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BRIEF COMMUNICATION

Gastrointestinal and blood parasite determination in the guanaco (*Lama guanicoe*) under semi-captivity conditions

Loreto Correa · Beatriz Zapata · Mauricio Soto-Gamboa

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Abstract The breeding of wild animals for commercial purposes is becoming more frequent nowadays. This situation has led to an increase in contact rates between wild and domestic animals, with subsequent reciprocal transmission of parasites. In this study, we characterized the gastrointestinal and blood parasites of a group of 15 semi-captive guanacos (*Lama guanicoe*). We characterized gastrointestinal parasites by analyzing fecal samples through the sedimentation–flotation technique and hemoparasites by using blood smears stained with Giemsa. We found several gastrointestinal parasites including Nematoda and protozoans. The most frequently found parasites were *Nematodirus* sp. and *Eimeria* sp. In contrast with previous studies, neither Cestoda nor *Fasciola* were found. The only hemoparasite detected was *Mycoplasma haemolamae*, a parasite already described in llamas and alpacas. We conclude that the most frequent gastrointestinal parasites of semi-captive guanacos were nematodes and protozoans. Also, the hemoparasite *M. haemolamae* seems to be prevalent among captive populations of South American camelids. Finally, captive guanacos share several parasites with the traditional livestock. Therefore, keeping captive or semi-captive guanacos without an adequate sanitary protocol might have adverse consequences to adjacent traditional cattle farming and/or for wild animals.

Keywords Guanaco · *Lama guanicoe* · Gastrointestinal parasites · Hemoparasite · *Mycoplasma haemolamae*

L. Correa · M. Soto-Gamboa (✉)
Instituto de Ecología y Evolución, Facultad de Ciencias,
Universidad Austral de Chile,
Isla Teja S/N, Casilla 567,
Valdivia, Chile
e-mail: mrsoto@uach.cl

B. Zapata
Departamento de Ciencias Biológicas Animales,
Facultad de Ciencias, Veterinarias y Pecuarias,
Universidad de Chile,
Santa Rosa 11735,
Santiago, Chile

Introduction

Breeding of wild animals in captivity usually involves the confinement of animals in reduced spaces. This leads to cohabitation of animals of sexes, different ages, and even different species. This type of management, where there is a high frequency of interactions between individuals, has an associated risk of increased stress, infectious diseases, and parasite transmissions (Hudson et al. 2002; Tait et al. 2002; Beldomenico et al. 2003). Thus, diseases that are rarely or never found under natural conditions may arise as epidemic outbreaks in captivity (Fowler 1998; Lamo 2011).

The guanaco (*Lama guanicoe*), a wild ungulate and the largest artiodactyl that inhabits the South American continent, is one of the four species of South American camelids. In Chile, it is found in fragmented populations along the Andes, insular territories, altiplano, Patagonia, and coastal areas (González and Bas 2000; Sarasqueta 2001; González et al. 2004b). It is a highly social species, monomorphic, that exhibits polygamy and resource-defense mating system (Sarno et al. 2004). Currently, guanacos are increasingly considered as an alternative to traditional livestock production. There are ca. 25,000 guanacos in Chile, and 600 of them are kept in captivity or in semi-captivity conditions in four centers dedicated either to scientific purposes or animal production (González et al. 2004b; Latorre and Bastres 2004). The commercial products obtained from guanaco are meat and fine fiber, which are mainly exported. The fiber is highly demanded due to its remarkable fineness (Tait et al. 2002;

Zapata et al. 2003; González et al. 2004a; 2004b; Zapata et al. 2004). However, studies of guanacos in captivity are scarce and mainly related to husbandry techniques. Currently, there is a paucity of studies involving epidemiology of captive guanacos, description of endoparasites associated with the species, or estimations of the risks of disease transmission to or from traditional livestock. In this context, our objective was to determine the gastrointestinal and blood parasites present in semi-captive guanacos and to determine the frequency of occurrence of each of the parasite species found.

Materials and methods

We conducted our study at the Experimental Station and Research Regional Center "Kampenaike" during the Austral summer of 2004. This station belongs to the Instituto de Investigación Agropecuaria and is located in the Magallanes region of southern Chile (52° 41′ S; 70° 54′ W), 70 km South of Punta Arenas. We studied a family group of 15 animals, including six juveniles and nine adults (one male and eight females). We collected fecal samples from the animals immediately after defecation. We obtained the samples from the upper part of the fecal pile to avoid contact with the ground. Samples were kept in individual plastic bags at −5°C until the time of analysis.

To search for gastrointestinal parasites, we used the sedimentation–flotation method (concentration technique) (Beldomenico et al. 2003; Cebra and Stang 2008). We soaked 5 g of fecal material obtained from each individual using tap water to obtain a fecal solution, and then we added 200 ml of water to each sample and decanted for 20 min. After this time, the supernatant was removed, and the sediment was re-diluted in 10 ml of tap water and decanted for 10 min. The supernatant was removed by manual aspiration, and the pellet was re-suspended using a saline-saturated (zinc sulfate) solution (specific gravity, 1.3). After homogenization, each solution was centrifuged (10 min at 2,500 rpm). Each tube was filled with the saline solution until a slight convex meniscus formed, and then a coverslip was placed on the top of the tube for 5 min and removed. Finally, the coverslip was placed on a microslide for parasite recognition. Microslides were read at ×100 and ×400 magnification using a light microscope belonging to the Veterinarian Pathology Institute, at the Veterinary Sciences Department of Universidad Austral de Chile.

We collected two blood samples from each guanaco during a regular husbandry activity on February 26, 2005. In the procedure, animals were restrained in resting position, with their eyes covered. Blood was collected by jugular venipuncture and deposited in 5-ml EDTA-coated Vacutainer tubes. We prepared three smears per animal. We fixed smears using methanol immediately after blood extraction and then stained with Giemsa at the Hematology laboratory of the Veterinary Medicine Department, Universidad de Chile. We chose the most homogeneous blood smear from each animal to perform the differential lymphocyte count. These smears were examined at ×1,000 magnification on a light microscope using oil immersion and blue-colored filter. We examined the thinnest area of each blood smear.

Results

Ten of 15 (66.7%) samples tested positive for parasites: three (20.0%) were positive to order Strongylida eggs, seven (46.7%) to *Nematodirus* eggs, and six (40.0%) to *Eimeria macusaniensis* protozoan oocyst (Table 1). Eggs of *Nematodirus* sp. were the most common parasite detected in the samples, being more abundant in juveniles than adults.

The differential leukocyte count obtained from the blood smear analysis varied between adults (Table 2) and juveniles (Table 3). Most adult females and juveniles presented alterations in the white line cell counts such as neutropenia, lymphocytosis, and monocytosis. The presence of several small round particles was detected in the peripheral areas of the cytoplasm of some erythrocytes, contiguous to the inner layer of the plasmatic membrane. These particles were also less frequently found stuck to the outer layer of the membrane. The particles were always observed in aggregated groups. Due to the characteristics of the particles, it suggests the presence of hemoparasite *Mycoplasma haemolamae* described in llamas and alpacas (Tornquist et al. 2010), which presents similar characteristics with *Mycoplasma haemofelis* of cats (Almy et al. 2006; Sykes 2010).

Discussion

The types and proportion of parasites detected in the fecal samples of the studied animals are partially consistent with those found in other centers where guanacos are kept under

Table 1 Summary of intestinal parasites eggs present in the studied animals

Animals	*Nematodirus* eggs	*Eimeria macusaniensis*	"Strongyle-type" eggs
Male	0/1	1/1	0/1
Females	2/8	2/8	1/8
Juveniles	6/6	4/6	1/6

The numerator represents the number of infected individuals and denominator, the total of studied animals

Table 2 Adult female differential leukocyte counts

Obtained values (females)	20–07	126	118	90–34	No ID	91–40	Reference values
% Neutrophils	46.2±2.9	39.7±1.9	43.5±3.1	42.0±4.8	34.7±3.1	43.5±4.4	61.5–73.7[a]
% Lymphocytes	22.0±0.5	31.0±3.0	29.5±2.6	24.5±2.8	32.8±4.8	28.2±1.8	7.8–24.7[a]
% Eosinophils	20.3±5.1	18.8±3.7	17.5±1.8	24.0±2.2	14.2±3.8	15.5±3.9	7.3–28.7[a]
% Monocytes	8.3±1.5	8.8±1.0	6.8±2.4	7.8±2.6	18.2±5.8	10.7±2.0	1.4–5.2[a]
% Basophils	0.8±0.6	0.0±0.0	0.3±0.6	0.7±0.3	0.0±0.0	1.2±0.6	0.0–1.4[a]
% Segmented	2.3±0.6	1.7±0.6	2.3±0.8	1.0 ±1.0	0.2±0.0	1.0±1.0	
N/L ratio	2.1±0.1	1.2±0.1	1.5±0.2	1.7±0.3	1.1±0.1	1.5±0.2	1.9–2.3/1[b]

Neutrophils/lymphocytes ratio and reference values. Data are expressed as mean±standard deviation

[a] Moore 2000

[b] Zapata et al. 2003

captive or semi-captive conditions. Flores et al. (2006) reported low prevalence of nematodes and eggs of *Fasciola* sp. and coccidia in captive guanacos in the Chubut province, Argentina. Similar results are reported from semi-captive and wild guanacos of Argentina (Robles et al. 2006; Issia et al. 2009). In contrast with previous studies, we did not detect *Fasciola* sp. The absence of *Fasciola* sp. in our study is explained by the climatic conditions (cool conditions) that impede its development (Alcaino and Apt 1989; Valenzuela and Quintana 1998). For example, *Lymnaea viatrix*—an intermediate host of *Fasciola* sp.—needs at least 2 months with environmental minimal temperatures over 10°C to complete their life cycle. Same conditions are necessary for the hatching of *Fasciola* eggs (Morales et al. 2000; Kleiman et al. 2007; Millan et al. 2008). These conditions do not occur in Magallanes where the mean annual temperatures reach 5°C to 6°C with a little difference between cool summer and winter (Roig 1984; Ruthsatz and Villagrán 1991).

We detected several nematode species including *Nematodirus* sp., which were frequently in the samples (46. 7%), and matched the findings of studies conducted in wild guanaco populations in Magallanes (Zapata et al. 2006). In contrast, studies conducted at lower latitudes have failed to detect *Nematodirus* spp. (Flores et al. 2006; Robles et al. 2006). We suspect that this pattern could be explained by different sanitary management or the fact that *Nematodirus* sp. presents a specific adaptation to low temperatures (van Dijk and Morgan 2008). In this sense, *Nematodirus* sp. larvae are able to survive the winter period as an egg shell capsule (Rose and Jacobs 1990; Stromberg 1997; Manfredi 2006), and therefore, the probability of prevalence is higher than other Trichostrongylides. It is not possible to rule out the presence of other nematodes due to our low sample size and the seasonality of the parasites.

Studies on llamas from northern Argentina reveal (Cafrune et al. 2006a) a higher diversity of parasites than those reported for guanacos in Patagonia (this study, Flores et al. 2006; Zapata et al. 2006) and llamas in Bolivia (Palacios and Stemmer 2006). For example, llamas have high prevalences of cestodes (17.0%), *Fasciola* sp. (21.6%), and nematodes such as *Lamanema* sp. (18.2%), Trichuris (70.5%), and *Capillaria* sp. (10.2%). In contrast, the most frequent parasites found in guanaco in our study (*Nematodirus* and strongyle-type eggs) as well as in studies of llamas in Bolivia (Palacios and Stemmer 2006) present

Table 3 Juveniles' differential leukocyte counts

Obtained values (juveniles)	13–4	14–4	15–4	9–4	12–4	8–4	Reference values	
% Neutrophils	44.7±1.3	46.2±1.3	40.2±2.5	38.0±1.0	38.5±2.0	32.3±0.3	47.2–62.3[a]	66.8[b]
% Lymphocytes	13.3±2.1	17.0±2.3	25.3±1.9	25.2±4.5	25.5±3.9	32.0±1.5	33.5–46.6[a]	29.1[b]
% Eosinophils	30.8±4.1	25.0±2.0	23.8±3.8	23.3±2.8	24.3±3.6	18.3±1.0	0.9–12.3[a]	
% Monocytes	10.2±2.4	10.2±2.0	9.7±0.3	13.3±1.3	10.7±1.3	17.0±2.8	1.7–6.1[a]	2.6[b]
% Basophils	0.2±0.3	0.2±0.3	0.5±0.5	0.2±0.3	0.3±0.3	0.3±0.3	0.0–1.0[a]	1.2[b]
% Segmented	0.8±0.8	1.5±1.5	0.5±0.5	0.5±0.5	0.7±0.6	0.7±0.8		1.8[b]
N/L ratio	3.5±0.6	2.7±0.4	1.5±0.1	1.5±0.4	1.5 ±0.3	1.0±0.1		2.2/1[b]

Neutrophils/lymphocytes ratio and reference values. Data are expressed as mean±standard deviation

[a] Moore 2000

[b] González and Bas 2000

the lowest prevalence among llamas (*Nematodirus* sp., 1.1%; strongyle-type, eggs, 3.4%). Studies performed in Vicuñas also differ from the results obtained in guanacos. Cafrune et al. (2006b) found a high prevalence of *Eimeria* sp. oocyts (79.8%) in fecal samples from Catamarca province in Argentina. However, the presence of nematodes was scarce (7.4%), and *Nematodirus* sp. was not detected in the samples.

The azurophilic spherical particles associated with the erythrocyte membrane resemble *M. haemolamae*. This species has been described in South America for Peruvian llamas and alpacas and in a Chilean alpacas herd (Tornquist et al. 2010). This hemoparasite is morphologically similar to *M. haemaofelis* described in cats and produces a subclinical anemia in most animals, but a few animals with immunosuppression, malnourished, or high parasite levels may develop the clinical form of the disease (Reagan et al. 1998; Moore 2000; Almy et al. 2006; Tornquist et al. 2010). *M. haemolamae* can be found at low quantities in blood of healthy animals and at higher quantities in blood of llamas and alpacas suffering from the juvenile immunodeficiency syndrome (Jain 1986; Middleton 1999; Moore 2000; Lascola et al. 2009). Furthermore, the presence of *M. haemolamae* could justify the generalized monocytosis observed in the differential leukocyte count.

Most of the gastrointestinal parasites found in this study are also present in livestock (Tait et al. 2002). In Argentinean and Chilean Patagonia, the most important livestock is represented by sheep. In Argentina, a 27,000 farm with more than 8.2 million sheep has been described (Robles 2007) and 2.2 million from Chile (INE 2007). Under this scenario, a narrow contact with wildlife is probable, and a cross-transmission of disease occurs (Beldomenico et al. 2003). If farms have sanitary procedures to prevent parasites, wild animals could act as hosts, and the prevalence of diseases is maintained in the environment. For example, *Teladorsagia* spp. and *Nematodirus* spp. are described as the most important nematodes of sheep in Patagonia (Robles 2007), and we may also find these parasites in guanacos too. It is unknown whether the *Nematodirus* sp. in guanacos corresponds to sheep forms; therefore, specific studies on the relationship between domestic and wildlife animal interactions are necessary.

Conclusion

Semi-captive guanacos of different ages from the Regional Research Center "Kampenaike" presented a variety of parasites. Nematode eggs especially *Nematodirus* sp. were frequently found. Oocysts were morphologically identified as *E. macusaniensis*. We did not find eggs of cestodes or trematodes. Our findings suggest the presence of *M. haemolamae* in blood samples from all animals analyzed. This could be the first record of the disease in guanacos. This parasite has been previously described for llamas and alpacas; therefore, our findings are not surprising. Despite the evidence presented here, the presence of *M. haemolamae* in guanaco should be confirmed using molecular methods.

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