A preliminary assessment of gastrointestinal parasites of the sun tailed monkey (*Allochrocebus solatus*) in a semi-free-ranging colony

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Running headline: Gastrointestinal parasites of *Allochrocebus solatus*

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Abstract

Background: The occurrence of gastrointestinal parasites in the sun-tailed monkey (*Allochrocebus solatus*) at the CIRMF primatology center is unknown. We therefore assessed the presence and richness (number of different parasite taxa) of gastrointestinal parasites in a semi-free ranging colony of *A. solatus*.

Methods: A total of 46 fecal samples were screened using a modified McMaster technique for fecal egg counts.

Results: In the 46 samples collected, seven taxa of gastrointestinal parasites, including protozoa and nematodes were identified. The most prevalent parasite was strongyles parasites (98%), followed by *Trichuris* spp. (72%), *Strongyloides* spp. (67%) and *Entamoeba coli* (65%). *Balantioides coli* (33%), *Endolimax nana* (25%) and Spirurid eggs (26%) were only found in a minority of the animals.

Conclusion: This study contributes new host records of gastrointestinal parasites in semi-free-ranging *A. solatus* and highlights the need to investigate the health of this species and implement proper precautions in the management of this colony.

Keywords: *Allochrocebus solatus*, gastrointestinal parasites, parasite richness, coprology, primates, endemism

Introduction

Understanding and monitoring the parasitological health of non-human primates is a crucial topic, as primates host a considerable variety of parasites that are infectious and potentially harmful to human health [1]. These parasites also constitute a major issue for the conservation, and ultimately the survival, of both captive and wild populations [2].

Gastrointestinal (GI) parasites also represent a potentially important selective force, which can infect various animals including primates. Some GI parasites are thought to be commensal, although their potential effects on the host are not yet known. Other parasites can negatively affect the host, by affecting host ecology [3], fitness [4], and reproduction [5, 6], increasing susceptibility to infection by other parasites [7], and ultimately decreasing survival [8]. The most common GI parasites infecting primates are protozoa and nematodes. For example, protozoa (*Entamoeba* spp.) have been recorded in apes (gibbons, orangutans and chimpanzees) and in many other primates [9–11]. Some nematodes have been reported in several populations of primates, including *Necator americanus* [12–15], *Oesophagostomum* spp. [10, 16–18], *Strongyloides* spp. [14, 19], *Ascaris* spp. and *Trichuris* spp. [19–21]. Patterns of GI parasites are influenced by several characteristics including the environment and the condition of the host [1]. Both habitat and the degree of human contact influence the prevalence and intensity of parasites [3].

Parasitological studies conducted in primates show that when humans and other primates share habitat there is an increasing possibility of sharing infectious diseases, which challenges public and primate health. For example, in the context of wildlife management in parks, zoological gardens or breeding centers, zoo keepers, veterinarians, researchers and caretakers face a potential zoonotic risk [22]. This is due to their phylogenetic proximity and highlights the need to improve the understanding of parasitic infections circulating in primates.

The sun-tailed monkey (*Allochrocebus solatus*) is endemic to Gabon, first described in 1988 [23] and declared a fully protected species by the Gabonese government in 1994. This species is Threatened [24] and listed on Appendix II of CITES. The sun-tailed monkey is easily recognizable by its reddish-brown back, white throat collar and long tail ending in bright yellow-orange [23, 25]. About 16% of its range is protected in Lopé, Waka and Birougou National Parks but its highest density is in the Foret des Abeilles, which is unprotected [25, 26]. Being semi-terrestrial, *A. solatus* is vulnerable to ground snares, making commercial hunting a growing threat [25], and *A. solatus* is no longer recorded at some villages in its range, because of widespread hunting [26, 27]. As a result, the population of *A. solatus*, which remains unknown may be subject to considerable decline. Furthermore, numerous potential threats such as habitat loss, mainly due to logging, and vulnerability to changing fruiting patterns under climate change could affect the geographic distribution of *A. solatus* [24], making *A. solatus* a species of immediate and critical conservation concern.

Allochrocebus solatus is little studied in the field because of its cryptic nature. Most of the available knowledge comes from the only captive colony of this species in the world, which is housed in a semi-free-ranging rainforest enclosure at the International Centre for Medical Research in Franceville (CIRMF). Previous studies of this species have described social and foraging behaviors, social organization, the degree of terrestriality, blood biochemical and hematological parameters [28–31]. No studies have been undertaken on the gastrointestinal parasites of this species. This study aimed to identify and quantify the prevalence and diversity of gastrointestinal parasites in this species for the first time.

Material and methods

Ethics Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study further complied with ethical protocols approved by the National Ethics Committee of Gabon and with the authorization of the Gabonese Ministries of Water and Forestry, Higher Education, Scientific Research and Innovation (N◦AR0031/09/MENESRESI/CENAREST/CG/ CST/ CSAR).

Study population

This study was performed on a semi free-ranging population of *Allochrocebus solatus* from 16 to 18 June 2019. The study population ranges in a 0.7-ha rainforest enclosure at the CIRMF primatology center, where they forage freely on natural leaves, fruits, roots, bark, seeds, stems, and insects [30, 31]. They are also provisioned in a feeding pen once or twice times a day with bananas, wild fruits, and soya-based homemade cake. Water is available ad libitum. This population was established between 1984 and 1998, with the introduction of wild individuals (five individuals: two males and three females). This study population was composed of ca. 35 individuals in 2011 and until today all are identified by a unique code tattooed on the inner [30].

Individual characteristics

Parasite prevalence, abundance of parasites in the feces, and number of taxa were examined with respect to sex and age. Exact dates of birth are unknown but the age of the founders and newborn was estimated based on body condition and patterns of tooth eruption and wear [32].

Sample collection

The study was conducted during the annual veterinary health checks. Individuals were darted and anesthetized by blowpipe intramuscular injections of ketamine (10 mg/kg body weight). They were then transferred to the CIRMF primatology center for medical examination and sampling. Two samples per individual were attempted before to be collected - one from the rectum during the capture and the other by trapping the individual briefly in the feeding pen the following day. A total of 46 fecal samples (1-5 g) from 26 individuals have been collected.

Parasitological Analyses

Gastrointestinal parasites were investigated using a modified McMaster technique for fecal egg counts [15]. Briefly, 4 g of stool was mixed with 56 ml of a saturate NaCl solution (obtained by adding 400g of NaCl to 1,000 ml of distilled water solution). Large debris were removed with a strainer and obtained a homogenized filtrate. An aliquot of this filtrate was placed into each chamber of McMaster counting slide and the number of eggs observed in each chamber using the 10X objective lenses. A measure of eggs per gram of feces were obtained by adding the number of eggs for both chambers and multiplying by 50. Nematodes found were identified based on egg shape, length, width, color, and contents according to previous reports [14, 33], while the identification of protozoa trophozoites and cystic stages was based on specifically morphological criteria like size, shape outline, cytoplasma, crystalloids and nuclei as previously done [34]. Furthermore, during fecal examination, all larvae found were identified microscopically base on head shape, tail, esophagus types and number of intestine cells [35, 36]. In this study, parasitic identification using microscopic analysis precludes precise determination at the species level, so parasite were identified to the species level when possible. For most of the parasites, the genus is likely to include several species, for this reason the abbreviation “spp” was used. Therefore, all the strongyles parasites were regrouped under that same label when they did not present any possibility of identification at the generic level the other.

Statistical analysis

The statistical software R version 4.0.2 for all statistical analyses and to create figures was used (R Core Team 2017). Descriptive statistics percentage, mean and standard deviation were used to summarize the data obtained. Percent prevalence was determined by multiplying the number of positive samples by 100 and dividing by the total number of samples. Parasite richness was defined as the number of parasite taxa found in a sample from one individual. Chi-square values were calculated to test for differences in presence of parasites between sexes and among age classes. Due to small sample sizes and unbalanced numbers of individuals by age and sex group, the effect of sex and age classes was tested on parasite richness using non-parametric Mann-Whitney U tests. Results were considered significant when p-value < 0.05.

Results

Overall prevalence

Overall, 98 % (n = 45) solatus were infected with gastrointestinal parasites. In the 46 fecal samples screened, seven parasites were found, of which four helminths, and three were protozoans (Figure 1). All the helminths species identified here were nematodes: unidentified strongyles parasites possibly belonging to different species, *Trichuris* spp., *Strongyloides* spp., and spirurid eggs (Figure 1). Strongyles parasites were the most common nematode (98 %) followed by *Trichuris* spp. (72 %), *Strongyloides* spp. (67 %) and spirurid eggs (26 %). In addition, the L3 larvae found during fecal examination may represent *Oesophagostomum* and *Strongyloides* genus (Figure 1).

Protozoans identified included a cyst morphologically similar to *Balantioides coli*, *Entamoeba coli* and *Endolimax nana* (Figure 1). Among them, *Entamoeba coli* had the highest prevalence (65 %) followed by *Balantioides coli* (33 %) and *Endolimax nana* (25 %) (Table 1)

Parasite species richness

Parasite species richness per fecal sample ranged from 0 to 7 species. Of the 46 fecal samples, 20 had 5-7 parasite species, 23 had 2-4 parasite species, 2 had a single parasite, and one had no parasites detected. The 26 individuals were infected with 0 to 7 parasite taxa (mean ± SD = 4.3 ± 1.8).

Effects of sex, age on prevalence and parasite richness

Neither the sex nor the age did not influence the presence of each parasite identified in the study (Table 2). Parasite richness was not associated with sex (Mann-Whitney U test, W = 218, p = 0.89) or age categories (W = 205.5, p = 0.29).

Discussion

This study represents the first report on gastrointestinal parasites of *A. solatus*. Seven parasites taxa (four nematodes and three protozoa) were identified. Most of these parasites have the potential to control and regulate host population growth, and affect specific interactions, especially during heavy infections [5].

Parasite richness

Parasite richness found (seven species) is almost the same that this reported for mandrill colony at CIRMF (7 species), partly due to the fact that mandrills and *A. solatus* are under the same conditions and neighbours [37]. Moreover, this number of parasite was substantially lower in this study than that reported for other cercopithecid monkeys. Specifically, 12 taxa were identified in mandrills (*Mandrillus sphinx*) from Bakoumba. Twenty one taxa were identified in the Tana River mangabey (*Cercocebus galeritus*) [38], 14 taxa in monkeys of Uganda's Kibale Forest [9, 10], 13 taxa in primates from Tanzania's Mahale National Park [39], 12 taxa in primates from Tanzania's Rubondo Island National Park [40], and 23 in the monkeys of Taï National Park, Cote d'Ivoire [14]. The low richness here could be due to the fact that more than one monkey species were investigated in many of these studies. In addition, it is also possible that *A. solatus* have smaller home ranges than monkey species at other study sites mentioned above. For example, it has been showed that Loskop vervets harbored lower parasites than other vervets because they face smaller home ranges than vervets at other study sites [41]. Moreover, the fact this colony is under proper veterinarians care (i.e., good nutritional, good management practices, good sanitary conditions) may contributed to this low parasite richness.

Diversity and prevalence

The overall prevalence of gastrointestinal parasites in this study was 98 %. This high prevalence encountered in this study could result from favourable climatic conditions. The warm and moist climate of tropical and subtropical countries provides the ideal environment for the survival of parasite eggs or larvae. This pattern may also result from several other factors such as the relatively high density of monkeys in the enclosure, which may cause constant re-infection, or the fact they spent a third of their time on the ground [30].

This study reports 7 species of gastrointestinal parasites, including four nematodes (strongyles parasites, *Trichuris* spp., *Strongyloides* spp., and spirurid eggs) and three protozoans (*Balantioides coli*, *Entamoeba coli* and *Endolimax nana*). The results showed that *A. solatus* is highly infected with strongyles parasites. Previous studies showed these nematodes are a large group of intestinal parasites and considered as the most harmful parasites in both monkeys and apes [12, 14, 15, 42, 43].

The diagnosis of strongyles are difficult because of the great similarities in terms of size, shape, character and appearance. However, the L3 larvae found may represent *Oesophagostomum* spp. despite the absence of a consistent coprocultures limiting the possibility to identify these parasites. *Oesophagostomum* genus is a common intestinal parasite frequently identified with a relatively high prevalence in baboons and vervets [12, 42], guenon [9, 10], macaque [44, 45], gorillas [46] and chimpanzees [18]. The potential presence of this nodular worm in *A. solatus* may be due to their diet and gut physiology [18, 39].

Trichuris spp. is a parasite with a simple and direct life cycle. This pinworm has been reported in numerous populations of primate across Africa [9, 10, 12, 41, 47] and in other regions [45]. *Trichuris* belongs to the soil-transmitted helminths, one of the most important groups of infectious agents with a high potential [48, 49]. Transmission occurs from oral ingestion of the infective eggs found in contaminated food, water and soil [12, 50]. Clinical manifestation can vary widely from asymptomatic to fatal infection [51]. Due to its wide distribution in non-human primates and humans, *Trichuris* could be involved in zoonotic transmission. *A. solatus* may facilitate the transmission of this parasite to animal keepers, and careful attention is required to avoid possible transmission to humans.

Strongyloides spp. infect a wide range of primates [14, 19, 39, 41]. Primates become infected by eating eggs or via skin penetration by infective larvae [52]. The high prevalence of *Strongyloides* eggs and larvae found in this study may be due to conditions that favor the development of the pre-parasite stages and the high frequency of contact between *A. solatus* and soil containing infective larvae. This is in accordance with results showing terrestrial *Papio* primates were likely to excrete more *Strongyloides* spp. larvae than arboreal *Cercopithecus* [12, 53]. The presence of this threadworm is also of public health interest because of its zoonotic nature, and again means that animal keepers should avoid possible transmission.

Spirurid eggs, have been recorded in primates across Africa [9, 41, 42]. This nematode is generally transmitted by ingesting an intermediate host, such as dung beetles or other arthropods or reptilian intermediate hosts. *A. solatus* frequently forage on the ground level and feed on many potential intermediate hosts for spirurids. This may explain what they are positive for this nematode.

Entamoeba coli is an amoeba that requires the ingestion of cysts and proliferation in the host intestine. This nonpathogenic *Entamoeba* has been mainly reported to infect primates [54–56]. *E coli* is distinguishable from other *Entamoeba* spp. due to the presence of eight nuclei. *Balantioides coli* is a ciliated protozoon that can parasitize a wide variety of animals including non-human primates and can also be responsible of a zoonotic transmission cycle. *B coli* is a lso considered as a pathogenic protozoa that can cause death in primates, particularly apes and humans [57]. As for *E coli*, hosts become infected by the ingestion of cysts and the parasite proliferates in the host intestine. This ciliate protozoan is also pathogenic to humans [58–60] and is widely distributed in primates especially in those experiencing extensive habitat overlap with humans [13, 61, 62]. The presence of *B coli* in *A. solatus* suggests that infection risk is a concern for animal keepers. *Endolimax nana*, a nonpathogenic intestinal protozoon was also detected. This amoeba parasitises a wide range of hosts, including humans and other primates [14].

Factors affecting parasitism

In this study, age and sex did not influence parasite prevalence or richness. This may be due to the preliminary results obtained from the small sample size. This is in contrast to several other studies that have investigated relationship between individuals trait on parasites in non-human primates. For example, in baboons, females excrete more parasite ova than males do [63]. In colobus, nematode prevalence was greater in male vs. female [10]. In the colony of mandrills at CIRMF prevalence of nematode eggs increased significantly with age in females, but not in males [37]. In mandrills from Bakoumba, not far from CIRMF age was associated with a specific decrease in nematode richness [34]. In Eastern chimpanzees Subadult had lower prevalence for most parasite species compared with adults in both years and also yielded a lower average parasite species richness [13].

Conclusion

This study contributes new host records for gastrointestinal parasites in a semi-free-ranging population of *A. solatus*. The results obtained emphasizes the need to plan a comprehensive longitudinal study of parasites found here and highlight the need for careful precaution during the management of this colony by animal keepers. This includes the need for basic hygiene standards, regularly deworming animals, and ensuring that the feeding pen is cleaned and disinfected daily.

Finally, due to the fact some parasites found here can be zoonotic and a potential health risk for animal keepers, molecular studies are needed to confidently distinguish species of gastrointestinal parasite and to test the possible transmission of the parasites to humans which do have close interaction with *A. solatus*

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Conflicts of Interest

The authors declare that they have no conflict of interest

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Tables

Tables 1: Prevalence of all parasites identified in 46 solatus fecal samples

Parasite species		No. Of positive samples	% prevalence
Entamoeba coli	Protozoa, amoeba	30	65
Endolimax nana	Protozoa, amoeba	12	26
Balantidium coli	Protozoa, ciliate	15	33
Trichuris spp.	Nematoda	33	72
Spirurid egg	Nematoda	12	26
Strongyloides spp.	Nematoda	31	67
Strongyles	Nematoda	45	98

Table 2: Prevalence of parasite species detected in *Allochrocebus solatus* by sex and age.

Parasite species by sex	**Male (n = 14)**	**%**	**Female (n = 32)**	**%**	***X^2***	**P-value**
Entamoeba coli	11	78,6	19	59,4	0.85	0.36
Endolimax nana	4	28,6	8	25,0	1.88e-31	1
Balantioides coli	4	28,6	11	34,4	0.002	0.97
Trichuris spp.	12	85,7	21	65,6	1.07	0.3
Spirurid egg	4	28,6	8	25,0	1.87e-31	1
Strongyloides spp.	7	50,0	24	75,0	0.45	0.5
Strongyles	14	100,0	31	97,0	0.24	0.7
Parasite species by age	**Adult (n = 28)**	**%**	**Young (n = 18)**	**%**	***X^2***	**P-value**
Entamoeba coli	18	64,3	12	66,7	1.26e-31	1
Endolimax nana	3	10,7	9	50,0	6.85	0.07
Balantioides coli	10	35,7	5	27,8	0.05	0.81
Trichuris spp.	20	71,4	13	72,2	1.26e-30	1
Spirurid egg	6	21,4	6	33,3	0.30	0.6
Strongyloides sp.	19	67,9	12	66,7	0.11	0.73
Strongyles	27	96,4	18	100,0	0.71	0.43

X^2 = chi-square; P-value = level of significance; P-value ≤ 0:05 indicates that the relationship is significant.

Figure legend

Figure 1: Gastrointestinal parasites identified in the study: (a) *Endolimax nana.* (b) *Trichuris* spp., (c) *Balantioides coli*. (d) Spirurid egg. (e, h) Strongyles parasites. (f) *Entamoeba coli*. (g) *Strongyloides* spp. (i) L3 larvae of *Oseophagostomum* genus. (j, k) L3 larvae of *Strongyloides* genus.

Source: The photo of the non-human primate was taken at the CIRMF primatology center by Camille Delaplace