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Endoparasitic infections in captive wild mammals under human care in San Luis Potosí, Mexico

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ABSTRACT

Objective: To determine the prevalence, richness and parasitic load in wild mammals of Tangamanga I and II Parks in San Luis Potosí, SLP. To assess whether infections are related to the type of feeding and weather seasons.

Design/methodology/approach: Analysis of fresh feces collected for three consecutive days at two sampling stations. Passive flotation techniques with sodium nitrate (qualitative) and McMaster (quantitative) were used.

Results: The overall prevalence observed was 36% (n=242). The parasitic richness is formed by protozoa: *Eimeria* sp., *Isospora* sp. and *Cystoisospora* sp.; by the cestode *Moniezia expansa*, nematodes: *Toxocara* sp., *Toxascaris* sp., *Ancylostoma* sp./*Uncinaria* sp., *Strongyloides* sp., *Trichuris suis* and Strongylid eggs. The parasite load in the case of protozoa was in the range of 0-8505 oocysts per gram of faeces, and 0-1400 eggs per gram of faeces in the case of helminths. Statistical analyses showed that the prevalence of parasites does not depend on the climatic season, and only in Tangamanga II Park is the prevalence dependent on the type of feeding (herbivores).

Study limitations/implications: The conservation method used limits the stool test techniques that can be employed (stool culture or sporulation).

Findings/conclusions: Endoparasitic infections can be a potential risk to the health of animals. In particular to those of great genetic value such as species threatened with extinction. In addition, the potentially zoonotic parasites observed pose a threat to the health of caregivers.

Keywords: wildlife, captivity, parasites, zoonoses.

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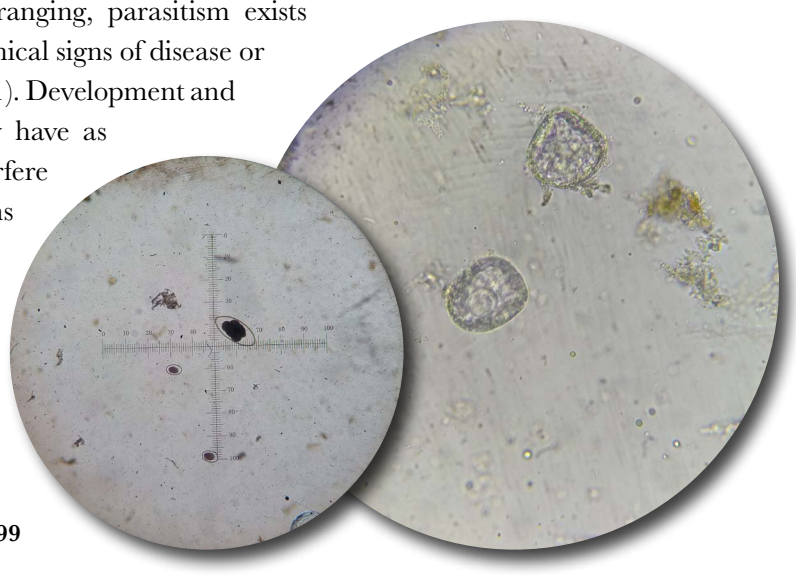
INTRODUCTION

Parasitism is the ecological relationship between one organism (the parasite) that benefits from causing harm, while another (the host) tolerates it. In wild animals of free ranging, parasitism exists naturally and often has no clinical signs of disease or parasitosis (Hossain *et al.*, 2021). Development and expansion of human activity have as intrinsic consequence to interfere with the balance of ecosystems and their populations, affecting them directly or indirectly. This leads to the creation of *ex situ* care centres for conservation or



management, such as the Management Units for the Conservation of Wildlife (UMA) (SEMARNAT, 2008) in Mexico; within them, wild mammals are kept in conditions of captivity (Sierra *et al.*, 2020). In that condition, they depend totally and exclusively on their keepers, therefore animal welfare must be a priority (OIE, 2021).

Animal-caregiver dependence implies that health conditions go beyond feeding; involving the management that the specimens receive, which include the cleaning and hygiene of their enclosures and food. This is of paramount importance when considering that intestinal parasites are one of the most important causes of disease and mortality in captive animals (Sierra *et al.*, 2020; OIE, 2021). In addition, there are potentially zoonotic parasites reported in wild mammals for example *Toxoplasma gondii* (Dărăbuş *et al.*, 2014). All of this is important in terms of public health, for animal keepers, as they work directly with animals and their excreta, and for the visitors of the zoo (Sierra *et al.*, 2020).

Therefore, this study was carried out with the aim of determining the prevalence, load and richness of intestinal parasites (protozoa and helminths) in the wild mammals of the UMA of the Tangamanga I and II Parks in San Luis Potosí, SLP. As well as to evaluate if parasites present are related to the type of feeding and the weather (seasons). Additionally, this is the first parasitological study carried out with the mammals of the Tangamanga I and II Parks; so, it is important to provide basic information necessary for decision-making by those responsible for the health and welfare of animals and workers.

MATERIALS AND METHODS

The study was developed in the UMA (DGVS-200-E-0055-SLP/98-SEMARNAT Registry) of the State Center for Culture and Recreation Tangamanga Park "Prof. Carlos Jonguitud Barrios"-CECURTI I and II. Tangamanga Park I (22° 07' 29.32" N and 101° 00' 01.74" W) is located in the west of the city of San Luis Potosí; while Tangamanga II Park (22° 10' 48.99" N and 100° 59' 05.78" W) in the north of the same city. The central area of the state of San Luis Potosí is characterized by a dry and semi-dry climate with rains in the months of June to September (average annual rainfall, 950mm). The average minimum temperature of the state is around 8.4 °C in January, while the average maximum temperature of the state is 32 °C in the month of May (INEGI, 2016).

The study was observational and included the analysis of stool samples from wild mammals in Parks I and II in two different seasons (dry: January-February; post rainy: October) during 2019. Fresh stool samples were collected, non-invasively, for three consecutive days in each season. The samples were collected directly from the floor (cement or wood) or soil (earth or grass) of the enclosure, collecting the portion that was not in direct contact with the substrate, to avoid possible contamination of the sample (Gallina, 2015). The collection of the samples was carried out in the morning before 10:00 AM.

In the field, the samples were collected, identified and stored in a thermal box (cooler) under refrigeration (5-10 °C) kept with frozen gels until transport to the laboratory, where they were stored in 5% formaldehyde solution until subsequent analysis. Parasites or sections of parasites that were observed in feces were collected and preserved in 70%

alcohol (Gallina, 2015) for subsequent analysis and taxonomic identification of genus using identification guides based on eggs and oocyst (Foreyt, 2001).

The sampling design varied according to the number of existing specimens and the type of enclosure in which the different species were found. The enclosures where there was only one specimen allowed the correct identification sample-specimen. In enclosures with two to five specimens, faecal samples were collected at random (simple randomized sampling), assuming that samples were collected from different individuals (100% of the population). In the case of enclosures with more than five specimens, as in the case of white-tailed deer in Tangamanga II Park, random faecal samples were collected (simple randomized sampling), assuming that samples were collected from different individuals among the 20% of the population (Daniel, 2017; Gallina, 2015).

In the case of llamas (*Lama glama*) which have the behaviour of defecating in pre-established places (dung deposits), samples were collected from deposits containing fresher faeces. Each day of collection the samples were taken from different deposits with the aim of increasing the probability of collecting the faeces of the three animals existing in each of the parks.

Passive flotation (Willis) techniques were used with sodium nitrate with 1.2 specific gravity and McMaster in the positive samples for gastrointestinal parasites. The McMaster technique allows the determination of the parasitic load, that is, the amount of eggs/oocysts per gram of faeces (Foreyt, 2001).

The information regarding the management of the specimens (feeding and cleaning) was obtained through interviews with the operational manager of the parks and also with the staff in charge of the daily care of the animals.

The statistical analysis method used was the χ^2 independence test considering a $p \leq 0.05$ (Daniel, 2017) used to evaluate, through a hypothesis test, whether the prevalence of parasites depends on the type of food of the animals studied, seasonality or both. In this study, parasite frequencies were obtained by type of mammalian feeding (carnivore, omnivore and herbivore) and by treatment of the environment where they live in relation to climatic variations (dry and post-rainy sampling season).

RESULTS AND DISCUSSION

All endoparasites found in the analyses have already been reported in wild mammals both in captivity (Dărăbuş *et al.*, 2014; Snak *et al.*, 2017; Sierra *et al.*, 2020; Hossain *et al.*, 2021) as of free-living (Mukul-Yerves *et al.*, 2014; Mino Botello *et al.*, 2016; Jones *et al.*, 2019).

The overall prevalence was 36% ($n=242$). The parasitic richness for the group of protozoa included oocysts of the genera *Eimeria* sp., *Isospora* sp. y *Cystoisospora* sp. For helminths, eggs were observed of the cestode *Moniezia expansa*, and nematodes of the genera *Ancylostoma* sp./ *Uncinaria* sp., *Nematodirus* sp., *Toxascaris* sp., *Toxocara* sp., of the Strongylid type, *Trichuris suis*, and larval eggs and larvae of the genus *Strongyloides* sp. The overall results are shown in Tables 1 and 2, and Figure 1.

A higher prevalence of protozoa in the dry season was found compared to the post rainy season (Figure 1). This can be explained by the fact that protozoan oocysts are

Table 1. Species sampled in Tangamanga I Park in the dry and post rainy seasons. Prevalence (%), richness and parasitic load (HPG=eggs per gram of faeces; OPG=oocysts per gram of faeces) observed.

Species	Total samples analyzed	Total positive samples	Prevalence (%)	Parasitic Richness	Parasitic Load
DRY SEASON					
Lama glama	9	5	56%	*Moniezia expansa*	50 - 1,050 EPG
				"Strongyles"	0 - 50 EPG
Odocoileus virginianus	9	6	67%	*Moniezia expansa*	0 - 50 EPG
				"Strongyles"	50 - 350 EPG
Potos flavus	3	0	-		
Procyon lotor	9	9	100%	*Eimeria* sp.	50 - 850 OPG
				Isospora sp.	0 - 50 EPG
Urocyon cinereoargenteus	6	0	-		
Panthera onca	6	2	33%	*Toxascaris* sp.	100 - 250 EPG
Lynx rufus	6	3	50%	*Toxascaris* sp.	150 - 200 EPG
				Toxocara sp.	0 - 50 EPG
Canis latrans	3	0	-		
Prevalence	51	25	49%		
Post RAINY SEASON					
Lama glama	12	5	42%	*Moniezia expansa*	100 - 150 EPG
				"Strongyles"	50 - 100 EPG
				Strongyloides sp.	0 - 50 EPG
				Nematodirus sp.	0 - 100 EPG
Odocoileus virginianus	6	4	67%	*Eimeria* sp.	50 - 100 OPG
				Moniezia expansa	0 - 50 EPG
				"Strongyles"	0 - 50 EPG
Potos flavus	3	0	-	-	
Procyon lotor	6	1	17%	*Strongyloides* sp.	0 - 50 EPG
Urocyon cinereoargenteus	12	6	50%	*Eimeria* sp.	0 - 8,050 OPG
				Cystoisospora sp.	0 - 550 OPG
Panthera onca	6	0	-	-	
Puma concolor	3	3	100%	*Ancylostoma* sp./ *Uncinaria* sp.	800 - 1,400 EPG
Lynx rufus	9	0	-	-	
Prevalence	57	19	33%		

forms resistant to certain unfavourable environmental conditions. In addition, we must consider the route faecal-oral transmission that occurs by the lack of hygiene and cleaning measures; as well as the types of floor and other objects existing in each enclosure where positive results were presented that may be acting as "shelters" for the oocysts (*e.g.* cracks or holes). Even the presence of parasitized and asymptomatic animals that maintain the reinfection cycle, or the combination of two or more of these factors (Sierra *et al.*, 2020; Hossain *et al.*, 2021).

Table 2. Species sampled in Tangamanga II Park in the dry and post rainy seasons. Prevalence (%), richness and parasite Load (HPG=eggs per gram of faeces; OPG=oocysts per gram of faeces) observed.

Species	Total samples analyzed	Total positive samples	Prevalence (%)	Parasitic Richness	Parasitic Load
DRY SEASON					
Pecari tajacu	3	1	33%	*Eimeria* sp.	0 - 50 OPG
Lama glama	10	3	30%	*Moniezia expansa*	50 - 300 EPG
Odocoileus virginianus	21	13	62%	"Strongyles"	50 - 1,300 EPG
				Eimeria sp.	50 - 350 OPG
Urocyon cinereoargenteus	6	0	-		
Lynx rufus	6	0	-		
Canis latrans	3	1	33%	*Eimeria* sp.	0 - 50 EPG
Bubalus bubalis	12	4	33%	*Eimeria* sp.	0 - 50 OPG
Prevalence	61	22	36%		
Post RAINY SEASON					
Pecari tajacu	3	3	100%	*Isospora* sp.	0 - 350 OPG
				Trichuris suis	0 - 50 EPG
				Oesophagostomum sp.	0 - 50 EPG
Lama glama	6	0	-	-	
Odocoileus virginianus	31	8	26%	*Eimeria* sp.	50 - 150 OPG
				Moniezia expansa	50 - 100 EPG
				"Strongyles"	50 - 1,150 EPG
Procyon lotor	3	3	100%	*Eimeria* sp.	200 - 600 OPG
				Strongyloides sp.	*
Urocyon cinereoargenteus	6	3	50%	*Ancylostoma* sp./ *Uncinaria* sp.	0 - 50 EPG
				Cystoisospora sp.	100 - 4,050 OPG
Lynx rufus	3	1	33%	*Strongyloides* sp.	*
Canis latrans	6	1	17%	*Ancylostoma* sp./ *Uncinaria* sp.	0 - 50 EPG
Bubalus bubalis	12	1	8%	"Strongyles"	0 - 50 EPG
Prevalence	70	20	29%		

Moniezia expansa was observed in both sampling seasons, since the route of infection is related to grazing, it may follow the fact that the lawn is constantly irrigated in the Park, which can favour the presence of the intermediate host, both in the dry season and post rainy season (Fassi-Fehri, 1987).

The presence of *M. expansa* in *Lama glama* and *Odocoileus virginianus* in Park I can be explained because both species share the same enclosure where they defecate and graze. This is consistent with the observation that in Park II the species do not share the enclosure and only the parasite was observed in *Lama glama*. It could also be explained by the movement of specimens from one park to another without proper preventive

measures; this is, without quarantine or deworming according to the information obtained in the interview.

The difference in parasitic loads could be explained by the differences in the conditions of the enclosures; therefore, in the possibilities of viability of intermediate hosts and reinfection. In Park I the enclosure has trees that provide many areas of shade and the grass receives frequent irrigation, always maintained green and abundant, which favours the establishment and proliferation of the oribatid mite and consequently the persistence of the reinfection cycle of the cestode. Whereas, in Park II there are not many shaded areas and there is practically no grass, prevailing a dirt floor that does not receive any type of irrigation; therefore, most of the feeding of the specimens is composed of hay fodder and grains, thus interrupting the biological cycle of the cestode.

Due to their direct biological cycle, infection with *Toxocara* sp. and *Toxascaris* sp. is via oral-faecal, so it is related to good hygiene and cleaning practices of the enclosures, although it may involve a paratenic host (Pariyar *et al.*, 2021). The positive results for *Toxocara* sp. and *Toxascaris* sp. observed in *Lynx rufus*, and *Toxascaris* sp. in *Panthera onca* in Park I could be explained by the presence of possible paratenic hosts, contamination or infection by street dogs or cats, contamination through fomites (Hossain *et al.*, 2021; Pariyar *et al.*, 2021) or by the exchange of animals between different enclosures without adequate hygiene or previous disinfection of the enclosure (OIE, 2021).

Parasitosis by *Toxocara* sp. and *Toxascaris* sp. are of relevant medical importance due to their complex extraintestinal larval migration that causes significant lesions in the organs of animals and also because they are potentially zoonotic, causing *larvae migrans* disease (Gakosso *et al.*, 2020). This becomes a public health problem and a potential risk to the health of the staff in charge of cleaning the enclosures of the animals (Sierra *et al.*, 2020).

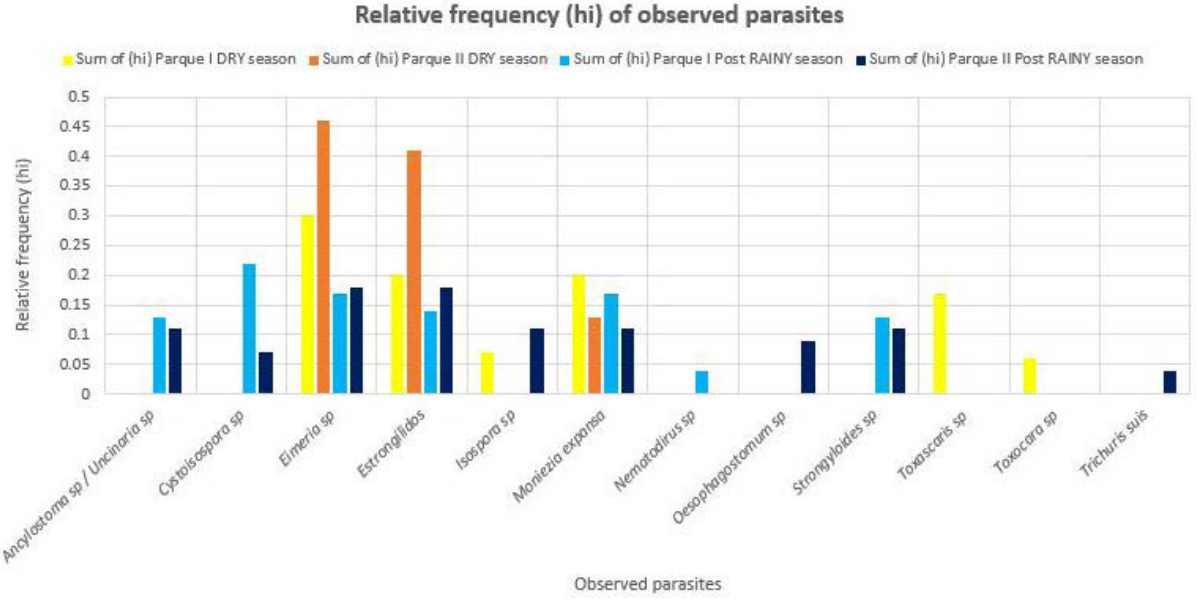


Figure 1. Graph of relative frequency (hi) of the parasites observed in the Tangamanga I and II Parks, in the sampling in dry and post rainy seasons.

The magnitude of the lesions in the organs and tissues of the animals is directly related to the magnitude of the parasitic load they present. However, when there are no preventive medicine protocols, the risk is that the animals do not present clinical signs and are in conditions of continuous reinfection, increasing the parasite load and consequently the number of larvae that shall migrate causing lesions (Klockiewicz *et al.*, 2019). Due to this, the existence of established biosecurity protocols is essential.

The Strongylids found in *Lama glama* and *Odocoileus virginianus* in Park I, both species with access to grazing which explains their infection and reinfection; also in the case of *Odocoileus virginianus* of Park II, despite the enclosure is mainly made of land, it is necessary to consider the movements of animals between the parks without the necessary preventive measures (OIE, 2021). Considering that the development and survival of L3 larvae depend on the temperature and humidity conditions of the environment, it was expected to find an increase in the prevalence and parasitic loads in post rainy season (Paixão *et al.* , 2018); nevertheless, the opposite was observed.

The positive results and parasitic loads in the dry season samples could be explained by the aforementioned lawn irrigation, and the presence of asymptomatic animals, which allow the infection-reinfection cycle to be established. All of this may be associated with inefficient management measures, as well as the absence of periodic stool test collection studies to detect asymptomatic animals, or deworming schedules with the use of specific drugs, and rotation of grazing sites in order to avoid the consumption of pastures contaminated with L3 larvae (Paixão *et al.*, 2018).

Strongyloides sp. larvae were found in some of the samples of *Procyon lotor* and *Lynx rufus* in Park II, and larval eggs in samples of *Procyon lotor* and *Lama glama* in Park I (Aranda *et al.*, 2013). Considering that one of the routes of infection is cutaneous, contaminated enclosures maintain the parasite cycle in those enclosures, in addition to its potentially zoonotic (Veraldi *et al.*, 2013).

Other results are reported as "*Ancylostoma* sp./*Uncinaria* sp." because it was impossible to distinguish both genera by the similarity of their eggs. They are phylogenetically related genera that cause similar clinical signs and lesions; both are present in Mexico. Therefore, similar treatment or prevention protocols can be used (Solorzano *et al.*, 2017). Both parasites are of direct life cycle and in general the route of infection is faecal-oral, although in *Ancylostoma* sp. they can also be lactogenic or cutaneous. Skin infection in humans is known as cutaneous larva migrans, so this genus is important for its zoonotic potential (Veraldi *et al.*, 2013). Therefore, enclosures with soil, grass or other vegetation, under favourable climatic conditions, allow and favour the development and permanence of the larvae of these parasites in the same way as enclosures with cement or wood floor that present porosities, cracks or holes that hinder their proper cleaning and disinfection. All of which may explain the results found in raccoon, grey fox, puma and coyote (Solorzano *et al.*, 2017; OIE, 2021).

The route of infection of *Trichuris suis* is oral through the intake of larvated eggs, which causes inefficient cleaning to contribute to the establishment and spread of the parasite (Hossain *et al.* , 2021). *Trichuris suis* is a typical parasite of pigs, but because of the similarities of their digestive systems, it is possible that they also parasitize collared peccaries (Jones *et al.*, 2019), which justifies the finding of the parasite in the faeces sample of the collared

peccary of Park II. In addition, the possibility of contamination through fomites must be considered (Pariyar *et al.*, 2021).

Regarding the genera of the parasites found, it is also observed that the relative frequency of parasites is different between both parks both in the dry season and in the *post* rainy season (Figure 1).

Prevalence results according to the sampling season were: 42% (n=112) in the dry season and 31% (n=127) in the post rainy season. Prevalence according to the park was: Park I, 49% (n=51) in the dry season and 33% (n=57) in the *post* rainy season (Table 1); in Park II, 36% (n=61) in the dry season and 29% (n=70) in the *post* rainy season (Table 2). Cases of multi parasitism, with the presence of two or more genera of parasites, were observed in both parks and in both sampling seasons.

Regarding weather season (dry and *post* rainy) it was observed that the prevalence of protozoa (coccidia) and helminths (cestodes and nematodes) did not depend on the season, both in Park I ($\chi^2_{(1,\ 0.05)}$=0.577; p=0.477) and in Park II ($\chi^2_{(1,\ 0.05)}$=0.278; p=0.598). Climate has an effect on parasites and their infectious stages (Paixão *et al.*, 2018), but in the parks evaluated this effect was not observed due to the management applied (frequent irrigation).

In regard to the type of feeding (carnivores, omnivores and herbivores) the highest prevalence of parasites was observed mainly in herbivores. Statistical analyses showed that, in both sampling stations, the prevalence of parasites in the specimens of Park I did not depend on the type of feeding (protozoa: $\chi^2_{(2,\ 0.05)}$=3,600; p=0.058 and helminths: $\chi^2_{(2,\ 0.05)}$=1.675; p=0.433). However, for the specimens of Park II the prevalence of parasites depended on the type of feeding (protozoa: $\chi^2_{(2,\ 0.05)}$=10.876; p=0.004 and helminths: $\chi^2_{(2,\ 0.05)}$=10.600; p=0.005).

CONCLUSIONS

Wild mammals in Tangamanga I and II Parks have endoparasitic infections caused by protozoa and helminths. However, although most animals do not show clinical signs of disease, there may be a potential risk to the health of the animals and the staff in charge of them, because potentially zoonotic parasites were found.

Weather seasons and the type of feeding of the animals of the study are related to the differences found in the prevalence, richness and parasitic load observed in the stool test results of the wild mammals of the Parks Tangamanga I and II.

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