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Brief Communication

Diagnosis of parasites in huemul (*Hippocamelus bisulcus*) feces from south and central Chile

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ABSTRACT

Objective. Identify parasites in huemul (*Hippocamelus bisulcus*) feces from natural protected areas of south–central Chile and evaluate differences in parasite prevalence between regions and seasons. **Materials and methods.** 200 fecal samples from huemul were collected during all seasons of the year from different localities of the Ñuble, Aysén and Magallanes regions between years 2006 and 2008. Samples were analyzed using flotation and sedimentation techniques and further observed with the microscope in order to detect parasites. Additionally, 124 samples were analyzed to determine the presence of *Fasciola hepatica*. **Results.** The 38.0% (n=76/200) of samples were positive to at least one parasite form, evidencing a prevalence of 11.8% (n=2/17) in Ñuble, 22.8% (n=28/123) in Aysén and a 76.7% (n=46/60) in Magallanes. *Moniezia* sp. eggs were found in the Ñuble region, meanwhile, Strongyle-type eggs, *Nematodirus* sp. eggs and *Eimeria* spp. oocysts were found in the Aysén and Magallanes regions. There was no evidence of *F. hepatica* during sample examination. Differences in parasitism were detected between seasons in Aysén and Magallanes ($p \leq 0.05$). **Conclusions**. Aysén and Magallanes regions showed a relatively higher prevalence and diversity of parasites than the Ñuble region. This could be related to differences in density of huemul populations and livestock in each region. Further parasitological surveys in huemul populations from central and southern Chile should be performed in the future. Studies involving the relationship between the huemul and livestock and the potential inter- and intraspecific transmission of parasites are also required.

Keywords*:* Cervidae, conservation, endangered, parasitology, wildlife (Source: ICYT, SKOS).

RESUMEN

Objetivo. Identificar parásitos en heces del huemul (*Hippocamelus bisulcus*) presente en áreas naturales protegidas del centro y sur de Chile y evaluar diferencias en la prevalencia de parásitos entre regiones y estaciones del año. **Materiales y métodos.** 200 muestras de heces de huemul fueron colectadas de distintas localidades de las regiones de Ñuble, Aysén y Magallanes a lo largo de todas las estaciones entre los años 2006 y 2008. Las muestras fueron analizadas utilizando las técnicas de flotación y sedimentación, para ser posteriormente observadas en el microscopio con el objetivo de detectar parásitos. Adicionalmente, se analizaron 124 de estas muestras para determinar la presencia de *Fasciola hepatica.* **Resultados.** El 38.0% (n=76/200) de las muestras analizadas fueron positivas a algún parásito, observándose una prevalencia de 11.8% (n=2/17) en Ñuble, 22.8% (n=28/123) en Aysén y un 76.7% (n=46/60) en Magallanes. En Ñuble se encontraron huevos de *Moniezia* sp., mientras que en Aysén y Magallanes se identificaron huevos tipo Estrongílido, *Nematodirus* sp. y ooquistes de *Eimeria* spp. No se evidenció *F. hepatica* en las muestras analizadas. Se detectan diferencias de parasitismo entre las estaciones ($p \leq 0.05$) en Aysén y Magallanes. **Conclusiones.** La diferencia en la prevalencia y diversidad parasitaria entre regiones incluidas en este estudio pueden estar relacionadas con la disimilitud en la densidad poblacional de huemules y la masa ganadera de cada región. Es necesario continuar con monitoreos parasitológicos en poblaciones de huemul en Chile sur y central y estudiar la relación de esta especie con el ganado y la posible transmisión inter- e intraespecífica de parásitos.

Palabras clave*:* Amenazado, Cervidae, conservación, parasitología, vida silvestre (Fuente: ICYT, SKOS).

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INTRODUCTION

The Patagonian huemul (*Hippocamelus bisulcus*) is a threatened deer species present in Andean-Patagonian areas dominated by low-density understory vegetation of Chile and Argentina (1). Huemul populations are small and scattered along its distributional range in Chile, being present in the Ñuble, Los Lagos, Los Rios, Aysen and Magellanes regions (1,2). Currently, this species is facing issues related with anthropogenic activity, such as habitat loss and fragmentation and the introduction of exotic diseases (2). TIn central Chile, the huemul is considered in critical risk of extinction due a continuous reduction of its population, with less than 50 individuals inhabiting natural areas of the Ñuble region (1). This situation might lead to consequences related with a reduced genetic variability, such as inbreeding and genetic drift (2). In contrast, huemul populations in southern Chile are characterized for having densities ranging from 2.2 to 6.9 individuals/km^2 in isolated areas (1). Weather and topographic conditions in this part of the country have limited human presence and agricultural activity, allowing huemuls to maintain more numerous and dense populations than their conspecifics inhabiting natural areas of central Chile (1).

The presence of livestock in natural protected and unprotected areas of central and southern Chile is a persistent threat for huemul populations, mainly because farm animals participate in the competition for food resources, modify the landscape and are an important source of infectious diseases and parasites (2). Previous reports indicate the presence of the parasites *Moniezia* sp., Strongyle-type, *Nematodirus* sp., *Dictyocaulus* sp., *Trichuris* sp., *Eimeria* spp., *Taenia hydatigena*, *Fasciola hepatica* and *Ostertagia* sp. in the Patagonian huemul**.**

The main objective of this study was to identify parasites present in huemul feces obtained from different protected natural areas of south and central Chile. Additionally, differences in parasite prevalence from samples collected in distinct regions of the country and seasons were also estimated.

MATERIALS AND METHODS

Sample collection. A total of 200 fresh fecal huemul samples were collected between years 2006 and 2008 from national reserves (NR) and parks (NP) located in three Chilean regions – 17 in Ñuble, 123 in Aysén and 60 in Magallanes (Figure 1). Samples were stored individually in hermetic plastic bags and carried in thermal boxes, which were maintained at 4°C in the Parasitology Laboratory, Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillan. A maximum of 5 days was considered from the moment that a sample was collected until it was analyzed in the laboratory.

Samples were obtained during all seasons of the year: 72 in fall (8 in Ñuble, 21 in Aysén and 43 in Magallanes), 51 in winter (3 in Ñuble, 42 in Aysén and 6 in Magallanes), 62 in spring (3 in Ñuble, 55 in Aysén and 4 in Magallanes) and 15 in summer (3 in Ñuble, 5 in Aysén and 7 in Magallanes). Samples from the Ñuble region were obtained in areas encompassed in the Andean zone of this region, particularly in Ñuble NR (37°00’45”S, 71°28’51”W), Ñuble river (36°39’12”S, 71°23’24”W) and Los Huemules de Niblinto NR (36°45’32”S, 71°27’04”W). This district possesses a cold-temperate climate with deciduous *Nothofagus* forests. In Aysén, samples were obtained in proximity to the Nef river (47°06’55”S, 72°55’32”W), Tortel (47°48›32»S, 73°29›13»W), Villa O’Higgins (48°27’140”S, 72°36’47”W), Bernardo O’Higgins NP (including the Bernardo and Huemules valleys) (49°48’33’’S, 74°29’06’’W) and Lago Cochrane NR (47°14’14”S, 72°32’14”W). These locations are characterized by forests of *Nothofagus pumilio* and *Nothofagus dombeyi* and Patagonian steppe. Samples from the Magallanes region were obtained from the Torres del Paine NP (50°57’38’’S, 73°24’51”W) and Katraska valley, located in the Bernardo O’Higgins NP (48°42’54”S, 74°00’37”W). This area possesses a cold-temperate climate with evergreen forests and peatbogs.

Sample analysis. All samples collected (n=200) were analyzed in search of parasite eggs and oocysts using the sedimentation and simple flotation techniques.

The simple flotation technique consisted in mixing 10g of fecal sample with 10mL of 10% formalin. The mix was left stand for 30 minutes, then strained through two layers of dampened surgical gauze and introduced into a 15mL glass conical tube until it was nearly filled with saline solution. The mix was centrifuged for 10 minutes at 1.500 rpm and the supernatant was disposed. The tube was once again filled with saline solution and the centrifugation process was repeated in order to wash the sample and obtain a sediment of approximately 1mL. The sediment was resuspended with 12mL of zinc sulfate solution and centrifugated for 2 minutes at 1.500 rpm. The tube was positioned vertically in a rack and completely filled with zinc sulfate so that an inverted meniscus was formed. Finally, a cover glass was placed on top of the tube for 10 minutes to capture parasite forms.

The sedimentation method followed the same steps applied during the simple flotation technique until the first centrifugation was done. After this step, the sediment

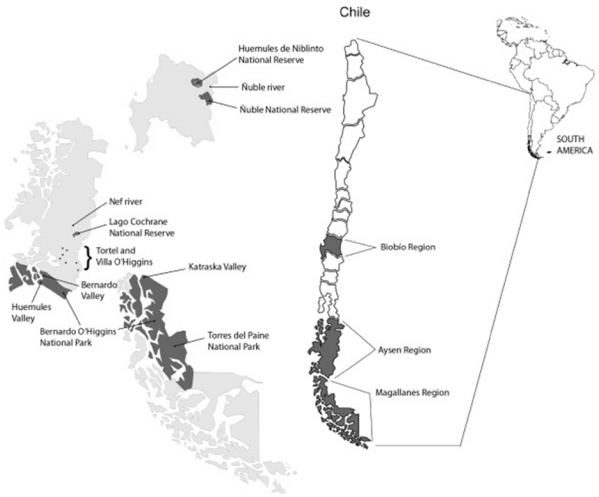

Figure 1. Map of Chile with the locations where huemul (*Hippocamelus bisulcus*) fecal samples were collected.

obtained was resuspended in 7mL of 10% formalin and 4mL of ethyl acetate was added. The mix was then shaken for 30 seconds and centrifugated for 10 minutes at 1.5000 rpm. The supernatant was disposed and two drops of sediment were each placed on separate slides. One of the slides was stained with iodine stain and the other one was left unstained (6). Additionally, 124 samples were evaluated in search of *F. hepatica* using the sedimentation technique previously described with the addition of 3 drops of malachite green stain (1%) to the sediment (7).

Statistical analysis. Parasite prevalence was calculated and defined as the percentage of fecal samples with at least one parasite species detected in relation to the total number of analyzed samples. Absolute values of prevalence were compared in each season through the statistical parameter chi-square with Yates correction and considering a significance level of p≤0.05 and 95% confidence.

Ethical considerations. This research work was authorized by the Chilean Livestock and Agricultural Services (SAG, in Spanish) and approved for the Bioethics Committee of the Facultad de Ciencias Veterinarias of Universidad de Concepción.

RESULTS

From the 200 samples analyzed, the 44% (88/200) were positive to at least one parasite form. Among these, parasites identified belonged to Strongyle-type eggs, *Nematodirus* sp. eggs (Nematoda), *Moniezia* sp. eggs (Cestoda) and *Eimeria* spp. oocysts. Mixed infections with Strongyle-type eggs and *Nematodirus* sp. were observed in 4 samples from Aysén and 6 samples from Magallanes, meanwhile, infections with Strongyle-type eggs, *Nematodirus* sp. and *Moniezia* sp. were identified in only 2 samples from Aysén. The presence of *F. hepatica* was not observed in samples analyzed. Details about the prevalence of different parasites in fecal samples is indicated in the Table 1.

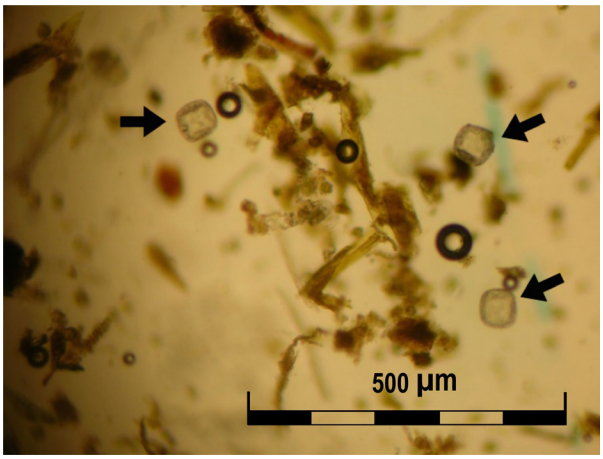

Figure 2. The presence of eggs of *Moniezia* sp. could be observed in huemul (*Hippocamelus bisulcus*) feces.

Table 1. Prevalence of parasite eggs and oocysts in fecal samples of huemul (*Hippocamelus bisulcus*) collected from different regions of Chile.

Parasites	Region			
	Ñuble (n=17)	Aysén (n=123)	Magallanes (n=60)	Total (n=200)
Strongyle-type	0	20.3 (25)	73.3 (44)	34.5 (69)
Nematodirus sp.	0	2.4 (3)	3.3 (2)	2.5 (5)
Moniezia sp.	11.8 (2)	0	0	1 (2)
TOTAL	11.8 (2)	22.8 (28)	76.7 (46)	38.0 (76)

Number of positive samples ( ).

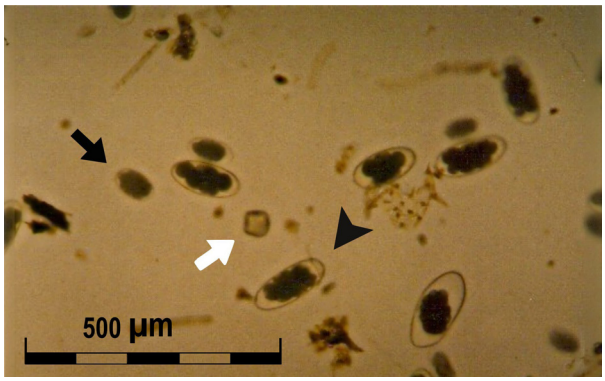

Figure 3. The presence of eggs of Strongyle-type eggs (black arrow), *Moniezia* sp. eggs (white arrow) and *Nematodirus* sp. eggs (arrow head) could be observed in huemul (*Hippocamelus bisulcus*) feces.

Regarding seasonality in the occurrence of parasite infections, parasite forms were detected in all seasons of the year, with a higher prevalence during fall and spring (Table 2). Mixed infections with Strongyle-type eggs and *Nematodirus* sp. were observed in 6 samples obtained in fall and 4 samples in spring, meanwhile, infections with Strongyle-type eggs, *Nematodirus* sp. and *Moniezia* sp. were identified in 2 samples from spring. The analysis of parasite prevalence between seasons for the Aysén and Magallanes regions revealed significant statistical differences between all seasons (p≤0.05).

Table 2. Prevalence of parasite eggs and oocysts in fecal samples of huemul (*Hippocamelus bisulcus*) collected from different seasons of the year.

Parasites	Season				
	Fall (n=40)	Winter (n=6)	Spring (n=19)	Summer (n=11)	TOTAL (n=76)
Strongyle-type	95.0 (38)	100 (6)	84.2 (16)	81.8 (9)	90.8 (69)
Nematodirus sp.	2.5 (1)	0	15.8 (3)	9.1 (1)	6.6 (5)
Moniezia sp.	2.5 (1)	0	0	9.1 (1)	2.6 (2)

Número de muestras positivas ( ).

DISCUSSION

Results obtained indicate a higher prevalence and diversity of parasites in samples from Aysén and Magallanes. This could be related with the humid weather and/or higher density of huemuls present in this area (1,2), which could favor their interaction with domestic and wild ungulates and lead to the dissemination of parasites to the huemul. The presence of *Nematodirus* sp., an internal parasite commonly present in sheep, was detected in both regions. Severe infections with *Nematodirus* sp. could result in weight loss, dehydration, submandibular edema and death in lambs (8), however, to this date there are no reports indicating pathological consequences of this parasite in the Patagonian huemul. Strongyle-type eggs were highly prevalent and identified during all seasons (Table 2), which could be related with the ability of these type of eggs to resist low temperatures and humidity (9)

The absence of eggs in the 56% of the samples could be consequence of an extended transportation period, resulting in a sub-estimation of those parasite forms that are less resistant to the environment. The technique used during this study is also a limitation because it only allows to identify parasite forms at the genus level. For this reason, it is recommended for future studies to apply methods that allow for the recognition of species.

The low detection and diversity of parasites observed in the Ñuble region could be a result of the low animal load present in the environment, as the area under study was considered for many years as a "livestock-free zone". It is also important to consider the extremely low density of huemuls in the area, a factor that could contribute to a less efficient dissemination of parasites between individuals and diminish the probability of infection (1,2). In the Ñuble region, only the presence of *Moniezia* sp. eggs was documented, a genus of parasites previously described in huemuls from this area (5). *Moniezia* sp. eggs were found only in fall and summer seasons. Detailed studies are required to evaluate if the increase in precipitation and temperature during these seasons could influence the dissemination of this parasite on the field or promote a raise in density of oribatid mites (intermediate hosts for *Moniezia*) in these locations, which could affect the dissemination of *Moniezia* sp. in the area. On the other hand, it is important to consider that the low diversity of parasites found in the Ñuble region could be related with a bias in the study resulting from the low number of samples analyzed. In consequence, it is not possible to dismiss the presence of parasites previously described in the area, such as coccidia and Strongyle-type eggs (3).

Fasciola hepatica was not detected during this study, but has been previously reported in huemuls held in captivity (4). In Chile, fascioliasis is highly prevalent between the Coquimbo and Araucan*í*a regions, which contrasts with the scenario present in southern areas of the country, particularly in Magallanes, where the parasite has not been reported (10). The absence of this parasite in *Ñuble* region could be a consequence of the non-existence of an intermediate host of *F. hepatica* in the areas included in this investigation, hypothesis that needs to be tested.

In conclusion, this study provides information about the parasites present in huemul populations distributed in central and southern Chile. Information gathered in this investigation will result relevant for the conservation of the Patagonian huemul, as populations are strongly affected by anthropogenic disturbances. No study has evaluated the pathological effects that *Moniezia spp.*, *Eimeria* spp. and *Nematodirus* sp. may have on the huemul, which could be of great relevance in the current context of continuous competition for resources with livestock and the influence of anthropogenic factors, particularly for the huemul population present in Ñuble. It is important to highlight the need to keep performing parasitological surveys in huemul populations, especially for those sharing its habitat with domestic animals. This will allow to better understand the mechanisms involved in the transmission of infectious and parasitic agents between livestock and huemuls.

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