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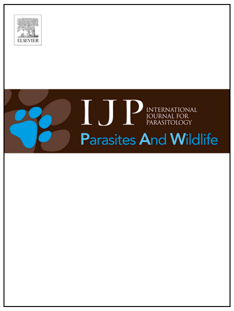

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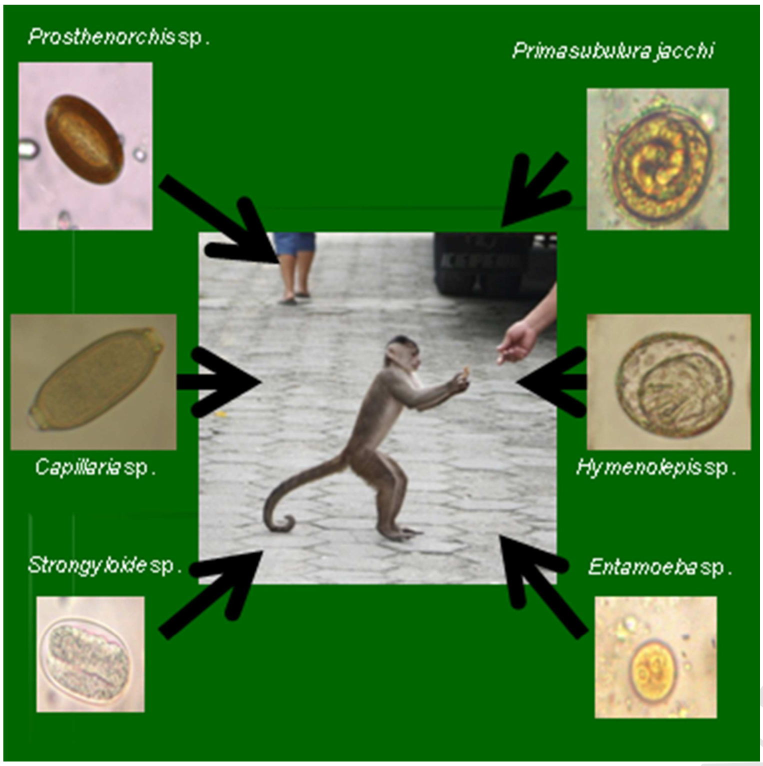

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Gastrointestinal Parasites in Captive and Free-ranging *Cebus albifrons* in the Western Amazon, Ecuador.

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Abstract

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Currently, there is a lack of surveys that report the occurrence of gastrointestinal parasites in the white-headed capuchin monkey (*Cebus albifrons*). We therefore assessed the presence and richness (= number of different parasite genera) of parasites in *C. albifrons* in wildlife refuges (n = 11) and in a free-ranging group near a human village (n = 15) in the Ecuadorian Amazon. In the 78 samples collected (median of 3 samples per animal), we identified a total of 6 genera of gastrointestinal parasites, representing protozoa, nematodes, acantocephalan and cestodes. We observed a high prevalence (84%) across the 26 individuals, with the most prevalent parasite being *Strongyloides* sp. (76.9%), followed by *Hymenolepis* sp. (38.5%) and *Prosthenorchis elegans* (11.5%). We found *Entamoeba histolytica/dispar/moskovskii/nuttalli* and *Capillaria* sp. in only a minority of the animals (3.8%). In addition, we observed unidentified strongyles in approximately one-third of the animals (34.6%). We found a total of 6 parasite genera for the adult age group, which showed higher parasite richness than the subadult age group (5) and the juvenile age group (3). Faecal egg/cyst counts were not significantly different between captive and free-ranging individuals or between sexes or age groups. The free-ranging group had a higher prevalence than the captive group; however, this difference was not significant. The only genus common to captive and free-ranging individuals was *Strongyloides* sp. The high prevalence of gastrointestinal parasites and the presence of *Strongyloides* in both populations support results from previous studies in *Cebus* species. This high prevalence could be related to the high degree of humidity in the region. For the free-ranging group, additional studies are required to gain insights into the differences in parasite prevalence and intensity between age and sex groups. Additionally, our study demonstrated that a serial sampling of each individual increases the test sensitivity.

Keywords: *Cebus albifrons*, prevalence, parasite richness, faecal egg/cyst counts, Amazonian Ecuador

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1. Introduction

Gastrointestinal parasites are commonly found in non-human primates (NHPs) in both captive and wild populations. Over the last decades, wildlife is commonly infected with gastrointestinal parasites (Ghandour et al., 1995; Eckert et al., 2006; Bezjian et al., 2008). However, habitat fragmentation is considered as impacting primate conservation because it could increase parasite richness and parasite load, affecting primate host ecology (Vitazkova and Wade, 2007), including host survival and population density (Chapman et al., 2005), reproduction (Beltran-Bech and Richard, 2014) and behaviour (Nunn and Altizer, 2006; Wren et al., 2010). Parasites are also considered to be a threat to public health (Daszak et al., 2000; Gillespie et al., 2008), as NHPs, either captive or free-ranging, are reservoirs of many human pathogens (Chapman et al., 2006; Vitazkova and Wade, 2006; Friant, 2007; Johnson-Delaney, 2009). Habitat fragmentation, along with bushmeat hunting and tourism, results in altered interfaces between animals and people (Homsy, 1999; Wolfe et al., 2005) that can facilitate the transmission of parasites from NHPs to humans and vice versa (Munene et al., 1998; Muriuki et al., 1998; Nizeyi et al., 2002; Gillespie and Chapman, 2006; Goldberg et al., 2008; Kowalewski et al., 2011). Parasitological studies conducted in NHPs revealed that primates with less human contact have a lower prevalence and intensity of parasites compared to groups with more human contact (McGrew et al., 1989). However, the type of parasite varies according to habitat. For example, the presence in NHPs of certain human parasites (e.g., *Giardia duodenalis*) is found at sites with more intense human contact (Vitazkova and Wade, 2006). In captive NHPs destined for the animal trade or as pets, the lack of relevant knowledge among pet owners, zoo keepers and veterinarians (Renquist and Whitney, 1987) may affect cross-species parasite transmission. A recent study demonstrated possible zoonotic transmission

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of Entamoeba nuttalli between NHPs and zoo keepers in 5 zoos (Levecke et al., 2015). Thus, it is important to study parasites in NHPs with different levels of contact with humans.

Most parasitological studies have been focused on Old World NHPs (Lilly et al., 2002; Hahn et al., 2003; Ocaido et al., 2003; Legesse and Erko, 2004; Gillespie et al., 2005; Muehlenbein, 2005; Chapman et al., 2006; Friant, 2007; Hopkins and Nunn, 2010; Howells et al., 2011). When studies did include New World NHPs, they were primarily *Alouatta* sp. (Stoner and González-Di Pierro, 2005; Cristóbal-Azkarate et al., 2010; Kowalewski et al., 2011; Milozzi et al., 2012; Behie et al., 2014; Maldonado-López et al., 2014). To broaden the scope of research, we chose to conduct our study on a *Cebus* species*.* The white-fronted Capuchin, *Cebus albifrons*, is distributed in several countries of South America. In Ecuador, there are two subspecies (*Cebus albifrons* and *Cebus albifrons aequatorialis)*. The former species, the subject of our study, is found in the western Amazon. Its conservation status is of Least Concern (de la Torre et al., 2008) and it is also listed in Appendix II of CITES (Convention on the International Trade in Endangered Species of Wild Fauna and Flora). Unfortunately, there are only a few studies on the ecology, behaviour and health of this species (Defler, 1979; Pozo R., 2004; Derby, 2008; Cervera and Donati, 2012). *Cebus albifrons* is a middle-sized primate found in high density biomass regions in the forest (Tello, 2003). Habitat fragmentation and hunting for the pet trade represent major threats for its populations (Nuñez-Iturri and Howe, 2007).

NHP parasite diversity is not very well known for *Cebus albifrons.* For free-ranging capuchins, only a few reports are found in the literature (Dunn and Lambrecht, 1963; Ewing et al., 1968; Phillips et al., 2004). In Ecuador, there is only one study, that of lymphatic filariasis in the coastal region (García-Solorza and Levi-Castillo, 1954). For captive capuchins, a single study has been reported from Peru (Guerrero M et al., 2012).

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There are, however, published studies on the gastrointestinal parasites in other species of *Cebus* (see Appendix for more details).

To understand how parasitism is affected by host-intrinsic and -extrinsic factors, we correlated parasite prevalence, parasite richness (number of parasite genera), and faecal egg/cyst count with sampling effort, host sex and age, and between captive and free-ranging groups of capuchins with different levels of contact with humans. This study also creates baseline data for parasites of this species.

2. Methods

2.1. Study Site and Study Groups

This study was done in Tena (Napo) and Puyo (Pastaza), two main cities in Western Amazon. The monthly mean precipitation is 324 mm for Puyo and 407 mm for Tena. The daily mean temperature for Puyo is 21.1 °C and is 23.8 °C for Tena, according to the Instituto Nacional de Meteorología e Hidrología (INAMHI, 2014). We examined gastrointestinal parasites in two populations, one captive and one free-ranging. The captive population was studied in three wildlife refuges in Puyo. Most NHPs from wildlife refuges have been donated by families or confiscated by the police during roadside checks. The average size of the enclosures (n = 5) in these wildlife refuges was 76.5 m^2, resulting in an average animal density of 0.15 per m^2. Prior to this study, deworming was not established as a standard procedure. These animals had frequent contact with the animal caretakers and tourists, who regularly fed them. The free-ranging population (Table 1) lived in the small town of Misahualli (Tena, Napo) (1° 2' 7.0" S, 77° 39' 59.4" W). The home range of this group is

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approximately 2.04 ha. The home range (range in which animals were seen in 95% of the observations) was calculated based on following the group for a 3-month period (from March to May in 2012) and recording the group position every hour with a GPS Garmin eTrex 30 (Garmin International Inc., Olathe, KS, USA). The data were mapped and analysed using ArcView 3.2 with the “animal movement” extension using the 95% fixed Kernel method (Hooge and Eichenlaub, 2000).

Sampling

Between March 2011 and May 2013, we collected a total of 78 faecal samples from a total of 26 habituated individuals, 11 captives and 15 free-ranging. For the free-ranging group (see Table 1), individuals were followed daily from 8 am to 6 pm for three months (March-May). All animals were individually identified to facilitate serial sampling (3 samples per animal taken at least 24 h apart to increase the test diagnostic sensitivity) (Stoner, 1996). We collected the samples immediately after defecation (Chapman et al., 2012) to avoid a possible contamination by parasites in the environment (mean time between defecation and sample collection = 2.4 min). We stored the faecal samples in 50 ml Falcon tubes in 10% phosphate buffered formalin at 4 °C to prevent eggs from hatching as well as to prevent deterioration of the parasite eggs and cysts. We labelled Falcon tubes with the animal ID, time and date.

Table 1. Study population of *Cebus albifrons* sampled in the Amazonian region in Ecuador

2.2. Faecal analysis

We analysed all samples at the International Centre of Zoonoses at the Central University of Ecuador. We processed one gram of stool using a formol-ether concentration method for each sample. Briefly, we filtered

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1 g of sample through a gauze mesh into a sterilized urine sample collection cup with 10 ml of distilled water. The filtered solution was then centrifuged for 1 min at 426 g. After the supernatant was discarded, the sediment was suspended in 5 ml of 10% formalin. A total of 1.5 ml of diethyl ether was added. The suspension was thoroughly mixed by shaking it. The suspension was centrifuged for 2 min at 239 g. One drop of the concentrate was transferred to a microscope slide, which then was covered by a 22 mm x 22 mm coverslip. A second drop was transferred to a separate slide. Before dropping the coverslip, a drop of Lugol's iodine was added to facilitate the detection of protozoa. Parasites were identified on the basis of egg colour, shape and contents using a standard light microscope and 100X – 400X magnification. The identification of parasites was based on Flynn (1973), Thienpont et al. (1986) and Hasegawa (2009).

2.3. Statistical Analysis

We calculated the prevalence as the number of infected individuals with a particular parasite divided by the total number of individuals. Parasite richness is defined as the number of parasite genera found in one individual. For each individual, we counted the number of unique parasite genera observed across the 3 samples. We counted the number of eggs and cysts in a total of 6 slides (2 slides per sample x 3 samples per animal). We used the total number of eggs and cysts counted in one individual as a surrogate for the intensity of infection (Stoner, 1996; Gillespie, 2006). We used several statistical tests to analyse the results: 1) Fisher's exact test to determine significance of differences in parasite prevalence between the groups and sexes; 2) Chi Square test to assess differences in prevalence in each age group; 3) Mann-Whitney U test to assess differences in parasite richness and intensity (as estimated by egg and cyst counts); and 4) Kruskal-Wallace test to assess differences in parasite richness and age groups. Finally, we studied the impact of

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serial sampling on the qualitative (absence/presence) and the quantitative (faecal egg/cyst counts) assessment of gastrointestinal parasite infections. The McNemar test was used to assess whether increasing the sampling effort increases the prevalence. Similarly, we applied the Wilcoxon signed rank test to verify whether the parasite richness and intensity of infection change when one, two or three consecutive samples were examined. All statistical tests were done using Statistica (Dell Statistica, Tulsa, OK, USA). A significance level of alpha <0.05 was set for all statistical analyses.

3. Results

3.1. Parasite richness, prevalence, and intensity of infection

We detected a total of 6 parasite genera across the 26 animals, including representatives of protozoa (*Entamoeba histolytica/dispar/moskovskii/nuttalli*), nematodes (*Capillaria* sp., *Strongyloides* sp., and unidentified Strongyles), Acantocephalan (*Prosthenorchis elegans*) and cestodes (*Hymenolepis* sp.) (Fig. 1). Of the 26 individuals tested, 22 (84%) harboured at least one parasite genera. Parasite prevalence in free-ranging NHPs was greater than in captive animals (15/15 vs. 7/11, P = 0.02), whereas sexes (males: 18/22, vs. females: 4/4, P = 0.49) and age groups (juveniles: 4/5 vs. subadults: 9/9 vs. adults: 9/12, χ^2 = 2.57, p = 0.277) were equally frequently parasitized. Free-ranging animals harboured more parasite genera (4) than those living in captivity (3) (U = 137.00, P <0.05), and males (6) more than females (2) (not significantly different, U = 54.5, P = 0.5). Adults (6) harboured more parasite genera than juveniles (3) and subadults (5) (H = 7.247, P = 0.03).

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Fig 1. From left to right in each row: *Hymenolepis* sp., *Capillaria* sp., *Strongyloides* sp., *Prosthenorchis elegans*, Strongyle (unidentified), *Entamoeba histolytica/dispar/moskovskii/nuttalli*. (40x)

The prevalence of each of the different genera across the two groups, the sex and the age is reported in Table 2.

The most prevalent parasite was *Strongyloides* sp. (20/26), followed by *Hymenolepis* sp. (10/26). *Capillaria* sp. (2/26) and *Entamoeba histolytica/dispar/moskovskii/nuttalli* (1/26) were only found in a minority of the animals. Important differences in parasitic infection were observed between the captive animals (n = 11) and free-ranging ones (n = 15). *Entamoeba histolytica/dispar/moskovskii/nuttalli* and *Prosthenorchis elegans* were only observed in the captive NHP, whereas *Hymenolepis* sp., *Capillaria* sp. and unidentified strongyles were only observed in the free-ranging animals. *Strongyloides* sp. was observed in both groups but was more prevalent in free-ranging animals (P = 0.032). Similar differences across the sex and age groups can be noted. *Strongyloides* sp. (P = 0.676) and Strongyles (unidentified) (P = 0.504) were found across both sexes, while all other parasites were exclusively found in males. Across the three age groups, only *Strongyloides* (χ^2 = 1.4, P = 0.481), Strongyles (unidentified) (χ^2 = 4.4, P = 0.105), and *Hymenolepis* (χ^2 = 5.4, P = 0.06) were observed. *Capillaria* spp. (χ^2 = 0.5, P = 0.751) and *Prosthenorchis elegans* (χ^2 = 1.7, P = 0.411) were not found in juveniles. *Entamoeba histolytica/dispar/moskovskii/nuttalli* was only observed in adult animals (χ^2 = 1.2, P = 0.545).

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The mean egg counts ranged from 3 to 156. *Prosthenorchis elegans* had the highest egg output (156) followed by *Strongyloides* sp. (22) (Table 2). Faecal egg and cyst counts were not significantly different between sexes and age groups for all parasite genera. Significant differences in egg counts were only observed between captive (0) and free-ranging animals for *Hymenolepis* sp. (4.3) (U = 137.5, P < 0.05) and Strongyles (unidentified) (5.6) (U = 137.5, P < 0.05).

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Table 2. Prevalence and egg/cyst count of parasites 6 genera detected in *Cebus albifrons* in the Ecuadorian Amazon by group, sex and age.

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3.2. Impact of sampling effort on parasite richness, prevalence and intensity of infection

Overall, more parasites were detected when more samples were examined, both in terms of parasite richness (Fig. 2) and prevalence (Fig. 3). For example, when only one sample was examined, 3 parasite genera were found in 3 out of 26 individuals. When examining the second and third samples, 6 and 9 individuals were found, respectively, with 3 parasite genera ($Z = -3.1$, $P < 0.05$). An increase in prevalence was observed in general ($N = 26$, $P = 0.031$), and specifically for two parasites, *Strongyloides* (1 sample: 12/26 vs. 2 samples: 16/26 vs. 3 samples: 20/26, $P = 0.008$) and Strongyles (unidentified) (1 sample: 4/26 vs. 2 samples: 7/26 vs. 3 samples: 10/26, $P = 0.031$). For *Hymenolepis* spp. (1 sample: 5/26 vs. 2 samples: 9/26 vs. 3 samples: 10/26, $P = 0.063$) and the remaining 3 parasite genera, no difference in prevalence was observed.

Fig 2. Cumulative richness from the first sample to the third sample for the 26 animals sampled.

Fig. 3. Cumulative prevalence of parasites in each sample.

Faecal egg and cyst count

The faecal egg count did not increase significantly with serial sampling for *Strongyloides* sp. ($Z = -1.8$, $p = 0.068$), *Hymenolepis* sp. ($Z = -0.1$, $p = 0.919$) and Strongyles (unidentified) ($Z = -1.8$, $p = 0.062$). In the case

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of the other parasites, only a few individuals (1 to 3) tested positive and counts did not increase significantly. For example, for *Prosthenorchis elegans,* the number of eggs increased across the three samples, but not significantly (Z = -1.6, p = 0.109) (Fig. 4).

Fig. 4. Cumulative egg and cyst count of parasites from each sample.

3. Discussion

3.1. Parasite Genera Found (Prevalence and zoonotic potential of each genus)

We identified 6 parasite genera in our surveys that contribute to the parasite ecology of *C. albifrons*, with *Strongyloides* sp. and *Hymenolepis* sp. being the most prevalent. NHP locomotion on the ground increases the possibility of *Strongyloides* transmission when the filariform larvae developed in the soil come in contact with the skin. Studies of terrestrial NHPs found a high prevalence of *Strongyloides* sp. of 44% and 60.7% (Ocaido et al., 2003; Bezjian et al., 2008) in baboons and 81.2% in captive orangutans with ground-dwelling behaviour (Mul et al., 2007). *Cebus albifrons*, however, is an arboreal primate. Specific information on *C. albifrons* in their natural habitat is lacking, but *C. capucinus* was reported to spend only 5% of their time on the ground (Rose, 1994). Both the captive and the free-ranging groups in our study spent an average of 70% of their observed time on the ground (Ramirez-W. and V.-Curicama, unpublished data). Thus, the high prevalence of *Strongyloides* found in our study may be due to the predominantly terrestrial locomotion. One study in the Tambopata area of Peru showed a lower prevalence (18%) of *Strongyloides* in *C. albifrons*

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(Phillips et al., 2004) than in our population; however, the Tambopata group was 40 km away from any human settlements, and ground-dwelling behaviour was not reported for this group. A long-term study would help us correlate *Strongyloides* sp. prevalence and the time spent on the ground for both the captive and the free-ranging groups. Given its high level of infection, the free-ranging group of *C. albifrons* may nevertheless represent a threat to other communities they encounter. For the captive group, the high prevalence of *Strongyloides* sp. might be associated with several factors: high densities in the cages, which causes a constant re-infection, and lack of environmental enrichment, both of which can result in animals spending more time on the ground, which, as described above, can increase the possibility of transmission. In captive *C. apella*, *Strongyloides* sp. was also the most prevalent (Santa Cruz et al., 2000). Even though we did not identify the species of *Strongyloides* in our samples, *Strongyloides cebus* is the only *Strongyloides* to infect neotropical primates (Little, 1966) and has been observed in *Cebus* species (Guerrero M et al., 2012) and in *Lagothrix cana* (Tenorio Mati et al., 2013). In marmosets (*Callithrix penicillata*) inoculated subcutaneously with third stage infective larvae of *Strongyloides stercoralis,* the parasite established and was maintained for up to approximately 10 months (Tenorio Mati et al., 2014). According to Darling (1911), however, the only way of infection would be through the oral mucosa because *S. stercoralis* does not infect *Cebus* through the skin. As NHPs are social and mouth contact between individuals is common (e.g., during grooming), the oral mucosa might be an infection route. The prevalence of Strongyles (unidentified) may be due to our inability to identify these species because there is a morphological similarity in the eggs of certain strongyles genera, such as *Oesophagostomum* sp., *Necator* sp. and *Ancylostoma* sp., and coprocultures were not performed in order to differentiate those genera (Little, 1981).

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Cestodes are known to infect neotropical primates (Pacheco et al., 2003; Müller, 2007; Parr et al., 2013b). *Hymenolepis* has an indirect life cycle associated with rats and arthropods, including beetles (Evans et al., 1998) and cockroaches (Johnson-Delaney, 2009), as intermediate hosts and which are present in human settlements (Michaud et al., 2003). *Hymenolepis* sp. was only present in the free-ranging group. Montenegro (2011) found *Hymenolepis* sp. in *C. flavius*, with a prevalence of 16.7%, which is lower than in our study; however, those animals were in a private reserve away from human settlements. The diet of *Cebus albifrons* is very generalized (Terborgh, 1983), including arthropods. We believe this is one of the reasons for a high prevalence of *Hymenolepis* sp. in the free-ranging group. *Hymenolepis nana* and *Hymenolepis diminuta*, two cestodes that infect humans (PAHO, 2003), might also infect *Cebus*. In the case of *Hymenolepis nana,* this species has a direct lifecycle, thus increasing the prevalence of *Hymenolepis* sp. *Hymenolepis cebidarum* also infects cebids (Dunn, 1963). Unfortunately, there is not enough published information about this species. Pacheco et al. (2003) found and measured several eggs of *Hymenolepis* sp. in free-ranging *Callicebus nigrifons*. They concluded that these animals were infected with several species of *Hymenolepis* that could only be identified at necropsy.

Prosthenorchis elegans was at a high prevalence in this study and was found only in captive individuals. The reason probably is that it is transmitted by cockroaches and other insects, specifically found in high density captive facilities of wildlife refuges because of the food accessibility, and can become a parasite reservoir for NHPs. *Prosthenorchis* sp. has been reported to infect *C. capucinus* (Yamashita, 1963; Parr et al., 2013b). *Prosthenorchis elegans* has been reported in *C. albifrons* (Garner et al., 1967; Cárdenas-Callirgos et al., 2010) and from other neotropical primates, both captive (Wolff et al., 1990; Berrocal and Naranjo, 1999; Pérez García et al., 2007; Pissinatti et al., 2007; Pérez et al., 2008) and free-ranging (Berrocal and Naranjo,

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1999; Phillips et al., 2004; Pinto et al., 2008; Müller et al., 2010; Wenz et al., 2010; Tavela et al., 2013). However, most of these studies are case reports in which surgical interventions are usually described, but there are no available data on the prevalence and the intensity of infection. Only Monteiro et al. (2010) report the prevalence of parasites in two neotropical primates, one similar to those found in this study. This parasite is sometimes of medical importance in captive NHPs, causing death (Arrojo, 2002; Guerrero M et al., 2012; Perez et al., 2013). Currently there are no successful medical treatments available, although surgical removal of the parasites has been reported in other host species (Wolff et al., 1990; Pérez et al., 2008).

The other parasites with a low prevalence in this study were *Capillaria* sp. and *Entamoeba histolytica/dispar/moskovskii/nuttalli*. *Capillaria* sp. was reported to infect *Cebus capucinus* (Foster and Johnson, 1939) and *Alouatta palliata* (Helenbrook et al., 2015) with a prevalence of 38%.

Entamoeba histolytica/dispar/moskovskii/nuttalli is transmitted directly through a faecal – oral route (Taylor et al., 2013). The presence of this species complex may suggest a zoonotic transmission; however, because they are morphologically indistinguishable, only molecular characterization can detect transmission between humans and wildlife (VanderWaal et al., 2014). This species complex has not been previously reported in *C. albifrons*. However, *E. histolytica* has been found in the New World NHPs *Ateles belzebuth hybridus*, *Leontopithecus chrysomelas*, *Saguinus oedipus* (Verweij et al., 2003), although differentiation from *E. nuttalli* was not possible at that time, and *Alouatta caraya* and *Lagothrix lagotricha* (Regan et al., 2014), as well as the Old World NHPs *Papio hamadryas, Cercopithecus aethiops,* and *Pan troglodytes verus* (Ghandour et al., 1995; Legesse and Erko, 2004; Levecke et al., 2010; Howells et al., 2011). The prevalence of *E. histolytica* in captive New World NHPs at the Twycross Zoo is low (11,8% for *Alouatta caraya* and 16,7% for *Lagothrix*

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lagotricha) (Regan et al., 2014). The low prevalence in our study is not necessarily a cause for public health concern, because we have not identified the Entamoeba of this species complex, but it is important to know if NHP populations can host naturally acquired *E. histolytica* (the human variant) infections. Several human and non-human genotypes have been identified in captive animals (Verweij et al., 2003; Tachibana et al., 2007); thus, caution is warranted and the use of molecular tools to identify the human and the NHP strains (Tachibana et al., 2013) and to identify the possible transmission between humans and NHP or vice versa is important (Levecke et al., 2015).

3.2. Parasite richness data

The parasite richness we found (6) is similar to the other two studies of capuchins in Peru, 5 parasites for the Pucallpa Zoo (*Paratriotaenia oedipomidatis*, Trichostrongylidae, Oxyuriodea, *Crytosporidium spp*, and *Balantidium coli*) (Guerrero M et al., 2012), and 3 for the Tambopata group (*Ascaris sp., Trichuris trichiura* and *Schistosoma mansoni*) (Phillips et al., 2004). None of these genera were found in our study. In our study, significantly higher parasite richness is found in the free-ranging group than in the captive group. The high contact between tourists and NHPs observed during a behavioural study (Ramirez-W., unpublished data) might be associated with this parasite richness. Rates of contact with people can be associated with oral and faecal transmission routes. For example, diapers left by tourists can come in contact with NHPs. Additionally, the monkeys drink from a creek contaminated by faeces coming from the houses nearby, likely with *E. histolytica/dispar/moskovskii/nuttalli* and other parasites.

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3.3. The relationships of prevalence and species richness with habitat, sex and age

Unfortunately, seasonality was impossible to analyse because these two regions (Puyo and Tena) have the highest humidity and precipitation rates in Ecuador, and there is no variation in patterns of climate data throughout the year. However, humidity has been demonstrated to influence the prevalence of some parasites (Stuart et al., 1990; Wren et al., 2015). For example, a humid environment contributes to the development of larvae from *Strongyloides* (Elsheikha and Khan, 2011; Armon and Cheruti, 2012), and may partly explain the high prevalence of *Strongyloides* reported here for Puyo and Tena.

Both groups are, or were, in contact with humans, and other studies have demonstrated that this may increase the possibility of parasite transmission (Chapman et al., 2005; Kowalewski and Gillespie, 2009). The free-ranging group from Misahualli has similar conditions to the *Cebus capucinus* group from the Manuel Antonio National Park, where monkeys eat food provided by tourists (Chinchilla et al., 2007). Captive NHPs eat food from the ground, and wild NHPs also eat food from the ground and soak food in water found in tree holes, water that could be contaminated by infective parasite ova and larvae (Stoner, 1996). Additionally, NHPs are social (Defler, 1979) which makes parasite transmission easier (Côté and Poulin, 1995). Primate locomotion on the ground increases the possibility of parasite transmission when larvae are in contact with the skin, as is the case for *Strongyloides* sp., or when cysts are ingested; for example, *Entamoeba* sp. The high prevalence of *Hymenolepis* in the free-ranging group might be associated with a ramdomly dispersed defecation and frequent ground dwelling behaviour. Despites the low prevalence of eggs of both *Hymenolepis nana* and *H. diminuta* in the soil (Kumar Rai et al., 2000), a frequent ground dwelling behaviour could enhance the probability of contamination when individuals walk from one tree to another.

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Parasite prevalence in the captive group might be linked to contaminated water and human excrement exposure, to the small area where the captive individuals are held and to constant exposure to infection. For the free-ranging group, the home range area is small and they are fed by tourists and locals. These conditions are similar to those in captivity, with similar risks of parasite transmission related to the poor habitat quality (Wolfe et al., 2005; Goldberg et al., 2008; Rwego et al., 2008). Nunn and Tae-Won Dokey (2006) found a positive correlation between parasite richness and range use. Crowding in a small area results in the repeated use of this area and a high risk of faecal contamination. Monkeys in these conditions can come into contact with directly transmitted parasites such as *Entamoeba* sp. cysts, and they also have a greater probability of encountering intermediate hosts of indirectly transmitted parasites (e.g., *Hymenolepis diminuta*, *Prosthenorchis elegans*). Moreover, there is a positive correlation between parasite richness and host density (Altizer et al., 2003). NHPs crowding in a small area might have a greater chance of reinfection by directly transmitted parasites (e.g., Strongyles). In Costa Rica, home range sizes for a *Cebus* group are greater (Parr et al., 2013a) and parasite richness is lower than in the Misahualli Group. Unfortunately, the other study of capuchins in Manuel Antonio, Costa Rica, does not report data on home range size (Chinchilla et al., 2007) (Table 3).

Table 3. Home range area and density of the Misahualli Group and of a group of *Cebus* in Costa Rica and their PSR.

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In the free-ranging group, a similar rate of parasite richness was found for both males and females. This result must be viewed with caution because more males were sampled, and *Hymenolepis* sp. was found only in males. Rose (1994) observed males of *C. capucinus* consuming more invertebrates than females, and therefore males might be more at risk of becoming infected given the lifecycle of *H. diminuta*. In rats experimentally infected with this species, the intensity of infection was higher in males than females, which could be attributed to the presence of testosterone, lowering the immune function (Addis, 1946).

The egg output shows another disparity regarding gastrointestinal parasites in males and females as reported by Wenz et al. (2010), who found a mean egg output per 100 µl of concentrated sediment for *Strongyloides stercoralis* of 2.5 for males and 1 for females. These figures are lower than the FEC figures found in our study. However, their methods differed from ours, and the groups mentioned in their study are arboreal. In our study, the egg output is higher in males, other than for the unidentified strongyles.

A high rate of contact between juveniles and subadults may not be a significant predictor for infections with parasites (MacIntosh et al., 2012; Gómez et al., 2013). Juveniles and subadults might be under more stress than adults because of their lower status and their immune function is not entirely developed (Müller-Graf et al., 1996). Moreover, in young baboons, it is thought that parasites, particularly nematodes, use a transmammary route of transmission (Müller-Graf et al., 1996). Contrary to these findings, our study showed there were no significant differences in prevalence with age or sex. Also in our study, parasite richness was higher in adults than subadults and juveniles. These results may be due to status in the group hierarchies. Adults have been in contact with the environment much longer and consume more food, increasing the risk of infection (Nunn et al., 2003).

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3.4. Impact of sampling effort on parasite richness, prevalence and intensity of infection

In our study, as has been shown before (Muehlenbein, 2005), the number of parasite genera increases when three samples are taken. The detection of parasites depends on their biology, especially their fecundity and how often they release eggs. For example, *Hymenolepis diminuta* is a parasite that releases up to 250 000 eggs per day. This could be a reason why *Hymenolepis* was detected in the free-ranging group from sample 1 to sample 3 in infected individuals. In a study on *Callicebus nigrifrons*, Pacheco et al. (2003) sampled four individuals infected with *Himenolepis* sp. and found in three a regular pattern of egg numbers in their samples. Thus, serial sampling of identified hosts increases the accuracy of prevalence data. Milozzi et al. (2012) found a similar prevalence of parasites (86.8%) when sampling three times. Regarding the intensity of infection, our study did not find a significant increase in egg/cyst counts when sampling three times. This might be because samples were not taken with the same interval between them, nor were the samples taken at the same time each day. To see whether the parasite has a release pattern of eggs or not, this method should be applied on consecutive days at the same hour.

This study reports on the parasite richness as well as the prevalence and the egg/cyst count of gastrointestinal parasites found in 26 individuals. The parasite distribution in this study reflects the presence of new parasites for *Cebus albifrons.* The multiple samples and the formol-ether concentration method is an

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accurate method with high sensitivity for most parasites, except for *Giardia sp*., which is commonly found in captive groups (Levecke, 2010). However, FECC did not increase with sampling effort. These findings suggest an impact of the human population on the free-ranging group. The free-ranging group had an overall higher prevalence and parasite richness than the captive group. It is possible that the free-ranging-group might be a source of infection for *Hymenolepis* sp. and *Capillaria* sp. Further research is needed to observe whether our findings further relate to biodiversity conservation, primate behaviour, the life cycle of parasites, and human health, since each parasite is affected by a range of ecological factors, such as humidity, temperature, behaviour, density, arthropod consumption, and water sources. The captive population where we found the *Entamoeba histolytica* complex species needs a deeper screening to identify the human and the NHP strains. In addition, coprocultures are necessary to differentiate unidentified strongyles. This study presents baseline data for future research on gastrointestinal parasites for *Cebus albifrons* in Ecuador.

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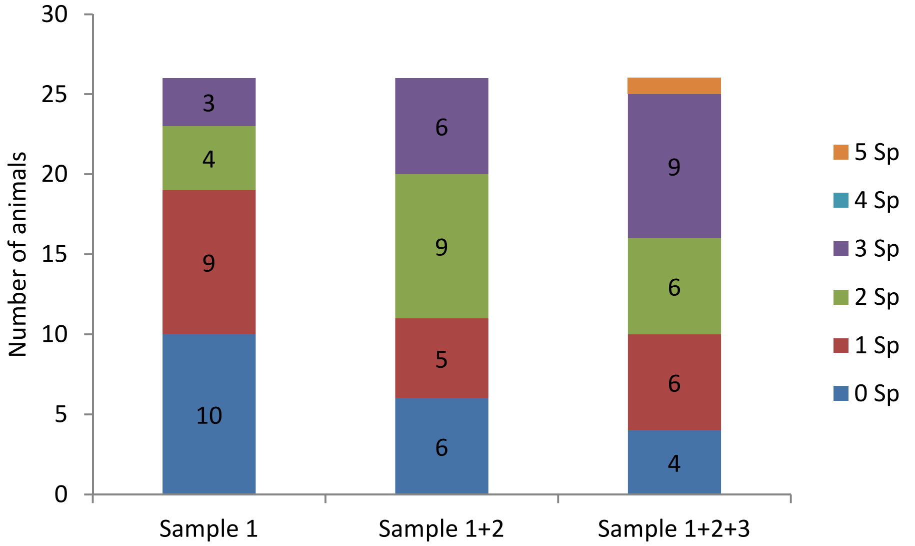

Fig. 2. Cumulative richness from the first sample to the third sample for the 26 animals sampled.

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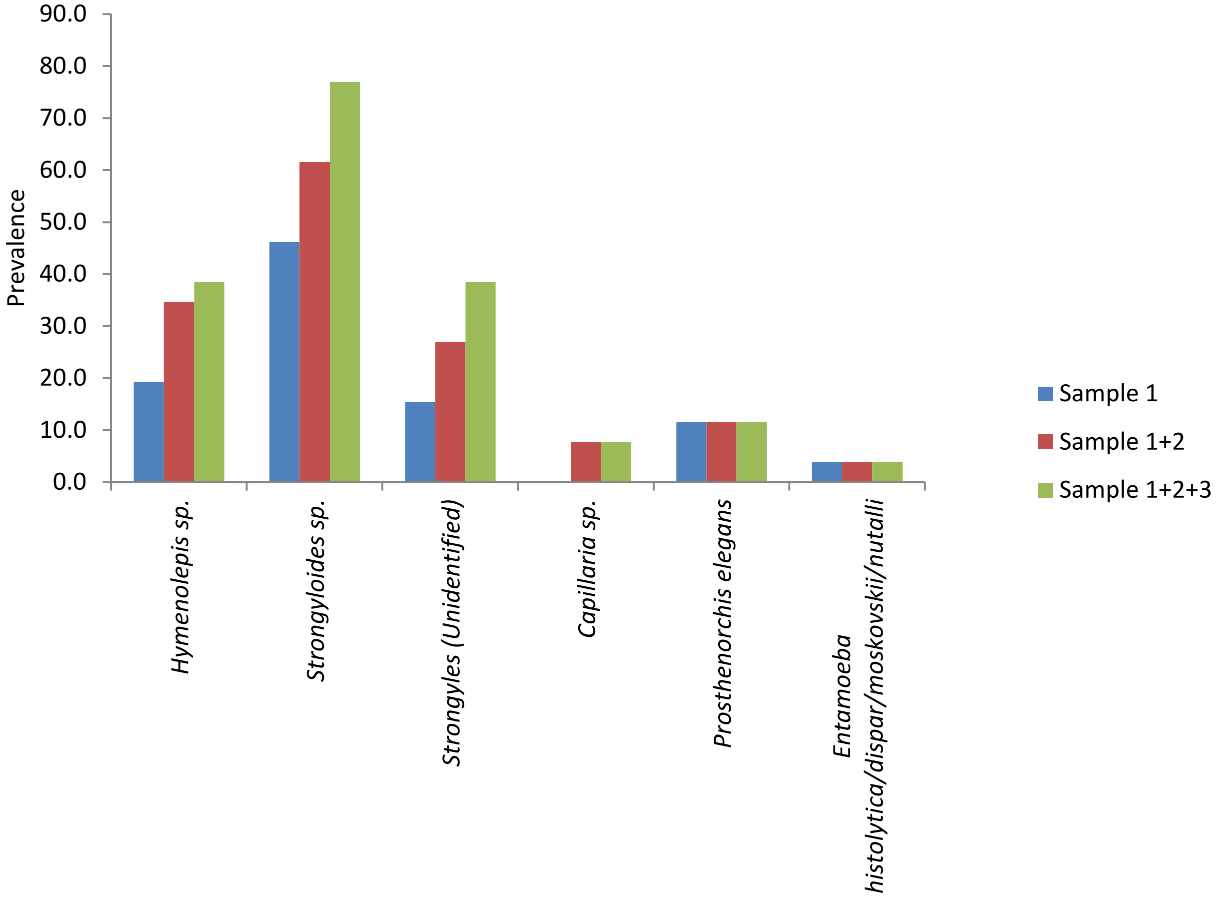

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698 Fig. 3. Cumulative prevalence of parasites from each sample.

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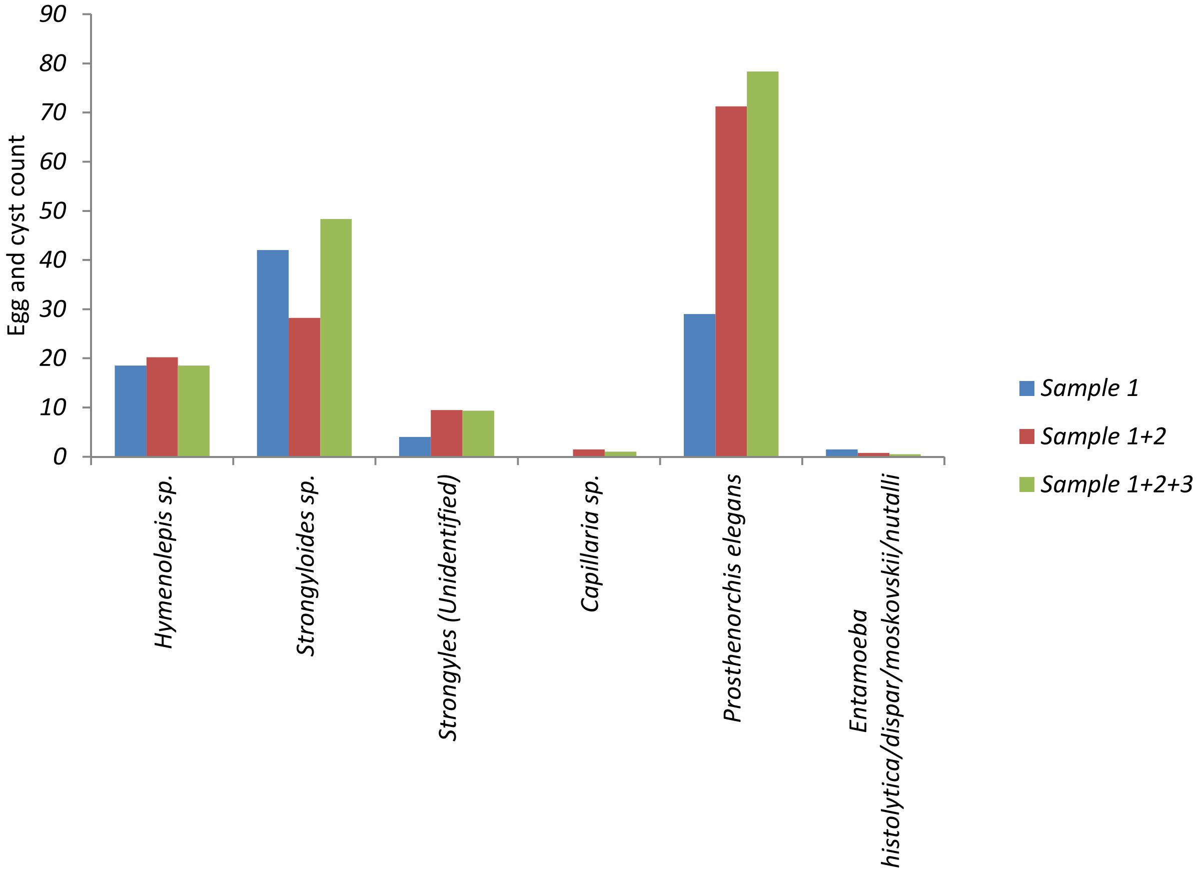

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701 Fig. 4. Cumulative egg and cyst count of parasites from each sample.

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Table 1. Study population of *Cebus albifrons* sampled in the Amazonian region in Ecuador

	Captive Population N = 11	Free-Ranging Group N = 15
Age		
Adults	7	5
Subadults	2	7
Juveniles	2	3
Sex		
Males	11	11
Females	0	4

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Table 2. Prevalence and egg/cyst count of 6 parasite genera detected in *Cebus albifrons* in the Ecuadorian Amazon by group, sex and age.

		Parasite Genera											
		Cestode		**Nematoda**						**Acantocephala**		**Protozoa**	
	Number of animals	*Hymenolepis* sp.		*Strongyloides* sp.		Strongyles (unidentified)		*Capillaria* sp.		*Prosthenorchis elegans*		*Entamoeba histolytica/dispar/moskovskii/nuttalli*	
		Prevalence	Mean Egg Count	Prevalence	Mean Egg Count	Prevalence	Mean Egg Count	Prevalence	Mean Egg Count	Prevalence	Mean Egg Count	Prevalence	Mean Cyst Count
Group													
Captive	11	0	0	6 (54.5)	50.8	0	0	0	0	3 (27.7)	156	1 (9.1)	3
Free-Ranging	15	10 (66.6)*	4.3	14 (93.3)*	9.7	9 (60.0)*	5.6	2 (13.3)	3	0	0	0	0
Sex													
Males	22	10 (45.4)	4.3	17 (77.3)	23.3	7 (31.8)	3.1	2 (9.1)	3	3 (13.6)	156	1 (4.0)	3
Females	4	0	0	3 (75.0)	14.6	2 (50.0)	15.5	0		0	0	0	0
Age													
Adult	12	2 (16.6)	9	8 (66.6)	15.7	2 (16.6)	4.5	1 (8.33)	3	1 (8.3)	17	1 (8.33)	3
Subadult	9	6 (66.6)	11.8	8 (88.8)	5.2	4 (44.4)	2.6	1 (11.1)	1	2 (22.2)	226.5	0	0
Juveniles	5	2 (40.0)	11	4 (80.0)	68.25	3 (60.0)	11.3	0	0	0	0	0	0

*($p < 0.05$)

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Table 3. Home range area and density of the Misahualli Group and of a group of *Cebus* in Costa Rica and their PSR.

	Number of Individuals	Home Range (ha)	Density (indiv/ha)	PSR
Ecuador				
Misahualli	15	5.38	2.78	2.5
Costa Rica				
Santa Rosa				
CP	24-26	80	0.3	1.36±0.76
EX	8-11	125	0.088	1.22±0.94
GN	33-35	188	0.186	1.27±0.84
LV	20-23	174	0.13	1.27±0.84

Source: Costa Rica data adapted from Parr et al. (2013a). PSR = Parasite species richness

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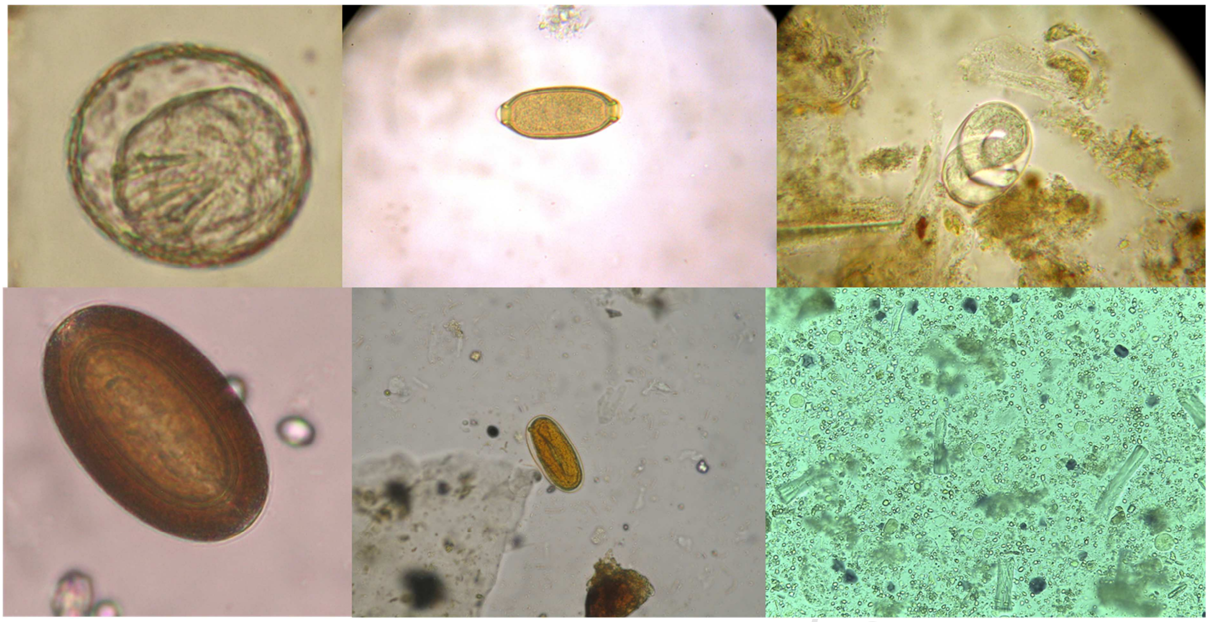

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Highlights

- This study brings new information about primate parasites to Ecuador.
- We analyze the prevalence, richness and FEC among the two habitats, sex and age.
- Adults harbour more species of parasites than subadults and juveniles.
- Sampling effort was essential to find a good number of parasites species.
- *E. histolytica/dispar* and *P. elegans* were found only in captive individuals.

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