (*Eimeria* spp. and *Sarcocystis* spp.) and *Mycoplasma haemolamae* have been described in llamas, alpacas and captive vicuñas in their original South America habitats (Cafrune *et al.*, 2001; Palacios *et al.*, 2003; Flores *et al.*, 2003; Correa *et al.*, 2012; Carletti *et al.*, 2013; Cafrune *et al.*, 2014). Very few reports refer to the parasite fauna of wild SAC´s. Beldomenico *et al.* (2003), Karesh *et al.* (1998) and Moreno *et al.* (2015) reported the presence of *Haemonchus, Nematodirus, Marshallagia, Ostertagia, Trichostrongylus, Cooperia, Oesophasotomun, Chabertia, Capillaria, Skrjabinema, Trichuris, Strongyloides, Dictyocaulus, Eimeria* and *Sarcocystis* species in wild guanacos from Cabo Dos Bahias Wildlife Reserve (Chubut, Patagonia, Argentina) and La Payunia Reserve (Mendoza, Argentina). Correa *et al.* (2012), reported the existence of several gastrointestinal nematodes, protozoa and *Mycoplasma haemolamae* in guanacos in captivity in Chile.

In wild vicuñas from Pampa Galeras, Perú, Bouts *et al.* (2003), reported the presence of *Nematodirus, Trichuris, Capillaria, Strongyloides, Lamanema chavezi* and *Eimeria punoensis*. In free-ranging vicuñas from Bolivia, Beltran-Saavedra *et al.* (2011), reported a 100% prevalence of *Coccidia* spp., 87.5% prevalence of nematodes and 3.1% prevalence of cestodes, with 87.5% of mixed infections (without specifications). The latter two studies on wild vicuñas were carried out on the northern subspecies (*V. vicugna mensalis*). Our team had no access to published data on the southern wild subspecies from Argentina, so we have assumed that this work is the first of its kind on wild *Vicugna vicugna vicugna*.

The objective of this study was to conduct a parasite screening to detect the presence of nematodes and coccidia in the faeces of wild southern vicuñas (*V. vicugna vicugna*) captured in Santa Catalina, Jujuy, in the Argentinean Altiplano.

Materials and Methods

A total of 416 free-ranging vicuñas (from a total population of approximately 700) were captured during November in three successive years: 2012, 2013 and 2014. There were two capture events each year as part of one of the activities of the "Local Management Plan: Conservation and

sustainable use of wild vicuñas (*V. vicugna vicugna*) in fields of the Cooperative of Agronomics Producers of Santa Catalina". This plan had been approved by the Biodiversity Direction of the Environmental Secretary of the Jujuy province (Resolutions No. 121 and 122/2012). Santa Catalina (65° 08´W; 22° 08´S) is a small town in the province of Jujuy on the Argentinean-Bolivian border of the Andean Puna, at 3800 meters above sea level. Wild vicuñas were captured following animal welfare protocols for the species (Gimpel and Bonacic, 2006; Bonacic, C *et al.*, 2012; Arzamendia *et al.*, 2010). We recorded sex and age estimated as adults (older than 3 years old), young (1 and 3 years) and Crias (less than 1 year old).

Faecal samples were obtained directly from the rectum of wild vicuñas using gloves and Vaseline® and were placed in individual plastic bags prior to the shearing process. Samples were kept refrigerated for five days pending their analysis at INTA Parasitology Laboratory. The faecal egg output was determined according to the modified McMaster technique for cattle and sheep (Robert and O'Sullivan, 1949). Five grams of the faeces were briefly placed in a mortar and suspended in 100ml of saturated solution of sodium chloride (specific gravity 1.2). Samples were disintegrated with the assistance of a pylon and contents were poured through a tea strainer into a

beaker. A magnet was placed on a magnetic stirrer. The supernatant was removed by aspiration and then transferred to a 4 cell counting chamber (modified McMaster chamber INTA), each with a 0.5 cc capacity. Eggs per gram (EPG) of faeces were observed at light microscopy (100X). Eggs were identified to genus level. The detection of oocysts was also based on the same technique used to determine number of oocysts per gram of faeces (OPG). Faecal cultures for larvae were performed using the Corticelli-Lai (1963) technique. Briefly, a pool of faeces were grown with an equivalent amount of vermiculite and placed in petri dishes of 10cm in diameter. These petri dishes were then placed in larger petri dishes of 15 cm diameter and water was added to generate a humid chamber. The dishes were kept in an oven at a temperature of 27 °C and 75% humidity for 10 days. After this, the dishes were inverted to allow most of the larvae to migrate to the water. Subsequently, sedimentation and siphoning steps were used to concentrate the larvae suspension on a slide and observations were taken using an optical microscopy. OPG and EPG were evaluated by ANOVA, followed by Bonferroni post-ANOVA test (Statistix 8, Analitical Software, Tallahasse, FL, USA). Significance was established at *P<0.05*. ANCOVA analysis was conducted to study frequency and shedding variables, with the

capture years (2012, 2013 and 2014) used as the covariates.

Results

A total of 150 vicuñas out of the 416 captured were sampled. This corresponded to 36.05% of the captured animals and 21.4% of the total population in the management area. Most of the captured vicuñas were observed to be in optimal health condition, with a media body score of 2.5 (Cebra et al. 2014). Sampled animals did not show any diarrhea, conjunctival pallor, submandibular edema (bottle jaw) or weakness during handling. No mortalities

occurred during the management or capture events. We identified four groups of parasites by faecal analysis (order *Strongyloides-type* (GIN) eggs, *Nematodirus* spp.; coccidian oocytes and cestodes).

Faecal egg counts showed that 40.66% (61/150) of the vicuñas had GIN eggs in their faeces and 7.33% (11/150) were OPG positive. Table 1 summarises the weather information regarding media temperature, rainfall and the presence and frequency of nematodes eggs and coccidian oocytes in wild vicuñas captured during 2012-2014.

Table 1. Weather information[1] and frequency of nematodes and coccidia in vicuñas captured during November 2012, 2013 and 2014 in Jujuy province, Argentina.

Date	Temp ºC	Rainfall (mm)	Rainy days	Age[2]	EPG[3] Frequency (%)	OPG[4] Frequency (%)
Nov. 2012	14.08	0.67	13	Crias (n=6)	33.3 (2/6)	16.6 (1/6)
				Young (n=30)	20 (6/30)	23.33 (7/30)
				Adults (n=7)	0	42.8 (3/7)
Nov. 2013	13.77	0.58	11	Adults (n=62)[2]	40.3 (25/62)	0
Nov. 2014	14.24	0.819	9	Crias (n=8)	50 (4/8)	0
				Young (n=4)	50 (2/4)	0
				Adults (n=33)	66.6 (22/33)	0
Total	*P*=0.2619	*P*=0.6764	150		40.6 (61/150)	7.33 (11/150)

[1]Meteorological information from "La Quiaca Meteorological Weather Station (Jujuy)", provided by Instituto de Clima y Agua, INTA Castelar; [2]Age, Crias: < 1year; young: 1-3 years; Adult: > 3 years; [3]EPG: eggs per gram; [4]OPG: oocysts per gram.

The range of *Strongyloide*s-type egg shedding was 20 to 160 EPG of faeces. Only one vicuña (adult male) was found to have a maximum of 300 EPG during November 2014. Table 2 displays the number of vicuñas with parasite eggs and oocysts by age. Adults showed a higher frequency and shedding, with a mean EPG of 55.5, and this count varied across the years (n = 102, F_2= 5.58, *P*= 0,005), with a significant lower count of eggs in the 2012 sampling (Bonferroni, *P<0.05)*. We could not see a differences between sexes (n = 102, F_1 = 0.15, *P*= 0.699). The average count of *Strongyloides*-type eggs for crias was 20 EGP and 37.5 EGP for young vicuñas. The faecal culture analysis was negative. Seven vicuñas were positive for *Nematodirus* sp. (7/150; 4.66%) and this parasite was detected more frequently in crias (Table 2). The detected range of OPG was 20 to 140 unidentified oocysts, with a higher shedding in young individuals (Table 2).

Table 2. Number of vicuñas (%) with Strongyle-type GIN eggs, oocysts, *Nematodirus* sp. and cestodes, based on age and sex of captured vicuñas during sampling in the years 2012, 2013 and 2014, in Jujuy province, Argentina.

Age[1]	Number of vicuñas (%)			
	Strongyle-type eggs	**Coccidia oocysts**	**N*ematodirus* spp. eggs**	**Cestodes**
Crias	14.28 (2/14)	7.14 (1/14)	28.57 (4/14)	0
Males	0	0	3	
Females	2	1	1	
Young	23.52 (8/34)	20.58 (7/34)	2.94 (1/34)	0
Males	3	3	0	
Females	5	4	1	
Adults	44.11 (45/102)	2.94 (3/102)	1.96 (2/102)	0.96 (1/104)
Males	20	2	0	0
Females	25	1	2	1

[1]Age, Crias: < 1year; young: 1-3 years; Adult: > 3 years.

We observed mixed infestations (2/150; 1.33%) in two female vicuñas, a young animal (2012) with *Strongyloides*-type GIN eggs (EPG of 20) and *Nematodirus* sp. and an adult vicuña with an EPG of 80 and a presence of cestode eggs (2014). We did not find a significant difference in parasite presence based on the weather conditions (temperature and rainfall) among the three years studied (One way ANOVA - Bonferroni *P<0.05*) (Table 2).

Conclusion

In this first study of nematodes and coccidian parasites in wild vicuñas (*Vicugna vicugna vicugna*) from the Argentinian Puna, we discovered a number of infected vicuñas and a significantly lower count of EPG and OPG compared with the results of similar studies conducted in Bolivia, Perú and Argentina. The low number of vicuñas with presence of parasites detected in this study (40.66% of *Strongyloides* type-eggs, 4.66% of *Nematodirus* spp, 7.33% of coccidian oocystis and 0.66% of cestodes) contrasts with similar studies reported by others authors. Beltran-Saavedra *et al.*, (2011) described a prevalence of 100% for coccidia, 87.5% for nematodes and 3.1% for cestodes in wild vicuñas in Northern Bolivia. Meanwhile, in a parasite survey conducted on wild vicuñas in Pampa Galeras, Perú, the report revealed a 17.9% prevalence of *Nematodirus*, a 5.1%

prevalence of *Trichuris*, a 5.1% prevalence of *Capillaria,* a 12.8% prevalence of *Trichostrongylus*, a 2.6% prevalence of *Lamanema chavezi* and a 41% prevalence of *E. punoensis* of 41% (Bouts *et al.*, 2003). In our study, GIN eggs were identified to genus level (*Nematodirus* sp., coccidia and cestodes) and as *Strongyloides*-type eggs, covering *Trichostrongylus* sp., *Haemonchus* sp., *Ostertagia* sp*., Cooperia* sp., *Chabertia* sp., *Oesophagostomum* sp. and *Telodorsagia* sp due to morphological similarities (Davidson et al. 2015).

The non-larvae hatch from collected faeces could be associated with the low number of eggs shed, or due to the time lag between sampling and analysis in the laboratory in Buenos Aires.

Regarding coccidian analysis, the aim of this study was to observe the presence of oocysts, without specific identification of *Eimeria* spp. The low results reported here can be contrasted with those reported in captive animals by Cafrune *et al.*, (2014) at the Abra Pampa Experimental Station of the National Agricultural Technology Institute (INTA) in Jujuy. Using the same technique employed in our study, they observed a high prevalence of *Eimeria.* This level was observed to be between 90% and 100*%* of positive animals (adults and juveniles, respectively), with detection of *E. punoensis* 93.19%, *E. alpacae* 77.44%, *E. lamae* 34.46%, *E. macusaniensis* 38.72%

and *E. ivitaensis* 1.27%. Despite the unidentified oocysts observed in this study, the higher number of OPG positive in juveniles agree with the results reported above by Cafrune *et al*. We found two mixed infections in two females; a young animal with *Strongyloides*-type eggs and *Nematodirus* sp. and an adult with *Strongyloides*-type eggs and cestodes. This low percentage contrasts with data reported in wild vicuñas from Bolivia, in which 87.5% of the vicuñas demonstrated infestation by more than one parasite species (Beltran-Saavedra *et al.*, 2011).

No significant differences were observed for temperature and rainfall between October and November of 2012, 2013 and 2014 (average temperature and rain fall in 2012, 2013 and 2014 respectively: 14.1 ℃ and 0.67 mm; 13.8 ℃ and 0.58 mm; 14.2 °C and 0.82 mm). Despite this one way ANOVA (Bonferroni, *P<0.05*), the spring of 2014 was warmer and had more rainfall than the others seasons. These conditions could explain the higher prevalence (66.4%) of parasites observed during 2014.

This difference observed in captive and free-ranging vicuñas demonstrates the importance of natural selection as the mechanism to maintaining healthy and strong animals living in the wild. Lack of intervention with drugs and farming management allows natural forces to strengthen the population.

In captive vicuñas living under farming management protocols, vaccination and drug treatment increased soil contamination by parasites (Cafrune *et al.*, 2014). It is known that captive breeding programs used with wild species alter the reproductive biology and the natural selection mechanism. This in turn affects the immunocompetence of the captive animals due to short term or chronic stress, resulting in behavioural and physiological effects on the animals (Goddard *et al.*, 1998; Moberg, 2000; Tarlow and Blumstein, 2007; Teixeira *et al.*, 2007; Marcoppido *et al.*, 2011). Most importantly, this longitudinal study demonstrates the need for additional work in order to understand the potential role of wild SACs in the epidemiology of parasite infestation. This is crucial in order to initiate appropriate systems for sustainable management of domestic llamas and for conservation of wild vicuñas.

The EPG and OPG counts observed in this survey suggest low levels of parasitism in wild vicuña (*Vicugna vicugna vicugna*) living in their free-ranging natural environment (the southern Argentinian Altiplano) which does not represent a threat to the health of wild vicuñas or domestic livestock.

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Conflicts of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication.

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