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INTESTINAL PARASITES OF *Alouatta caraya* (PRIMATES, CEBOIDEA): PRELIMINARY STUDY IN SEMI-CAPTIVITY AND IN THE WILD IN ARGENTINA

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ABSTRACT: Endoparasitic infections are common in nonhuman primates and important factors in regulating their natural populations. Primates are particularly vulnerable to the infections of directly transmitted parasites because they often live in close social groups that facilitate their transmission. The main objective of this study was to provide baseline data on gastrointestinal parasites of semi captive and wild howler monkeys *Alouatta caraya* troops from Argentina. We collected 110 fecal samples from 38 howler monkeys from four troops, two of them kept in semi-captivity at the CRMAN, Córdoba, and two wild troops from Las Lomas, Corrientes. We identified six species of parasites: four Protozoa, *Giardia lamblia*, *Blastocystis hominis*, *Eimeria* sp. and *Entamoeba coli*; one Cestoda, *Bertiella mucronata* and one Nematoda, *Strongyloides* sp. Of the individuals sampled, 86.8% harbored at least one type of gastrointestinal parasite. Protozoa were found in 78.9% of hosts and helminths in 21.1%. We found significant differences in the prevalence of three protozoan species between study sites; and howlers from Las Lomas showed the highest prevalence. The differences found may be related to environmental conditions, where warmer and wetter regions (Las Lomas) favor the survival of infectious stages of some parasitic species.

RESUMEN: Parásitos intestinales de *Alouatta caraya* (Primates, Ceboidea): estudio preliminar en semi cautiverio y en vida silvestre en la Argentina. Las infecciones endoparasíticas son frecuentes en los primates no humanos y factores importantes que regulan sus poblaciones naturales. Los primates son particularmente vulnerables a las infecciones por parásitos de transmisión directa debido a que usualmente éstos viven en grupos sociales que facilitan su transmisión. El principal objetivo del presente estudio fue proveer información de los parásitos gastrointestinales que albergan tropas de monos aulladores *Alouatta caraya* en semi-cautiverio y en vida silvestre en Argentina. Colectamos 110 muestras de materia fecal de 38 monos aulladores pertenecientes a cuatro tropas, dos de ellas mantenidas en semicautiverio en el CRMAN, Córdoba y dos tropas silvestres en campo Las Lomas, Corrientes. Identificamos seis especies de parásitos: cuatro protozoos: *Giardia lamblia*, *Blastocystis hominis*, *Eimeria* sp. y *Entamoeba coli*; un cestode, *Bertiella mucronata* y un nematode, *Strongyloides* sp. El 86.8% de los individuos muestreados presentaron al menos un tipo de parásito gastrointestinal. Se encontraron protozoos en el 78.9% de los animales y helmintos en el 21.1%. Hallamos diferencias significativas en la prevalencia de tres especies de protozoos entre los sitios de estudio, donde los aulladores de Las Lomas mostraron la mayor prevalencia. Las diferencias encontradas pueden estar relacionadas con

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las condiciones ambientales, donde regiones más cálidas y húmedas (Las Lomas), favorecen la supervivencia de estadios infecciosos de algunas especies de parásitos.

Key words: *Alouatta caraya*, Argentina, Helminths, Protozoa.

Palabras clave: *Alouatta caraya*, Argentina, Helmintos, Protozoos.

INTRODUCTION

Parasites represent an important and ubiquitous component of primates natural communities (Nunn et al*.,* 2003; Nunn and Altizer, 2006; Vitazkova and Wade, 2006). Primates are particularly vulnerable to the infections of directly transmitted parasites because they often live in close social groups that facilitate their transmission (Stoner, 1996; Stoner et al., 2005). The genus *Alouatta* is the most widely distributed of the New World monkeys, ranging from 18° N in Mexico to 27° S in Argentina, where the black and gold howler monkey, *Alouatta caraya* (Humboldt, 1812), reaches the southernmost limit of its geographic distribution in the provinces of Formosa, Misiones, Chaco, Corrientes and Santa Fe (Neville et al., 1988; Brown and Zunino, 1994). Among Neotropical primates, species of the genus *Alouatta* are some of the best studied in relation to parasite infections. For example, Stuart et al., (1990) found a high incidence of Strongylid infections in troops of *A. palliata* inhabiting riverine forests in Costa Rica, suggesting that high humidity would prolong larval survival. In Mexico, Trejo-Macias et al. (2007) identified six species of intestinal parasites (two unidentified trematodes, *Controrchis biliophilus*, *Trypanoxyuris* sp., *Parabronema* sp. and one Coccidia) in troops of *A. p. mexicana* inhabiting continuous and fragmented forests and found a higher prevalence of infection in fragmented habitats, related to a greater contamination from humans and domestic animals. For *A. pigra*, 16 species of endoparasites were reported in Belize and Mexico; six protozoans, six nematodes, two trematodes and two cestodes (Stoner and González-Di Pierro, 2005; Eckert et al., 2006; Vitazkova and Wade, 2006, 2007; Trejo-Macías et al., 2007), and in general they found an increased prevalence of nematodes and some protozoan during the wet season and in fragmented habitats. In the case of *A. seniculus*, Phillips et al. (2004) provided baseline information on the presence of three protozoan (*Chilomastix mesnili*, *Blastocystis hominis* and *Iodamoeba buetschii*) and two helminths (*Strongyloides* sp. and *Trichuris* sp.) in south-eastern Peru. Martins et al. (2008) surveyed troops of *A. belzebul* at five sites in the Brazilian state of Pará recovering 12 taxa of endoparasites, being relatively high the infection rates, and showing a marked variation both within and among populations. Finally, almost 11 endoparasites species were identified from *A. guariba* in forest patches next or within plantations and in close proximity to human settlements in different regions in Brazil, suggesting these factors increases the human-wildlife parasites exchange (Kowalewski and Gillespie, 2009).

Field studies conducted on wild populations of *A. caraya* in Argentina have provided information on different groups of intestinal parasites infecting this species such as nematodes, *Trypanoxyuris* sp*., Trypanoxyuris minutus*, *Strongyloides* sp.; unidentified Trematoda eggs and *Bertiella mucronata* (Cestoda) (Santa Cruz et al., 2000 a; b; Delgado, 2006; Kowalewski et al., 2010). *Giardia lamblia* (Syn. *G. intestinalis* [Lambl, 1859]; *G. duodenalis* Stiles 1902) was the only protozoan reported (Venturini et al., 2003; Kowalewski et al., 2010). *A. caraya* faces changes in its natural habitat such as, deforestation as well as illegal hunting, because it is the most commonly traded primate for the pet market in Argentina (Bertonatti, 1995; Chebez, 2009). The study of infection patterns in semi captive primates may help to provide an index on the health status of individuals.

Coprological surveys for intestinal parasites involve non-invasive procedures and provide data to be included in the analysis of ecological relationships between primates and their environment (Eckert et al., 2006; Gillespie, 2006).

In the framework of the study of behavior and intestinal parasites of black and gold howler monkeys, our main objective was to describe the intestinal parasites in semi captive and wild howler monkeys *A. caraya* troops (ACA) from the Centro de Reeducación del Mono Aullador Negro (CRMAN) and campo Las Lomas, respectively in Argentina.

MATERIALS AND METHODS

Study sites

In the present study, we surveyed four howler monkeys *A. caraya* troops. Two of the troops were held in semi captivity at the CRMAN; this 300 ha private-owned land is located in La Cumbre (30° 59' S; 64° 29' W), Córdoba Province, at 1420 m asl, in central Argentina. The local climate is temperate, with temperatures ranging from -8.8 °C to 32 °C in winter and 0.8 °C to 38.4 °C in summer; the average annual rainfall is 700 mm (Demaio and Medina, 1999). The landscape is a combination of native hilly forest (locally called "bosque serrano") and a matrix of grassland (*Festuca* sp. and *Stipa* sp.). In addition, the area has been extensively altered by human activities resulting in a modification of the natural forest and the introduction of exotic vegetation, which is suitable habitat for howler monkeys (Bruno et al., 2005; Bruno, 2011; Bruno and Mudry, 2011). The other howlers under study were two wild troops from campo Las Lomas, a 623-ha private-owned land, located in San Cosme District, Corrientes province, in the northeast of Argentina (27º 23'S; 58º 22'O). The local climate is subtropical, with a mean annual temperature of 21 °C and an average annual rainfall of 1200 mm (Rumiz et al., 1986). The landscape is characterized by a semi deciduous forest surrounded by a matrix of grassland (Carnevalli, 1994; Burkart et al., 1999; Zunino and Kowalewski, 2008). There are several seasonal lagoons surrounding some of the forest patches.

Subjects

We surveyed four troops of howler monkeys, two held in semi captivity (T2: 4 adults, 1 subadult, 2 juveniles and 2 infants; and T3: 4 adults, 1 subadult, 5 juveniles and 3 infants) and two wild troops (T. Sopranos: 4 adults, 1 subadult, 4 juveniles and 3 infants; and T. Mudo: 8 adults, 3 juveniles and 2 infants). We identified the howler monkeys based on body size, coat color, natural markings, wounds and facial features (Cabrera, 1958). At campo Las Lomas, we habituated the howlers to our presence during a three-month period. The CRMAN receives and houses illegally commercialized howler monkeys and particular donations (Bruno et al., 2005). After arrival, the individuals received veterinary assistance (including treatment for parasitic infections when needed). The monkeys are maintained in facilities for two to six years, and subsequently groups are formed and released in the forest patches (Bruno et al., 2005). Once released, they are only provided with a daily ration of supplementary food and fresh water, besides the natural vegetation of the patches (Bruno, 2011; Bruno and Mudry, 2011). We selected these two troops because their conformation is stable, and since the controlled release no management is involved except for the recapture of expelled or sick individuals. T2 was formed in 2000 and released in January 2001 and T3 was formed and released in 2003 (Bruno, 2011). Once released, the individuals did not receive any parasitic treatment.

Sample collection and analysis

We followed each troop from dawn to dusk and collected serial samples of fresh feces from 38 individuals of the four troops immediately after defecation to avoid contamination (preferably in the morning). We collected three fecal samples from each individual, resulting in a total of 110 samples. All fecal samples were taken from individually identified monkeys and stored in flasks containing 10% formalin solution. We processed all the samples in the CEPAVE-UNLP (Centro de Estudios Parasitológicos y de Vectores. Universidad Nacional de La Plata) via standard concentration by sedimentation (Ritchie, δ=1010) and by flotation (Sheather, δ=1200) techniques (Becerril Flores and Romero Cabello, 2004). The samples resulting from the application of these techniques were divided among three different slides, each for a different method, in order to search for parasitic forms (eggs, cysts and larvae), using temporary staining (Lugol's iodine), and examined under light microscopy at 100, 400 and 1000 x magnification (Olympus BX 51). Parasites were identified based on morphology, size, and appearance of eggs, cysts and larvae following standard references. We described parasite infections in terms of prevalence of infection and

species richness. Prevalence is the proportion of individuals hosts sampled infected with a particular parasite species (Stuart and Strier, 1995; Bush et al., 1997; Gillespie, 2006; Chapman et al., 2009). Richness (S) is defined as the number of unique intestinal parasitic species documented from the host's fecal samples (Chapman et al., 2006). Sorensen Similarity Coefficient (CS) was also calculated to assess parasite species composition between study sites (Morales and Pino, 1987). We calculated prevalence of infection for total intestinal parasite species and for each individual parasite species in howler monkeys for each study site. We performed a X^2 test to compare variations in parasite prevalence between the two study sites, with EPIDAT 3.1 and PASW Statistic 18 software. Statistical significance was assumed at $\alpha < 0.05$.

RESULTS

Species composition and total prevalence of howler parasites at CRMAN and Las Lomas

Howler monkeys were parasitized by six parasite species. Infections of protozoa included one nonpathogenic amoeba (*Entamoeba coli*), *Blastocystis hominis*, *Giardia lamblia*, and *Eimeria* sp. Helminths recovered included eggs of the cestode *Bertiella mucronata*, and the nematode larvae of *Strongyloides* sp. (**Table 1**). Of all individuals examined from both areas, 86.8% (33/38) were infected with at least one type of gastrointestinal parasite. In the CRMAN, the prevalence was lower (72.2%), whereas all individuals from Las Lomas were infected (100%). Protozoa were found in 78.9% of the animals; 55.5 % in the CRMAN and 100% in Las Lomas. Helminths were less prevalent and were found in 21% of all howlers sampled, 22.2% in the CRMAN and 20% in Las Lomas. In the present study, the two most prevalent species were *Blastocystis hominis* (73.7%) and *Giardia lamblia* (50%). Species richness (S) was higher in wild troops in Las Lomas (6 vs. 4) and parasite species composition in both areas showed a similarity of 80% (CS = 0.80).

Comparison of parasite prevalence between howlers from CRMAN and Las Lomas

The total prevalence of intestinal parasites was higher among howlers from Las Lomas than those of the CRMAN, and two species, *Eimeria* sp. and *Strongyloides* sp. were found only at Las Lomas. However *B. mucronata*, was more prevalent among howlers from the CRMAN (**Table 1**). Significant differences in prevalence of infection was found between sites for three species of protozoan (*Blastocystis hominis*: $X^2 = 21.37$, $p < .05$; *G. lamblia*: $X^2 = 18.24$, $p < .05$ and *Entamoeba coli*: $X^2 = 50.09$, $p < .05$) which were more prevalent at Las Lomas.

Table 1

Species composition and prevalence of infection (%) of howler parasites in Argentina.

Parasite species	Prevalence of infection (%)		
	CRMAN	Las Lomas	Both sites
Protozoa			
Blastocystis hominis	44.4	100.0	73.7
Giardia lamblia	27.7	70.0	50
Entamoeba coli	5.5	65.0	36.8
Eimeria sp.	0.0	15.0	7.9
Helminths			
Bertiella mucronata	22.2	10.0	15.8
Strongyloides sp.	0.0	10.0	5.3
Total protozoan infection	55.5	100.0	78.9
Total helminth infection	22.2	20.0	21
Number of individuals sampled	18	20	38
Number of samples collected	50	60	110

DISCUSSION

To our knowledge, this is the first report on gastrointestinal parasites from semi captive troops of howler monkeys *A. caraya* in Argentina. Five of the gastrointestinal parasites species identified in the four troops surveyed have been reported in previous studies for *A. caraya* in Argentina and Brazil: *Blastocystis hominis* in Corrientes, Argentina (Martínez et al., unpublished); *Giardia lamblia* also in the same region (Venturini et al., 2003; Kowalewski et al., 2010) and *Eimeria* sp. in Brazil (Godoy et al., 2004). Among helminths, the nematode *Strongyloides* sp. was described in Corrientes (Santa Cruz et al., 2000a), as well as the cestode *Bertiella mucronata,* which was also reported from Corrientes and Chaco, Argentina (Santa Cruz et al., 2000 a, b). In the present study, we found more species (six) of gastrointestinal parasites than previously reported in Argentina (Pope, 1966 [two species]; Santa Cruz et al., 2000b [two species identified]; Venturini et al., 2003 [one specie]; Delgado, 2006 [two species] and Kowalewski et al., 2010 [one specie]). However, the lower species richness of parasites in monkeys from the CRMAN compared to those from Las Lomas may be related to the fact that they were captured when they were infants, isolating them from their natural environment and preventing them from natural parasitic infection. Another possible explanation may be related to the environmental conditions in both study sites.

The prevalence of *Giardia lamblia* in wild howlers from Las Lomas was similar or higher than previous records for the same region (70 % *vs* 67 %) (Kowalewski et al., 2010) and from wild troops in Chaco, where Venturini et al. (2003) found only 3.6 % of infection and Kowalewski et al. (2010), 57 %. The prevalence of *Bertiella mucronata* found in wild howlers in our study, showed a higher prevalence of infection compared to findings by Delgado (2006) (20 % vs 5 %), but similar (20 % *vs* 21%) compared with Santa Cruz et al. (2000b) in wild troops from Isla Brasilera, Chaco.

We found no evidence of infection with *Cryptosporidium* sp. However, there is one record of *Cryptosporidium* sp in one howler monkey kept in semi captivity in Corrientes (Santa Cruz et al., 2002). In this regard, Kowalewski et al. (2010) suggested this pathogen is not a natural component of the howler parasite communities in Chaco and Corrientes. The results of our study based on the analysis of 110 samples over a period of one year could support the findings by Kowalewski et al. (2010).

We found significant variation in parasite prevalence between howlers from the two study sites. Protozoan infection was higher in wild monkeys from Las Lomas than those in semi captivity from the CRMAN. This could be related to the environmental differences between the two areas, where warmer and wetter regions (characteristic of Las Lomas) are more favorable for the survival of infectious stages of some parasitic species, thus increasing chances of infection. Factors such as high precipitation, humidity and low altitude seem to be associated with higher levels of parasite prevalence and richness (Stuart et al., 1998; Stoner et al., 2005; Kowalewski and Gillespie, 2009). Many of the frequently studied parasites of primates are transmitted orally, either when the monkey ingests contaminated water, plant foods, or by an intermediate host containing the infective stage of a parasite (Nunn and Altizer, 2006; Chapman et al., 2009). Natural infection by protozoans seems to occur through the ingestion of resistant cyst (Nunn and Altizer, 2006), which are found in soil, food, water or surfaces contaminated with feces from infected animals (Godoy et al., 2004). Among protozoa, *Giardia lamblia* and *Blastocystis hominis* are potential zoonotic gastrointestinal parasites (Kowalewski and Gillespie, 2009). *Giardia lamblia* is commonly found in *Alouatta,* while *Blastocystis hominis* was found in *Alouatta seniculus* (Stuart et al., 1990; Phillips et al., 2004: Chinchilla et al., 2005), *A. pigra* (Stoner and González-Di Pierro, 2005) and *A. caraya. Blastocystis hominis* and *Giardia lamblia* were present and prevalent in howlers from both study sites. It is important to note that the most prevalent parasites have a direct life cycle, which would facilitate their transmission. Most of the species of parasites found in the present work also parasitize humans and livestock (Nunn

and Altizer, 2006; Kowalewski and Gillespie, 2009; Vitazkova, 2009), and the fact that the howlers surveyed inhabit environments with frequent contact with human communities, may increase opportunities to cross-species transmission (Kowalewski and Gillespie, 2009). In addition, the permanent presence of humans and frequently concurrence of tourism in the case of the CRMAN and the regular presence of poachers (illegal hunters) and rural workers in Las Lomas, may contribute to human-wildlife disease exchange, and howler monkeys may serve as reservoirs for human infection.

Helminths are also commonly reported in wild primates (Nunn and Altizer, 2006). The occurrence of *Bertiella mucronata* in *A. caraya* has been previously reported in Argentina, (Pope, 1966; Santa Cruz et al., 1995; Santa Cruz et al., 2000 a, b). According to Stuart et al. (1998) the infection is probably related to the accidental ingestion of oribatid mites bearing cysticercoids. Howler monkeys are primarily folivorous, and there are no reports that they deliberately consume arthropods. The low prevalence of infection of *B. mucronata* in our study may be a result of the low consumption of invertebrates in their diet. However, Bravo and Zunino (1998) found the presence of arthropod larvae within some of the seeds consumed by *A. caraya*. It is likely that howler monkeys may become infected when accidentally ingesting the intermediate hosts while foraging on leaves, fruits, or drinking contaminated water. The higher prevalence of *B. mucronata* in monkeys of the CRMAN compared with those from Las Lomas, may be due to environmental factors, such as low relative moisture and higher solar irradiation that could favor the persistence of intermediate hosts. Also, howlers could be ingesting the infected arthropods accidentally during terrestrial travel. We registered frequent terrestrial locomotion and travel, often over defecation sites, only in howlers from the CRMAN, thus increasing the opportunity for transmission of the infective stage. *Strongyloide*s sp. occurs in tropical and subtropical regions, although it can be found in more temperate ones, where is associated with wetter areas (Nunn and Altizer, 2006). The larvae are eliminated in the feces as L1 and need humid soils to develop into the infective L3 stage. In our study, *Strongyloides* sp. was present in wild howlers from Las Lomas, where the high humidity may favor its development.

The results of our study may contribute to the baseline knowledge of intestinal parasites infecting howler monkeys and aid in the understanding of patterns of parasitism in *A. caraya*. In turn, coprological surveys of endoparasites will allow us to non-invasively estimate the health of individuals, detect the presence of parasites of zoonotic importance and assess whether there is any risk for other wild populations in case of translocation.

CONCLUSIONS

This study is a contribution to a baseline knowledge of endoparasites infecting black and gold howler monkeys, *A. caraya,* in Argentina. We found wild troops of howler monkeys from Las Lomas to have greater species richness of parasites and higher prevalence of protozoan infection. Differences in the microenvironment between the two study sites may account for the variation in parasite prevalence in individuals of *A. caraya*. Further analysis of the association between socioecological variables and indices of parasite infection will provide valuable information of the patterns of parasitism in semi captive and wild howler monkeys in the two study sites.

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