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► **To cite this version:**

Serge-Ely Ely Dibakou, Barthélémy Ngoubangoye, Larson Boundenga, Stephan Ntie, Cyr Moussadji, et al.. Preliminary assessment of gastrointestinal parasites of the sun-tailed monkey (*Allochrocebus solatus*) in a semi-free-ranging colony. *Journal of Medical Primatology*, 2022, 51 (3), pp.127-133. 10.1111/jmp.12581 . hal-04505967

HAL Id: hal-04505967

<https://hal.science/hal-04505967>

Submitted on 15 Mar 2024

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

3
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15
16 Running headline: Gastrointestinal parasites of *Allochrocebus solatus*

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21

22 **Abstract**

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

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

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

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

37

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

40

41 **Introduction**

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

46 Gastrointestinal (GI) parasites also represent a potentially important selective force, which
47 can infect various animals including primates. Some GI parasites are thought to be commensal,
48 although their potential effects on the host are not yet known. Other parasites can negatively affect
49 the host, by affecting host ecology [3], fitness [4], and reproduction [5, 6], increasing susceptibility
50 to infection by other parasites [7], and ultimately decreasing survival [8]. The most common GI
51 parasites infecting primates are protozoa and nematodes. For example, protozoa (*Entamoeba* spp.)
52 have been recorded in apes (gibbons, orangutans and chimpanzees) and in many other primates
53 [9–11]. Some nematodes have been reported in several populations of primates, including *Necator*
54 *americanus* [12–15], *Oesophagostomum* spp. [10, 16–18], *Strongyloides* spp. [14, 19], *Ascaris*
55 spp. and *Trichuris* spp. [19–21]. Patterns of GI parasites are influenced by several characteristics
56 including the environment and the condition of the host [1]. Both habitat and the degree of human
57 contact influence the prevalence and intensity of parasites [3].

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

64 The sun-tailed monkey (*Allochrocebus solatus*) is endemic to Gabon, first described in
65 1988 [23] and declared a fully protected species by the Gabonese government in 1994. This species
66 is Threatened [24] and listed on Appendix II of CITES. The sun-tailed monkey is easily
67 recognizable by its reddish-brown back, white throat collar and long tail ending in bright yellow-
68 orange [23, 25]. About 16% of its range is protected in Lopé, Waka and Birougou National Parks
69 but its highest density is in the Forêt des Abeilles, which is unprotected [25, 26]. Being semi-
70 terrestrial, *A. solatus* is vulnerable to ground snares, making commercial hunting a growing threat
71 [25], and *A. solatus* is no longer recorded at some villages in its range, because of widespread
72 hunting [26, 27]. As a result, the population of *A. solatus*, which remains unknown may be subject
73 to considerable decline. Furthermore, numerous potential threats such as habitat loss, mainly due
74 to logging, and vulnerability to changing fruiting patterns under climate change could affect the
75 geographic distribution of *A. solatus* [24], making *A. solatus* a species of immediate and critical
76 conservation concern.

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

85

86 **Material and methods**

87 ***Ethics Statement***

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

93

94 ***Study population***

95 This study was performed on a semi free-ranging population of *Allochrocebus solatus* from 16 to
96 18 June 2019. The study population ranges in a 0.7-ha rainforest enclosure at the CIRMF
97 primatology center, where they forage freely on natural leaves, fruits, roots, bark, seeds, stems,
98 and insects [30, 31]. They are also provisioned in a feeding pen once or twice times a day with
99 bananas, wild fruits, and soya-based homemade cake. Water is available ad libitum. This
100 population was established between 1984 and 1998, with the introduction of wild individuals (five
101 individuals: two males and three females). This study population was composed of ca. 35
102 individuals in 2011 and until today all are identified by a unique code tattooed on the inner [30].

103

104 ***Individual characteristics***

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

108

109 ***Sample collection***

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

116

117 *Parasitological Analyses*

118 Gastrointestinal parasites were investigated using a modified McMaster technique for fecal egg
119 counts [15]. Briefly, 4 g of stool was mixed with 56 ml of a saturate NaCl solution (obtained by
120 adding 400g of NaCl to 1,000 ml of distilled water solution). Large debris were removed with a
121 strainer and obtained a homogenized filtrate. An aliquot of this filtrate was placed into each
122 chamber of McMaster counting slide and the number of eggs observed in each chamber using the
123 10X objective lenses. A measure of eggs per gram of feces were obtained by adding the number
124 of eggs for both chambers and multiplying by 50. Nematodes found were identified based on egg
125 shape, length, width, color, and contents according to previous reports [14, 33], while the
126 identification of protozoa trophozoites and cystic stages was based on specifically morphological
127 criteria like size, shape outline, cytoplasm, crystalloids and nuclei as previously done [34].
128 Furthermore, during fecal examination, all larvae found were identified microscopically base on
129 head shape, tail, esophagus types and number of intestine cells [35, 36]. In this study, parasitic
130 identification using microscopic analysis precludes precise determination at the species level, so
131 parasite were identified to the species level when possible. For most of the parasites, the genus is
132 likely to include several species, for this reason the abbreviation “spp” was used. Therefore, all the

133 strongyles parasites were regrouped under that same label when they did not present any possibility
134 of identification at the generic level the other.

135 136 *Statistical analysis*

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

146

147 **Results**

148 *Overall prevalence*

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

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

159

160 *Parasite species richness*

161 Parasite species richness per fecal sample ranged from 0 to 7 species. Of the 46 fecal samples, 20
162 had 5-7 parasite species, 23 had 2-4 parasite species, 2 had a single parasite, and one had no
163 parasites detected. The 26 individuals were infected with 0 to 7 parasite taxa (mean \pm SD = 4.3 \pm
164 1.8).

165

166 *Effects of sex, age on prevalence and parasite richness*

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

170

171 **Discussion**

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

176

177 *Parasite richness*

178 Parasite richness found (seven species) is almost the same that this reported for mandrill colony at
179 CIRMF (7 species), partly due to the fact that mandrills and *A. solatus* are under the same
180 conditions and neighbours [37]. Moreover, this number of parasite was substantially lower in this
181 study than that reported for other cercopithecoid monkeys. Specifically, 12 taxa were identified in
182 mandrills (*Mandrillus sphinx*) from Bakoumba. Twenty one taxa were identified in the Tana River
183 mangabey (*Cercocebus galeritus*) [38], 14 taxa in monkeys of Uganda's Kibale Forest [9, 10], 13
184 taxa in primates from Tanzania's Mahale National Park [39], 12 taxa in primates from Tanzania's
185 Rubondo Island National Park [40], and 23 in the monkeys of Tai National Park, Cote d'Ivoire
186 [14]. The low richness here could be due to the fact that more than one monkey species were
187 investigated in many of these studies. In addition, it is also possible that *A. solatus* have smaller
188 home ranges than monkey species at other study sites mentioned above. For example, it has been
189 showed that Loskop vervets harbored lower parasites than other vervets because they face smaller
190 home ranges than vervets at other study sites [41]. Moreover, the fact this colony is under proper
191 veterinarians care (i.e., good nutritional, good management practices, good sanitary conditions)
192 may contributed to this low parasite richness.

193

194 ***Diversity and prevalence***

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

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

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

214 *Trichuris* spp. is a parasite with a simple and direct life cycle. This pinworm has been
215 reported in numerous populations of primate across Africa [9, 10, 12, 41, 47] and in other regions
216 [45]. *Trichuris* belongs to the soil-transmitted helminths, one of the most important groups of
217 infectious agents with a high potential [48, 49]. Transmission occurs from oral ingestion of the
218 infective eggs found in contaminated food, water and soil [12, 50]. Clinical manifestation can vary
219 widely from asymptomatic to fatal infection [51]. Due to its wide distribution in non-human
220 primates and humans, *Trichuris* could be involved in zoonotic transmission. *A. solatus* may
221 facilitate the transmission of this parasite to animal keepers, and careful attention is required to
222 avoid possible transmission to humans.

223 *Strongyloides* spp. infect a wide range of primates [14, 19, 39, 41]. Primates become
224 infected by eating eggs or via skin penetration by infective larvae [52]. The high prevalence of
225 *Strongyloides* eggs and larvae found in this study may be due to conditions that favor the
226 development of the pre-parasite stages and the high frequency of contact between *A. solatus* and
227 soil containing infective larvae. This is in accordance with results showing terrestrial *Papio*
228 primates were likely to excrete more *Strongyloides* spp. larvae than arboreal *Cercopithecus* [12,
229 53]. The presence of this threadworm is also of public health interest because of its zoonotic nature,
230 and again means that animal keepers should avoid possible transmission.

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

236 *Entamoeba coli* is an amoeba that requires the ingestion of cysts and proliferation in the
237 host intestine. This nonpathogenic *Entamoeba* has been mainly reported to infect primates [54–
238 56]. *E coli* is distinguishable from other *Entamoeba* spp. due to the presence of eight nuclei.
239 *Balantioides coli* is a ciliated protozoon that can parasitize a wide variety of animals including
240 non-human primates and can also be responsible of a zoonotic transmission cycle. *B coli* is a Iso
241 considered as a pathogenic protozoa that can cause death in primates, particularly apes and humans
242 [57]. As for *E coli*, hosts become infected by the ingestion of cysts and the parasite proliferates in
243 the host intestine. This ciliate protozoan is also pathogenic to humans [58–60] and is widely
244 distributed in primates especially in those experiencing extensive habitat overlap with humans [13,
245 61, 62]. The presence of *B coli* in *A. solatus* suggests that infection risk is a concern for animal

246 keepers. *Endolimax nana*, a nonpathogenic intestinal protozoon was also detected. This amoeba
247 parasitises a wide range of hosts, including humans and other primates [14].

248

249 ***Factors affecting parasitism***

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

260

261 **Conclusion**

262 This study contributes new host records for gastrointestinal parasites in a semi-free-ranging
263 population of *A. solatus*. The results obtained emphasizes the need to plan a comprehensive
264 longitudinal study of parasites found here and highlight the need for careful precaution during the
265 management of this colony by animal keepers. This includes the need for basic hygiene standards,
266 regularly deworming animals, and ensuring that the feeding pen is cleaned and disinfected daily.
267 Finally, due to the fact some parasites found here can be zoonotic and a potential health risk for
268 animal keepers, molecular studies are needed to confidently distinguish species of gastrointestinal

269 parasite and to test the possible transmission of the parasites to humans which do have close
270 interaction with *A. solatus*

271

272 **Acknowledgements**

273 Many thanks to all personal of Primatology Center of Centre Interdisciplinaire de Recherches
274 Médicales de Franceville (CIRMF) for their incredible assistance during the sanitary control and
275 their help in collecting the fecal samples.

276

277 **Conflicts of Interest**

278 The authors declare that they have no conflict of interest

279

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445 **Tables**446 **Tables 1:** Prevalence of all parasites identified in 46 solatus fecal samples

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

447

448

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

Parasite species by sex	Male (n = 14) %		Female (n = 32) %		X²	P-value
<i>Entamoeba coli</i>	11	78,6	19	59,4	0.85	0.36
<i>Endolimax nana</i>	4	28,6	8	25,0	1.88e-31	1
<i>Balantioides coli</i>	4	28,6	11	34,4	0.002	0.97
<i>Trichuris</i> spp.	12	85,7	21	65,6	1.07	0.3
Spirurid egg	4	28,6	8	25,0	1.87e-31	1
<i>Strongyloides</i> 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) %		X²	P-value
<i>Entamoeba coli</i>	18	64,3	12	66,7	1.26e-31	1
<i>Endolimax nana</i>	3	10,7	9	50,0	6.85	0.07
<i>Balantioides coli</i>	10	35,7	5	27,8	0.05	0.81
<i>Trichuris</i> spp.	20	71,4	13	72,2	1.26e-30	1
Spirurid egg	6	21,4	6	33,3	0.30	0.6
<i>Strongyloides</i> sp.	19	67,9	12	66,7	0.11	0.73
Strongyles	27	96,4	18	100,0	0.71	0.43

450 X² = chi-square; P-value = level of significance; P-value ≤ 0:05 indicates that the relationship is significant.

451

453 **Figure legend**

454

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

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