FIRST RECORDS OF GASTROINTESTINAL PARASITES IN WOOLLY MONKEYS *(LAGOTHRIX LAGOTHRICHA)* IN COLOMBIA, FROM WILD, CAPTIVE AND REINTRODUCED INDIVIDUALS

Camilo Quiroga-González[1], Elisa Jiménez[1], Nelson F. Galvis[1], Mónica A. Ramírez[1] Mario Ortiz[2], Camila Gonzalez[2] and Pablo R. Stevenson[1]

[1] Laboratorio de Ecología de Bosques Tropicales y Primatología (LEBTYP). Departamento de Ciencias Biológicas. Facultad de Ciencias. Universidad de los Andes Bogotá, Colombia. E-mail:ca.quiroga158@uniandes.edu.co
[2] Centro de Investigaciones en Microbiología y Parasitología Tropical (CIMPAT). Departamento de Ciencias Biológicas. Facultad de Ciencias. Universidad de los Andes, Bogotá, Colombia. E-mail:mario-or@uniandes.edu.co

Abstract

Interest about parasites in vertebrate populations during translocation and reintroduction programs is increasing; thus, a description of parasites in captivity infecting animals to be relocated is necessary. This study aimed to characterize the communities of gastrointestinal parasites in woolly monkeys (*Lagothrix lagothricha*) from captive and wild individuals, as well as the change in parasite prevalence in four individuals during a reintroduction process. To accomplish this goal, we used a fecal flotation technique to analyze the collected samples. In captivity, 95 % of the screened samples were infected with at least one parasite, while only 77 % of wild primate samples showed infection, indicating higher prevalence in captive vs. wild individuals. Overall, wild and captive woolly monkeys shared many groups of parasites (Strongylidae, Oxyuridae and Entamoebidae) and we found a trend of lower parasite prevalence after release in captive individuals. Our data showed a consistent difference between captive and reintroduced individuals suggesting that variables related to diet, overcrowding and human presence may be the most important factors explaining parasite communities.

Keywords: conservation ex-situ, woolly monkeys, nematodes, intestinal parasites.

Resumen

El interés sobre el impacto de los parásitos en las poblaciones de vertebrados durante los programas de translocación y reintroducción ha aumentado últimamente; esto hace necesario una descripción de los parásitos que infectan a los animales en cautiverio que van a ser reubicados. El objetivo de este estudio fue el de caracterizar las comunidades de parásitos gastrointestinales en monos churuco (*Lagothrix lagothricha*) de individuos cautivos y silvestres, así como el cambio en la prevalencia de parásitos en cuatro individuos durante un proceso de reintroducción. Para lograr este objetivo, utilizamos una técnica de flotación fecal para analizar las muestras colectadas. En cautiverio, el 95 % de las muestras examinadas estaban infectadas con al menos un parásito, mientras que solo el 77 % de las muestras de primates silvestres mostraron infección, lo que indica una mayor prevalencia en individuos en cautiverio frente a los silvestres. En general, los monos churuco salvajes y en cautiverio compartieron muchos grupos de parásitos (Strongylidae, Oxyuridae y Entamoebidae) y se encontró una tendencia de menor prevalencia después de la liberación de los individuos. Nuestros datos mostraron una diferencia consistente entre individuos cautivos y reintroducidos, lo que sugiere que variables relacionadas con la dieta, hacinamiento y la presencia humana pueden ser los factores más importantes que explican las comunidades de parásitos.

Palabras clave: conservación exsitu, churucos, nematodos, parásitos intestinales

Introduction

Primates are one of the most important taxonomic groups in terms of conservation challenges, due to the danger of extinction most of them face (Chinchilla et al., 2005; Estrada et al., 2017). Non-human primates are particularly susceptible to parasitic infections because they can get infected from other animal parasites, in spite of not being the primary host (Johnson-Delaney, 2009). In addition, primates are vulnerable to parasitic infections due to the social structure, which facilitates their transmission (Freeland, 1983). Habitat fragmentation and population size can also compromise the population's health and increase the prevalence and richness of parasites (Gillespie and Chapman, 2008; Püttker et al., 2008). That is the reason why the prevalence of parasites usually increases in smaller areas where the possibility of re-infection is higher caused by an unusual increase in primate crowding (Müller, 2007). This is the scenario in zoos and rescue centers, where high population densities and poor sanitation favor high prevalence of parasites (Guerrero et al., 2012).

Information about gastrointestinal parasites in Old-World primates is well known (Gillespie et al., 2005; Opara et al., 2010; Petrezelkova et al., 2010), on the contrary, in South America information gaps remain and an enormous

sampling effort is needed (Hopkins and Nunn 2007). Woolly monkeys (*Lagothrix lagothricha*) are among the less well-known species and to our knowledge, in Colombia there are no studies describing gastrointestinal parasites either from wild or captive populations. These primates are critically endangered according to IUCN (2008) due to hunting pressure and habitat fragmentation that have reduced the natural populations of this species to local extinction in some areas (Stevenson and Aldana, 2008). In this context, the aim of this study is to provide a first characterization of gastrointestinal parasite communities in Colombian woolly monkeys (*Lagothrix lagothricha*) from captive and wild individuals, and to characterize the parasite community in four reintroduced individuals. We first evaluated if there were differences in the prevalence of gastrointestinal parasites between captive and wild individuals to evaluate if they shared the same parasites. Then, we compared these results with parasites found in reintroduced individuals into the wild, to assess the potential parasite loss or gain after release. We expected a higher density of zoonotic parasites in captive individuals, given their proximity to humans and their limited home range. Similarly, we expected a higher prevalence of parasites in captive primates compared to released ones as a result of changes in population density and diet.

Methods

Study Sites

Fecal samples from captive primates were collected between June and November 2015 in two different enclosure sites in Colombia. The first site was Fundación Bioandina located at Mesitas, Cundinamarca (4° 34'36.603"N, 74° 27' 3.944"W). This captivity site is located at an altitude of 990 m a.s.l. with a mean annual precipitation of 1,561 mm, and temperature ranges between 17.3–26.8 °C. (Fig. 1).

Here we found five individuals, two adult females, one adult male, one sub-adult female and one juvenile female belonging to two subspecies (*Lagothrix lagothicha lugens* and *Lagothrix lagothricha lagothricha*). The individuals were found in two small enclosures located side by side permitting contact between them. The other site was Pereira`s Centro de Atención y Valoración (CAV), Risaralda (4° 48' 17.176"N, 75° 47' 1.687"W). This captivity site is located at an altitude of 1,411 m a.s.l. with a mean annual precipitation of 2,441 mm. Temperature ranges between 14.7–26.8 °C (Fig. 1). Here we found four individuals, two adult males, one adult female, and one juvenile female, also from both subspecies. All the individuals were located in a big enclosure enriched with sticks and planks to encourage their locomotion.

Samples from wild individuals were obtained between September 2010 to April 2013 from two groups of wild woolly monkeys (*L. l. lugens)* inhabiting a pristine forest in Parque Nacional Natural Cueva de Los Guacharos (PNNCG), Huila, Colombia (1° 33' 0" N, 76° 7' 59.998" W). This National Park is located in a montane forest with an extension of 9,000 hectares with a mean altitude of 2,000 m a.s.l. and a mean annual precipitation of 3,100 mm; temperature ranges between 12–20 °C (Fig. 1). Here we found two habituated woolly monkey groups varying in size from 18 to 27 individuals.

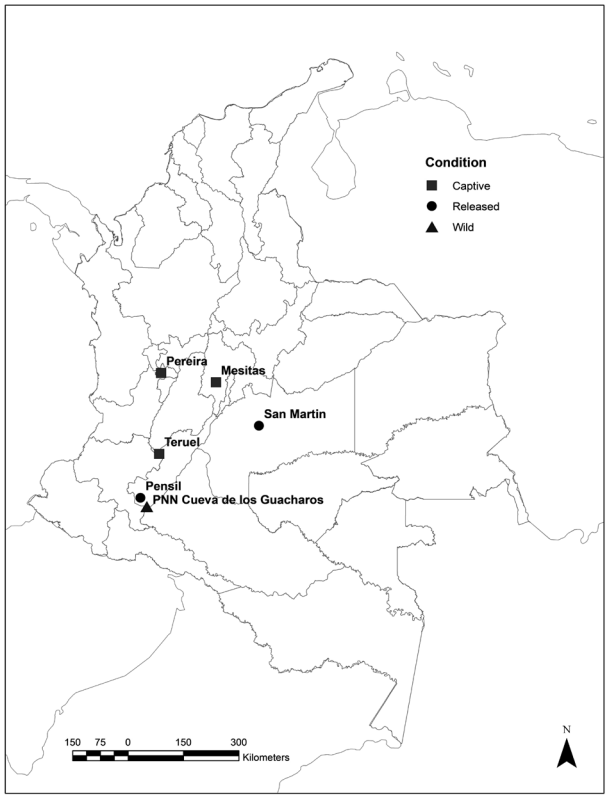

Figure 1. Map showing the different study sites where primates were sampled. The shape of the figures corresponds to the three different conditions of the sampled individuals.

Regarding reintroduced primates, fecal samples from two captive individuals were taken between March and July 2017 from a captivity site at Teruel's CAV, Huila (2° 49' 53.93"N, 75° 50' 0.775"W). This captivity site is located at an altitude of 910 m a.s.l. with a mean annual precipitation of 1635 mm; temperature ranges between 19.1–30.3 °C (Fig. 1). The individuals shared a big enriched enclosure (260 m^3) with 10 other primates. They were fed twice a day with a mixture of fruits and vegetables that does not correspond to their diet in natural habitats. Individuals were released in August 2017 in a biological corridor located at El Pensil, Huila (1° 45' 43.949"N, 76° 17' 11.68"W). This forest is located at a mean altitude of 1,850m a.s.l. with a mean annual precipitation of 2,284 mm, and temperature ranges between 12–20 °C (Fig. 1). Fecal samples were collected from the moment of release through March 2018. The other two individuals were sampled in captivity at Teruel between March of 2018 and November of the same year and released in a Biological Reserve located in San Martín, Meta (3° 31' 6.24"N, 73° 24' 11.88" W). This release site is

located at a mean altitude of 280 m a.s.l. and a mean annual precipitation of 2,513 mm; temperature ranges 25 – 28 °C (Fig. 1). Fecal samples were obtained from the time of release through April 2019.

Sample collection

Fecal samples were collected immediately after defecation and to avoid environmental contamination, only 2 grams of primate feces not in direct contact with the ground were sampled in 15 ml Falcon tubes filled with 10 % formaldehyde. Tubes were shaken to maximize the contact surface between the sample and formaldehyde. For each sample, study site, date, time, individual name and sex were recorded. The samples were kept at room temperature until transport to Laboratorio de Ecología de Bosques Tropicales y Primatología (LEBTYP) at Universidad de Los Andes, Colombia where they were stored until processing.

Sample processing

Samples were processed at Centro de Investigaciones en Microbiología y Parasitología Tropical (CIMPAT) at Universidad de los Andes, Colombia. For parasite identification, we followed the fecal flotation method suggested by Gillespie (2006), using a saline solution calibrated with a pycnometer at a specific gravity of 1.28. One gram of each preserved sample was placed in a 15 ml Falcon tube filled 2/3 with distilled water and the sample was homogenized. Then the sample was manually centrifuged for 10 minutes, the supernatant was discarded, and the fecal matter was re-suspended with the saline solution filling the tube to form an inverted meniscus where a cover slip was placed. After centrifuging manually for 10 minutes, the cover slip was removed and analyzed under a microscope using 4x, 10x and 40x magnification. Eggs, cysts and larvae were counted and measured with a micrometer. We used a drop of dilute Lugol's iodine solution (20 %) to facilitate the identification of protozoan cysts. Photos of representative individuals were taken.

Data analysis

Information of parasite prevalence was defined as the number of samples infected with one parasite group divided by the total number of samples taken in each study site. We performed Chi-square analysis between the prevalence of each group of parasites to identify the difference in parasite communities between study sites.

Results

A total of 185 samples were collected and analyzed. From these, 43 belonged to wild woolly monkeys, 41 to captive and 101 to reintroduced (56 taken when still in captivity and 28 after release into the wild at Pensil and 17 at San Martín). A great variety of gastrointestinal parasites was found: six Nematode families (Trichostrongylidae, Oxyuridae, Ancylostomatidae, Ascarididae, Strongylidae and Trichinellidae), eggs belonging to the class Cestoda and Trematoda, and one protist belonging to the family Entamoebidae (Fig. 2).

In captive individuals at Mesitas and Pereira, we found that 95 % of the samples had at least one parasite individual (egg, cyst or larvae) and 90 % had polyparasitism. We identified five groups of parasites: four members of the phylum Nematoda (Trichostrongylidae, Strongylidae, Trichinellidae and Oxyuridae) and one protist of the family Entamoebidae.

Families with higher prevalence were Oxyuridae (83 % of samples in Mesitas), and Trichstrongylidae (80 % in Mesitas and 88 % in Pereira).

We found significant differences between samples taken from primates in captivity sites ($X2 = 76.1$, df = 5, $p < 0.01$) due to the fact that Pereira was the only study site where family Trichinellidae was recorded. When prevalence of parasitic families between the two captivity sites was compared, we only found significant differences in the Oxyruridae, for which Mesitas had a greater prevalence ($X2 = 9.96$, df = 1, $p < 0.05$) (Fig. 3).

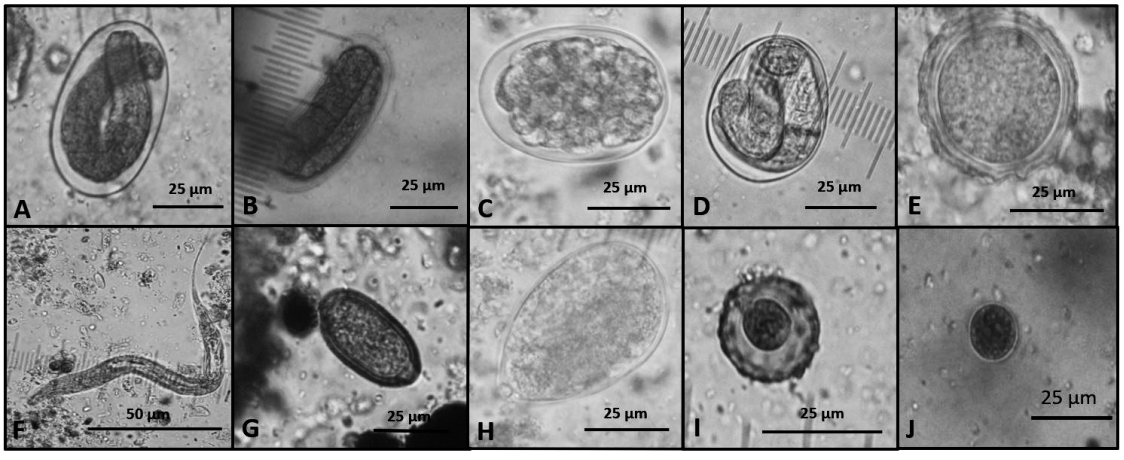

Figure 2. Gastrointestinal parasites found in captive and/or wild woolly monkeys. (A) Trichostrongylidae, (B) Oxyuridae, (C) Ancylostomatidae, (D) Ascarididae, egg without cortex (E) Ascarididae, (F) Strongylidae, (G) Trichinellidae, (H) Trematoda, (I) Cestoda, (J) Entamoebidae.

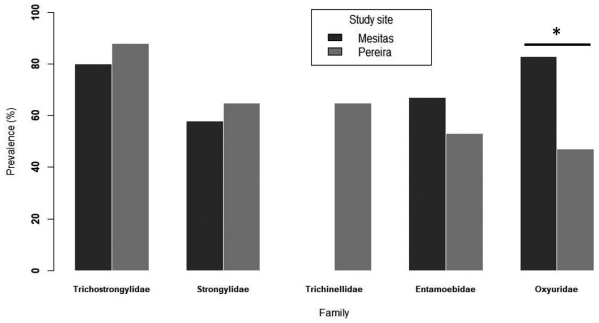

Figure 3. Prevalence of gastrointestinal parasites in woolly monkeys in each captive site. Mesitas (n = 24), Pereira (n = 17).

Regarding wild woolly monkeys at PNNCG, we found that 77 % of the samples analyzed had at least one parasite individual. We identified five groups of parasites: a protist from the family Entamoebidae, three nematode families (Oxyuridae, Ascarididae and Strongylidae) and one Cestode. We found that 46 % of the samples had Helminths and 42 % Protists.

When we evaluated the samples of the reintroduced individuals, we found eight groups of parasites, five helminths (Trichostrongylidae, Oxyuridae, Ascarididae, Ancylostomatidae and Strongylidae), one protist (Entamoebidae), one Trematode and one Cestode. In both reintroduction sites, we found significant differences between captive and released individuals (Pensil: $X2 = 57.8$, df = 8, $p < 0.01$ and San Martín: $X2 = 69$, df = 8, $p < 0.01$) where a prevalence reduction was shown in almost all parasite groups. Based on this result we separated the samples in captive and released categories for further analysis. We found an evident reduction of positive samples for almost all nematodes ($X2 = 31.692$, df = 8, $p < 0.01$) including families Trichostrongylidae, Oxyuridae, Ancylostomatidae and Ascarididae (Fig. 4).

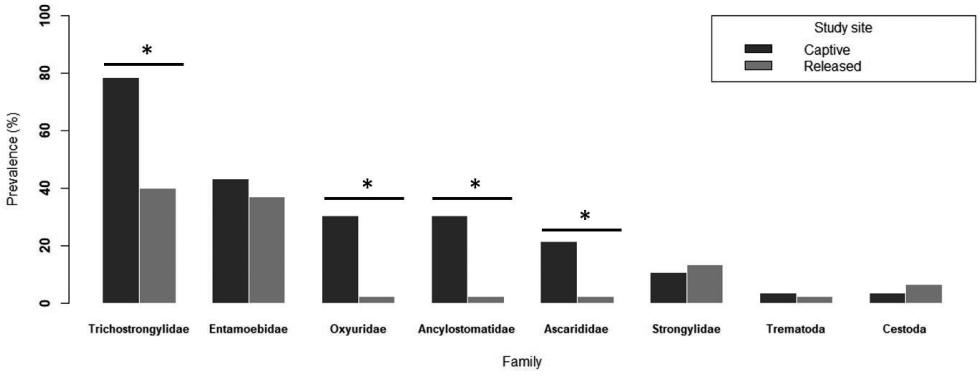

Figure 4. Prevalence of gastrointestinal parasites in woolly monkeys in captive (n = 56) and released (n = 45) individuals.

We observed that captive individuals had a higher prevalence of nematodes than wild and released ones but no differences in the prevalence of protists and cestodes were found. Also, we found that trematodes were present in captive and released individuals but not in the wild (Table 1).

Table 1. Prevalence (positive samples/total samples (*100)) of gastrointestinal parasites in captive individuals in Teruel, wild individuals at PNNCG and released individuals at Pensil and San Martín.

	Captive (n = 56)	Wild (n = 43)	Released (n = 45)
Nematodes	91 %	46 %	45 %
Protists	43 %	42 %	37 %
Trematodes	4 %	0 %	2 %
Cestodes	4 %	2 %	7 %

Discussion

For the first time, gastrointestinal parasites infecting captive and wild Colombian woolly monkeys were studied. As expected, many of the parasite families found have been reported in the same primate genus (Michaud et al., 2003; Larrañaga and Shanee, 2012; Pinto et al., 2013) but we found four new parasite records for these primates: Entamoebidae, Trichostrongylidae, Trematoda and Cestoda. We found differences between sites where primates were held in captivity since the family Oxyuridae had a higher prevalence in Mesitas compared to Pereira. This can be explained by the fact that these parasites are transmitted mainly by contact between individuals, and overcrowding in captivity can promote this behavior facilitating their transmission (González-Hernández et al., 2014). All parasites present in Mesitas and Pereira have been commonly reported in captivity sites or near urban areas in different primate species (Hasegawa et al., 2004; Soto-Calderón et

al., 2016) and have been reported in humans, which may suggest possible zoonotic infections (Yamashita, 1963; Legesse and Erko, 2004). Parasites found in captivity were similar to the ones found in the wild at PNNCG. Three of the five parasite families found there (Strongylidae, Oxyuridae and Entamoebidae) were found in wild woolly monkeys with the exception of Trichinellidae and members of the Trichostrongylidae family that only appeared in captivity. Ascarididae and Cestoda were the parasites found in the wild that were absent in captivity.

As for the reintroduced individuals, we found a tendency to reduction in parasite prevalence after being released. These differences between sites can be associated to higher rates of infection in the captivity sites due to overcrowding, since primates share the enclosure with other 10 individuals. The higher prevalence of family Trichostrongylidae, Oxyuridae, Ancylostomatidae and Ascarididae may be because these parasites are geohelminths (Bethony et al., 2006; Botero and Restrepo, 2015) and infect the primates when they come into contact with the floor. The presence of members of the Ascarididae family supports this idea, since these parasites need a maturation time in the floor before being infective (CDC, 2010). On the contrary, released individuals have better chances to explore higher forest strata and avoid contact with these parasites. The higher prevalence of Oxyuridae may be explained by the reasons we mentioned before that these parasites are transmitted mainly by contact between individuals. A similar situation has been reported in spider monkeys, where the number of grooming interactions was positively correlated to the presence of *Strongyloides* and *Trichostrongylus* (Rimbach et al., 2015). Fewer encounters with conspecifics can then reduce the prevalence of parasites in released individuals when compared to those in captivity.

Another factor that may be influencing differences in parasite prevalence is primate diet. Many authors have reported a negative relation between the consumption of some plants and parasitic infections (Huffman et al. 1997; Stoner and González-Di Pierro 2006). Many of the plant families that primates consume in the wild and were not consumed in captivity belong to the families Moraceae, Rubiaceae, Araceae and Lauraceae, which have been found to have deworming effects (Waller et al. 2001; MacIntosh and Huffman 2010). These plant families are consumed by woolly monkeys in high and low lands (Stevenson et al. 1994 and Ramirez et al. 2014), perhaps a switch on the diet in reintroduced individuals promoted a reduction in some parasite prevalence's similar to the ones in wild individuals.

Our data showed a consistent difference between study sites suggesting that diet, overcrowding and human presence may be the most important factors explaining parasite communities in woolly monkeys in Colombia. We do not consider environmental variables to be playing an important role, since captivity sites were different among them, but similar in parasite communities; the same tendency was found in wild and released individuals. Due to the fact that some parasites were found in captive and released individuals, but not in the wild ones, we support the idea of restoring parasite-host balance before releasing the individuals as Armstrong and Seddon proposed (2008). To achieve this, it is convenient to reduce the number of individuals in an enclosure and limit the contact of the individuals with the ground to reduce infection rates and zoonotic infections. Additionally, it may be useful to provide the primates with deworming plants, mainly those common in the diet of wild woolly monkeys prior to reintroduction.

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