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GASTRO-INTESTINAL PARASITES OF FREE-RANGING RED-HANDED HOWLERS (*ALOUATTA BELZEBUL*) IN EASTERN AMAZONIA

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Abstract. Few parasitological studies are available for Neotropical primates, and little is known of the endoparasites of the red-handed howler, *Alouatta belzebul*. In the present study, data were collected at five sites in eastern Amazonia, representative of the region's fragmented anthopogenic landscape. Fecal samples were collected from six to fourteen different groups at each site (n = 212 samples), fixed in MIF, and analyzed in the laboratory using the Lutz and Faust methods. Overall, 77.4% of samples presented at least one endoparasite taxon, and twelve taxa were identified – five protozoans and seven helminths – although only one to species level (*Trypanoxyuris minutus*). Up to five taxa were recorded in a single sample, although 72% of positive samples contained no more than two taxa. Between six and twelve taxa were recorded at a given site, and infection rates varied from 67.5% to 86.0%. However, no clear relationship was found between the number of taxa and infection rates, and variation within one population was similar to that found among populations. Both the number of taxa and infection rates were relatively high in comparison with most other platyrrhine species, although neither variable appeared to have had a negative effect on *A. belzebul* population density.

Key words: parasites, protozoa, helminths, *Alouatta belzebul*, population, Amazonia.

Resumo. Estudos parasitológicos em populações naturais de primatas neotropicais são ainda incipientes, havendo poucos dados disponíveis sobre o guariba-de-mão-ruiva, *Alouatta belzebul*. No presente estudo, dados foram coletados em cinco locais da Amazônia oriental, representativos da paisagem antrópica fragmentada da região. Amostras fecais foram coletadas de seis a quatorze grupos diferentes em cada local (n = 212 amostras), fixadas em MIF e analisadas em laboratório, utilizando as técnicas de Lutz e Faust. No total, 77,4% das amostras foram positivas para pelo menos um táxon de endoparasita, e foram identificados doze táxons, sete de helmintos e cinco de protozoários, embora apenas um até o nível de espécie (*Trypanoxyuris minutus*). Amostras individuais apresentaram até cinco diferentes táxons, embora 72% de amostras positivas tinham até dois táxons. Em cada local de coleta, o número de táxons identificados variou entre seis e doze e a taxa de infecção entre 67,5% e 86%. Entretanto, não foi encontrado qualquer padrão entre o número de táxons e taxas de infecção, e a variação encontrada dentro de uma população foi semelhante àquela encontrada entre populações. Tanto o número de táxons e as taxas de infecção foram relativamente altos em comparação com a maioria de outras espécies de platirríneos, embora nenhuma destas variáveis aparentava ter um efeito negativo sobre a densidade populacional de *A. belzebul*.

Palavras-chave: parasitas, protozoários, helmintos, *Alouatta belzebul*, população, Amazônia.

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INTRODUCTION

Infection by endoparasites appears to be a commonplace phenomenon in nonhuman primates, in which more than 250 species of helminths, primarily nematodes, have been identified (Diniz, 1997). While most individuals may tolerate a given parasite load, Stoner (1996) has suggested that endoparasitism in wild populations may have important negative effects on the distribution and density of some species. These effects may be exacerbated by anthropogenic habitat disturbance, which tend to alter demographic patterns significantly (Stuart & Strier, 1995; Gillespie *et al.*, 2005). However, detailed parasitological studies of wild primate populations are still few and far between, and relatively little, if anything is known of infection rates or host-parasite relationships in the majority of species.

The study of infection patterns in free-ranging populations may provide important insights into ecological and (co)-evolutionary processes, which may ultimately be relevant to the development of conservation strategies (Price *et al*., 1986; Málaga, 1989; Mendes, 1989; Stuart & Strier, 1995; Sorci *et al*., 1997). There is an interesting behavioral component in the case of the primates, which may utilize plants (Wrangham, 1995; Baker, 1996) or other substances (Knezevich, 1998) for their therapeutic effects. The use of nonrandom sites for defecation may also be a strategy directed at the avoidance of infection (Goodall, 1962; Freeland, 1980), one which may be especially important in the howler monkeys (Braza *et al.*, 1981; Gilbert, 1997; Martins, 1999).

In the present study, fecal samples were collected from free-ranging red-handed howlers, *Alouatta belzebul*, at five sites in eastern Amazonia, representing fragmented and isolated populations of the region's antropogenic landscape. Prior to this study, little was known of endoparasitism in the species (Muniz, 1994; Vicente *et al*., 1997), and nothing of infection rates in free-ranging populations. In addition to recording a large number of parasite taxa and relatively high infection rates in comparison with other howler species, the study revealed considerable variation both within and among populations.

METHODS

Study area

The study area was located on the right or east bank of the Rio Tocantins in the

Brazilian state of Pará° (3°43'-5°15'S, 49°12'-50°00'W), which is currently part of the Tucuruí reservoir, flooded in 1984/85 (Eletronorte, 2000). Five sites were selected for data collection, two in continuous forest on the margin of the reservoir and three on islands of varying size (180 to 484 hectares). The islands were formed during the flooding of the reservoir, and were contiguous with the margin prior to this.

The climate is typical of the region, with annual precipitation of 2000-3000 mm, and a pronounced dry season between July and November. The vegetation at the study sites is lowland Amazonian *terra firme* rainforest, either primary or disturbed primary (Eletronorte, 2000). The vertebrate fauna is relatively intact, and includes six other primate species: *Aotus infulatus*, *Cebus apella*, *Chiropotes satanas*, *Saguinus midas* and *Saimiri sciureus* (Ferrari *et al*., 2002).

Sample collection and processing

Samples of feces were collected during the dry seasons of 2000 (October) and 2001 (August and September). All samples were collected from the ground immediately after defecation, and were stored in 50 ml collection pots with one part feces to three parts MIF (Mercurochrome-Iodine-Formaldehyde) fixer solution (Neto & Corrêa, 1991; de Carli, 1994). Parasites found in the feces prior to fixing were retrieved carefully and washed in 0.9% saline solution or distilled water and fixed in 10% formaldehyde or AFA (Alcohol, Formaldehyde and Acetic Acid).

Six of the best-preserved specimens of adult worms that were fixed in AFA were prepared for diaphanization. This technique reveals the internal structures of the parasite, which are important for taxonomic identification.

Two routine techniques of laboratory analysis – Lutz's method and Faust's method – were used in the present study. Lutz's method, also known as the Hoffman, Pons & Janer method, is based on the spontaneous sedimentation of the feces in water. Faust's method involves the centrifugal flotation of feces in Zinc Sulfate solution. Both methods are qualitative, i.e. they detect the presence of the eggs and larvae of helminths, and protozoan cysts, but do not provide quantitative measures of infection (Gillespie, 2006). In the majority of cases, Lugol's solution was used to stain the eggs and cysts in order to facilitate their observation and identification.

The data were organized by group, site and parasite taxon. Infection rates (the number of positive samples/total number of samples x 100) were calculated for each site and parasite taxon, following Stuart *et al.* (1993).

RESULTS

The total of 212 feces samples were distributed evenly across sites (Table 1), although there the number of groups sampled per site did vary, reflecting population density and study conditions at each site (Martins, 2002). Just over three-quarters of the samples tested positive for the presence of endoparasites and, while there was some variation, infection rates were relatively high at all sites. The number of endoparasite taxa varied considerably among sites, however, doubling from 6 (site 4) to 12 (site 1).

Altogether, twelve taxa were identified (Table 2), although in only one case (*Trypanoxyuris minutus*) was it possible to identify the species (Figures 1 and 2). As some specimens were identified to only the family level or higher, it is possible that the total number of species may be underestimated. *Trypanoxyuris minutus* appears to be common in *Alouatta*, and was identified easily on the basis of the morphological characteristics of both eggs and adults.

In general, parasite taxa with high infection rates were also relatively widespread. Infection rates also tended to follow similar patterns at different sites. *Iodamoeba*, for example, returned the first or second highest rate at all five sites. The most divergent group was the nematode larvae, which were found in more than half of the samples collected at site 4 but, surprisingly, in only 7% at site 1.

The number of taxa observed in a given sample varied from one to five, but the latter value was recorded in a single sample from site 1. At other sites, no more than four taxa were registered in any one sample. Despite this, and the variety of taxa recorded at site 1, this site returned the lowest mean number of taxa per sample (1.8±1.0), although the mean varied little among sites, and the highest value was 2.2±0.9, at site 4. Overall, 72.0% of samples contained only one (39.7%) or two (32.3%) taxa.

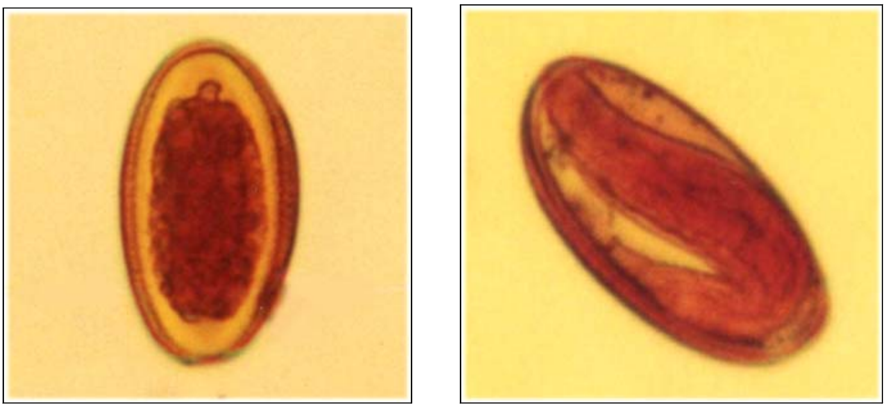
This same lack of any consistent pattern of variation extends to most other aspects of the results, such as the relationship between the richness of parasite taxa and infection rates (Figure 3). Perhaps the most surprising feature of the results is the contrast between sites 1 and 2, which, theoretically, represent the same *A. belzebul* population, given that they are located within the same area of continuous forest, and are separated by less than 5 km. Statistically, site 2 is strikingly similar to site 3 (Table 1), and even presents the exact same fauna of endoparasites (Table 2). In fact, even the infection rates recorded for most taxa, except amoebids, were remarkably similar at the two sites. These similarities appear to be no more than coincidence, however, given that the two sites were located at opposite extremes of the study area.

Table 1. General results for the collection and analysis of feces samples from eastern Amazonian *A. belzebul*.

		Number of:				
Site	Characteristics	Groups sampled	Samples collected	Positive samples	Infection rate	Endoparasite taxa identified
1	Continuous forest	9	43	37	86.0	12
2	Continuous forest	9	46	34	74.0	7
3	Island of 484 ha	12	40	29	72.5	7
4	Island of 360 ha	6	43	37	86.0	6
5	Island of 180 ha	14	40	27	67.5	9
All		50	212	164	77.4	12

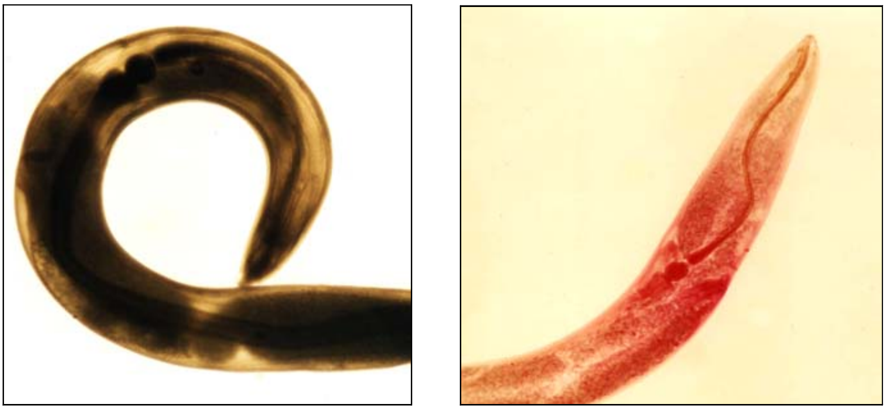
Table 2. Infection rates of endoparasites in eastern Amazonian *A. belzebul* by taxon and study site.

	Infection rate at:					
Taxon	Site 1	Site 2	Site 3	Site 4	Site 5	All sites
Protozoa						
Endolimax	7.0	6.5	7.5	0.0	7.5	5.7
Entamoeba	2.3	0.0	0.0	9.3	5.0	3.3
Giardia	4.6	0.0	0.0	0.0	0.0	0.9
Iodamoeba	60.4	37.0	45.0	58.1	35.0	47.2
Amoebidae	28.0	17.4	5.0	0.0	2.5	10.8
Helminths						
Ascaris	7.0	11.0	17.5	18.6	5.0	11.8
Fasciola	2.3	0.0	0.0	0.0	0.0	0.5
Trichuris	2.3	0.0	0.0	0.0	2.5	0.9
Trypanoxyuris minutus	28.0	19.6	20.0	25.6	40.0	26.4
Hymenolepididae	4.6	0.0	0.0	0.0	0.0	0.9
Ancilostomidae	4.6	6.5	10.0	23.3	17.5	12.3
Nematode larvae	7.0	41.3	37.5	53.5	25.0	34.0



(a) (b)

Figure 1. *Trypanoxyuris minutus*: egg with (a) germ cells, and (b) semi-formed larva. Both from Site 5, stained with Lugol's solution and observed at 400x.



(a) (b)

Figure 2. Anterior extremity of adult female *Trypanoxyuris minutus* collected from adult female *A. belzebul* at Site 1: (a) stained with Lugol's solution, and (b) prepared by the carmine chloride bleaching method (observed at 100x).

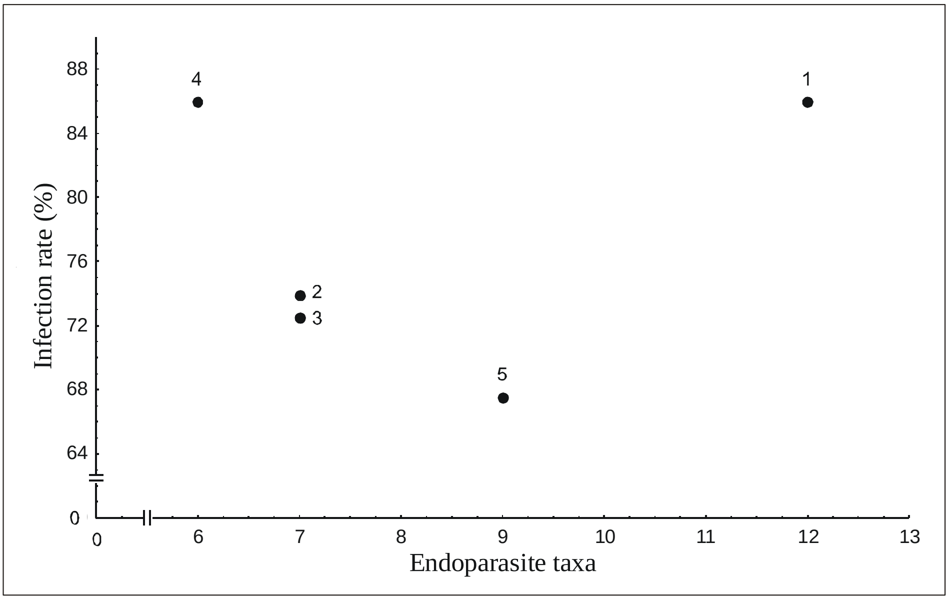


Figure 3. Relationship between the taxonomic richness of endoparasites and infection rates at the five *A. belzebul* study sites in eastern Amazonia.

DISCUSSION

This is the first detailed parasitological survey of this relatively poorly-known howler species which, with a total of more than two hundred fecal samples collected and analyzed, represents one of the most comprehensive studies ever carried out on a Neotropical primate species (Table 3). The results indicate relatively high infection rates and richness of the intestinal fauna in comparison with other studies and species. However, as in the present study, there is no systematic pattern of infection among studies. The highest infection rates found in free-ranging populations (Melo et al., 1997; Martins, 1999) did not appear to be related to the number of parasite taxa, for example, and the variation among populations of Alouatta fusca is striking.

Of the twelve taxa identified, only two – *Trypanoxyuris minutus* and *Ascaris* –had been recorded previously for *A. belzebul* (Stiles *et al*., 1929; Vicente *et al*., 1997). *Trypanoxyuris* appears to be relatively common in *Alouatta* (Coppo *et al*., 1979; Stuart *et al*., 1998). These, and most of the other helminths, may be pathogenic, as may the protozoans *Giardia* and *Entamoeba*, especially at high levels of infestation. However, host-parasite relations are poorly known in the platyrrhines, which makes it difficult to interpret the significance of infection rates in these populations.

The most common taxon overall – *Iodamoeba* – is considered to be a commensal amoebid, and rarely has a pathogenic effect on its host, although little is known of its action in non-human primates. In the case of *T. minutus*, the howlers' social behavior, and the habit of defecating together at specific sites (Braza *et al*, 1981; Gilbert, 1997; Martins, 1999) may have contributed to the relatively high rates of infection. This parasite generally causes little more than perianal itching and discomfort, however, and cases of serious intestinal lesions or secondary infections are relatively rare (Diniz, 1997).

Of the other protozoans recorded here, *Giardia*, *Entamoeba* and *Endolimax* can be pathogenic, especially at high rates of infestation, as can the helminthes *Trichuris*, ancilostomids and hymenolepidids. In general, however, these species are harmless, or asymptomatic. *Fasciola* may also cause itching, under some circumstances. However, parasite-host interactions in these taxa are still poorly known, especially in the case of the platyrrhines.

Obviously, more data will be required before host-parasite relationships in *A. belzebul* can be understood more fully. In the meantime, this first preliminary study of endoparasitism in the species has revealed a number of interesting patterns, such as relatively high infection rates and numbers of parasite taxa, and marked variation both within and among populations. For the time being, at least, these populations do not appear to be affected adversely by these patterns, given that densities were relatively high at all sites (Martins, 2002).

Table 3. Results of quantitative parasitological studies of Neotropical primates.

Species	Origin	Endoparasite taxa	Infection Rate (n samples)	Source
Alouatta belzebul	Free-ranging	12	77.4 (212)	Present study
Alouatta caraya	Captive	4	58.0 (88)	Coppo *et al.* (1979)
	Free-ranging	8	38.6 (44)	Cruz *et al.* (2000)
Alouatta fusca	Free-ranging	0	0.0 (62)	Stuart *et al.* (1993)
	Free-ranging	5	82.3 (51)	Martins (1999)
	Captive	4	23.6 (165)	Müller *et al.* (2000)
Alouatta palliata	Free-ranging	4	48.4 (155)	Stuart *et al.* (1990)
Aotus trivirgatus	Captive	2	66.7 (6)	Coppo *et al.* (1979)
Brachyteles arachnoides	Free-ranging	4	39.0 (128)	Stuart *et al.* (1993)
Callicebus personatus	Free-ranging	3	82.5 (17)	Melo *et al.* (1997)
Callithrix jacchus	Captive and free-ranging	11	68.2 (178)	Ximenes (1997)
Cebus apella	Captive	4	93.8 (16)	Coppo *et al.* (1979)

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