



Article Gastrointestinal Parasitic Infections in Non-Human Primates at Gabon's Primatology Center: Implications for Zoonotic Diseases

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Abstract: Parasites and infectious diseases pose significant threats to primate populations, especially in captive non-human primates (NHPs). This study aimed to assess the diversity and prevalence of intestinal parasites in NHPs at the CIRMF Primatology Center. A total of 97 fecal samples were analyzed using parasitological techniques, including sodium chloride flotation and modified Baermann sedimentation methods. An overall parasite prevalence of 93.81% (91/97) was observed. Sixteen groups of parasites with zoonotic potential were identified, comprising ten genera of nematodes (Trichuris, Enterobius, Hookworm, Trichostrongylus, Mammomonogamus, Spirure, Oesophagostomum, Schistosoma, Ascaris, and Strongyloides), three genera of protists (Eimeria, Balantioides coli/Buxtonella, and Entamoeba), one genus of cestodes (Hymenolepis), and two genera of trematodes (Dicrocoelium and Paramphistomum). High prevalences were noted for Oesophagostomum spp. (83.5%), Strongyloides spp. (52.58%), and Trichostrongylus spp. (50.52%). These findings underscore the potential role of the CIRMF Primatology Center in maintaining and facilitating the transmission of intestinal parasites with high zoonotic potential. The co-existence of human and NHP parasites in shared environments, such as zoos and research facilities, emphasizes the need for a holistic, One Health approach that addresses the interconnected health of humans, animals, and the environment. This study highlights the urgent need for collaborative strategies to mitigate the risks of zoonotic parasite transmission between NHPs and humans in captive settings.

Keywords: non-human primates; gastrointestinal parasites; prevalence; zoonotic diseases; CIRMF primatology center; one health approach

1. Introduction

Parasitic diseases pose a significant global health challenge [1–4]. Approximately 1.5 billion people are infected with intestinal parasites annually, leading to approximately 135,000 deaths [5]. This statistic highlights the critical need to address parasitic infections from both human and zoonotic perspectives. The close genetic similarity between humans



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and great apes, coupled with increased interactions through activities such as hunting, agriculture, logging, ecotourism, urbanization, and the domestication of non-human primates (NHPs) significantly elevates the risk of cross-species transmission of parasites [6–8].

Great apes infected with intestinal parasites not only face serious health issues like malnutrition, anemia, and secondary infections but also represent a threat to human populations [1,9]. These health impairments directly affect their survival and reproductive success, exacerbating the conservation challenges faced by already endangered great ape populations [10,11]. In settings like the Primatology Center, where humans and various NHPs species interact closely, the risk of pathogen exchange is significantly heightened [12], unlike in the wild where natural barriers reduce such interactions [13].

The Primatology Center of the Interdisciplinary Center for Medical Research in Franceville (CIRMF) and Gabonese sanctuaries are dedicated to the care and rehabilitation of orphaned NHPs and those rescued from illegal private ownership [14–16]. A dedicated team of caretakers, veterinarians, and nurses work tirelessly to ensure the proper nutrition and overall welfare of these animals. Despite these efforts, NHPs at the center remain susceptible to a range of infections, including viral, fungal, bacterial, and parasitic diseases [17–21]. Among these, gastrointestinal parasites (GIPs) are particularly prevalent posing significant health risks such as growth retardation, gastrointestinal disorders, abortions, and neurological problems [22,23].

While the establishment of protected areas, sanctuaries, and primatology centers are essentials for NHP conservation [24,25], these confined environments also facilitate the zoonotic and anthropozoonotic transmission of pathogens [1,26–28]. The SARS-CoV-2 pandemic serves as a stark reminder of the risks posed by close human–animal interactions. Given the ongoing decline in great ape population [29], the CIRMF Primatology Center plays a critical role in understanding the impact of gastrointestinal parasites on NHP health and their conservation. This study aims to assess the diversity of intestinal parasites at the center, highlighting their potential effects on both primate and human health. By doing so, it seeks to provide valuable insights that can inform and improve conservation efforts, ultimately contributing to the long-term survival of these endangered species.

2. Materials and Methods

2.1. Study Site and Sample Collection

This study was conducted at the Primatology Center of CIRMF, located in southeastern Gabon (Figure 1). The center features enclosures with natural ground for free-ranging animals as well as aviaries with cemented floors. Sampling collection took place between February to April 2023, from 9 a.m to 1 p.m. A total of approximately 97 fecal samples were collected from five (5) primate species. Table 1 shows the distribution of the NHP species sampled, their characteristics, and status under the International Union for Conservation of Nature (UICN).



Figure 1. Location of the primatology center.

Common Name	Species	Habitat	Male	Female	UICN Status	Protection Status in Gabon	Total
Macaque	Macaca rhesus	aviary	7	3	last concern 2015	-	10
Nictitans	Cercopithecus nictitans	aviary	1	3	endangered 2020	-	4
Chimpanzee	Pan t. troglodytes	aviary	14	11	endangered 2016	fully protected	25
Mandrill	Mandrillus sphinx	enclosure	20	20	vulnerable 2016	fully protected	40
Solatus	Allochrocebus solatus	enclosure	2	16	near threatened 2019	fully protected	18
Total			44	53			97

Table 1. Distribution of the population according to each sampled monkey species.

To ensure the precise and uncontaminated fecal collection from NHPs, animals were sequentially captured in the feeding zone. Each individual was identified by their tattoo or ear tag. Fecal samples were collected immediately after defecation, avoiding ground contact, and placed in labeled coprology containers indicating species, sex, and collection date. Samples were either analyzed on the same day or stored at room temperature for a later analysis within 48 h. A microscopic examination of eggs and cysts was conducted using a Leica DM2000 LED microscope equipped with a Leica DFC450 digital camera for image capture.

2.2. Microscopic Analysis

Fecal samples were processed immediately using flotation and sedimentation methods as previously described [30,31]. An average of 2 g of fecal matter was used. This amount was added to a saline solution, the concentration of which varied according to flotation or sedimentation (40% and 9%, respectively). The staining step with bromothymol was omitted to enhance the visualization of parasite eggs and oocysts. Larval forms were extracted using the Baermann method. Parasite identification was based on morphological characteristics, color, and content, following the guidelines of [32,33]. In this study, to differentiate between *Necator* and *Ancylostoma* eggs, we focused on the biological behavior of the eggs. Notably, *Ancylostoma* eggs typically hatch within 24 h post-emission [34,35]. Thus, any eggs identified 48 h after collection were classified as *Necator* spp. However, as this distinction is still uncertain, we decided to refer to the worms *Ancylostoma* and *Necator* as 'hookworms'. Although larval forms belonging to the *Strongyloides* and Enterobius genera have been observed, it was not possible to identify the corresponding species precisely. To assess the parasitic load, the protocol by [36] was followed, using 2 g of fecal matter. The calculation of the parasitic load was calculated using the following formula:

 $EPG = (Total number of eggs counted/Number of grids counted) \times (Total volume (mL)/Examined volume (mL) \times 50 (Dilution factor).$

2.3. Statistical Analysis

The data for this study were analyzed using R software (version 4.3.0). The prevalence of a given parasite was calculated as the ratio of the number of individuals positive for that parasite to the total number of individuals examined. To compare the prevalence of parasitic infections among different primate taxa, we employed the Kruskal–Wallis test, which is suitable for non-parametric continuous data. This test allowed us to determine if there were significant differences in infection rates between groups. Additionally, Fisher's exact test was applied to compare the number of infected and uninfected animals, providing a robust assessment of the impact of infections within various populations. The Shannon diversity index (H) and equitability (E) were also calculated to evaluate the parasitic diversity within the taxa, offering insights into the richness and evenness of the present parasitic species. Finally, a linear regression model was utilized to examine the influences of taxon, habitat, and sex on parasitic load, thereby identifying significant factors contributing to the variations in parasitic infections.

3. Results

3.1. Diversity and Distribution of Parasite Genera in Non-Human Primates at the CIRMF Primatology Center

This study identified 3 protists and 13 helminths in 5 examined NHP species (Figure 2 and Figure S1). All NHPs were infected with four common helminths: *Trichuris, Oesophagostomum, Trichostrongylus,* and *Strongyloides*. The distribution of the remaining nine helminths and three protists was as follows: Hookworm found in chimpanzee, macaque, mandrill, and solatus; *Schistosoma* in mandrill; *Mammomonogamus* in chimpanzee and mandrill; *Spirura* in macaque, mandrill, and solatus; *Enterobius* in chimpanzee, mandrill, and nictitans; *Hymenolepis* in solatus; *Dicrocoelium* in sacaque and solatus; *Paramphistomum* in solatus; *Balantioides coli/Buxtonella* in chimpanzee, macaque, mandrill, and solatus; *Eimeria* in mandrill; and *Entamoeba* in chimpanzee.



Figure 2. Parasitic structures identified in the feces of NHPs at the CIRMF Primatology Center (**A**), *Trichuris* sp. (**B**,**T**), *Oesophagostomum* sp. (**C**), *Trichostrongylus* sp. (**D**), Spirure (**E**), *Balantioides coli/Buxtonella* sp. (**F**), *Mammomonogamus* sp. (**G**), *Eimeria* sp. (**H**,**W**), *Strongyloides* sp. (**I**), *Dicrocelium* sp. (**J**,**U**), *Enterobius* sp. (**K**), *Hymenolepis* sp. (**L**), *Ascaris* sp. (**M**), *Entamoeba* sp. (**N**), *Schistosoma* sp. (**O**), *Paramphistomum* sp. (**P**,**V**,**Q**), Hookworm (**R**,**S**), unidentified eggs.

For all the parasites, species diversity, as reflected by Shannon's diversity index and equitability, indicated high parasite species diversity across all NHP species. However, the highest diversity (Shannon Index) and equitability were recorded in mandrills (H:2.92; E:1.81), followed by solatus (H:2.89; E:1.80), chimpanzee (H:2.47; E:1.53), and macaques (H:2.46; E:1.53) (Figure 3).



Figure 3. Shannon diversity index by host species.

3.2. Prevalence of Parasites in NHPs at CIRMF Primatology Center

The analysis of samples collected from non-human primates at the Primatology Center of CIRMF revealed an overall parasitic infection prevalence of 93.81%, providing an overview of the parasitic status within the studied population. The analysis of parasite prevalence revealed significant variations among different primate species, including chimpanzees, macaques, mandrills, nictitans, and solatus. In terms of overall prevalence, mandrills exhibited the highest rate (97.5%), followed by chimpanzees (96%), solatus (100%), nictitans (75%), and macaques (70%) (Table 2).

Groups	Taxa	Chimpanzee	Macaque	Mandrill	Nictitans	Solatus	Means
Groups Nematoda Cestoda Trematoda Protist	Trichuris sp.	16	20	7.50	25	33.33	16.49
	Oesophagostomum sp.	88	50	90	50	88.89	83.50
	Trichostrongylus sp.	60	20	60	25	38.89	50.52
	Hookworm	33.33	20	32.5	0	24	29.03
Nematoda	Schistosoma spp.	0	0	5	0	0	2.06
	Mammomonogamus sp.	4	0	2.5	0	0	2.06
	Strongyloides sp.	56	30	65	25	$\begin{array}{c} 33.33\\ 88.89\\ 38.89\\ 24\\ 0\\ 0\\ 38.89\\ 0\\ 5.56\\ 0\\ \hline 11.11\\ 11.11\\ 5.56\\ \hline 11.11\\ 0\\ 0\\ 0\\ \hline 100\\ \end{array}$	52.58
	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	0	1.03				
	Spirure	0	20	22.5	0	5.56	12.37
	Enterobius sp.	16	0	10.26	25	0	9.37
Cestoda	Hymenolepis sp.	0	0	0	0	11.11	2.06
	Dicrocelium sp.	0	10	0	0	Solatus 33.33 88.89 38.89 24 0 0 38.89 0 5.56 0 11.11 11.11 5.56 0 11.11 5.56 11.11 0 0 11.11 0 0 11.00 0	3.09
Trematoda	Paramphistomun sp.	0	0	0	0		1.03
	Balantioides coli/Buxtonella sp.	68	10	42.5	0	11.11	38.14
Protist	<i>Eimeria</i> sp.	0	0	7.5	0	0	3.09
	Entamoeba sp.	8	0	0	0	0	2.06
Percentage (%) of animals with at least one parasite	ith at least 96 70 97.5 75 100		-			
<i>p</i> -value	e (Kruskal–Wallis)		0.4	406			

Table 2. Prevalence of parasite by host species.

Upon closer examination, we also assessed the prevalence of each parasite species within each taxon (Table 2). Chimpanzees are distinguished by a particularly high prevalence of *Oesophagostomum* (88%) and *Balantioides coli/Buxtonella* (68%), indicating significant exposure to these parasites. In contrast, macaques show moderate prevalence levels for several parasites, including *Strongyloides* (30%) and Hookworm (20%). Mandrills also display high prevalence rates, particularly for *Oesophagostomum* (90%) and *Trichostrongylus* (60%). Nictitans exhibit varied prevalence, peaking at 25% for *Trichuris*. Finally, solatus are characterized by a notable prevalence of *Oesophagostomum* (88.89%) and *Strongyloides* (38.89%) (Table 2).

To identify the most prevalent parasite at the primatology center, we calculated the prevalence of each parasitic species in our study population sample without the distinction of taxon. *Oesophagostomum* spp. emerged as the most widespread parasite, infecting 83.51% of individuals. Other parasites, such as *Strongyloides* spp. (52.58%), *Trichostrongylus* spp. (50.52%), and *Balantioides coli/Buxtonella* (38.14%), were also detected at significant levels. Conversely, certain parasites, such as *Ascaris* spp. (1.03%) and *Mammomonogamus* spp. (2.06%), exhibited relatively low infection rates (Figure 4).

The following table illustrates the impact of gender, habitat, and host species on the degree of parasitism. The statistical analysis revealed no statistically significant differences (p > 0.05) in parasite infestation levels in relation to the factors under investigation. The infestation rate of females was similar to that of males (0.409), irrespective of habitat (p = 0.082) or host species (p = 0.406). The proportion of infected animals ranged from 70% to 100%, with the macaque group exhibiting the lowest level of infestation (50%), whereas all solatus were infected with at least one parasite (Table 3).



Figure 4. Prevalence of each parasite genera.

Table 3. Impact of	gender and	habitat factors	on parasitism	levels
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Factors	Classes	Sample (N)	Infected	Prevalence Rate (% \pm Standard Deviation)	Df	<i>p</i> -Value
Living farm	Enclosure	57	56	98.25 ± 0.70	1	0.082
	Aviary	40	35	87.5 ± 3.54	1	0.082
Sex	Male	44	41	93.18 ± 2.12	1	0.4096
	Female	53	50	94.33 ± 2.12	1	
	Chimpanzee	25	24	96 ± 0.70		
Hosts	Macaque	10	7	70 ± 2.12		
	Mandrill	40	39	97.5	4	0.406
	Nictitans	4	3	75 ± 0.70		
	Solatus	18	18	100		

3.3. Influence of Analyzed Variables on Parasite Load

The analysis of the linear regression model revealed significant influences of taxon and habitat on parasitic load.

The results indicate that mandrills and solatus exhibit significantly higher parasitic loads compared to chimpanzees, which serve as the reference group (Figure 5). Specifically, mandrills show a coefficient of 2510.2 (p = 0.00698), while solatus have a coefficient of 2139.9 (p = 0.02517). These findings suggest that these two taxa are more vulnerable to parasitic infections. Conversely, macaques display a significantly lower parasitic load, with a coefficient of -741.7 (p = 0.02851).

Regarding habitat, primates living in aviaries also demonstrate a high parasitic load, with a coefficient of 2882.6 (p = 0.00198). In contrast, the analysis did not reveal a significant effect of sex on parasitic load, as indicated by the coefficient of -322.4 (p = 0.10323) for males compared to females.



Figure 5. Influence of variables (hosts, sex, and species) on parasite load.

4. Discussion

Primatology centers are crucial for the understanding of parasite exchange dynamics, especially the transmission of parasites between primates and humans due to frequent interactions [27,37]. Our study aimed to elucidate the diversity of intestinal parasites in captive NHPs at CIRMF's Primatology Center. In the present study, we found a global parasite infestation prevalence of 93,81% (91/97) among the studied primates, which is notably higher than previous captive NHPs studied in Africa (67% to 76.2%) [18,38,39] and Asia (89.6%) [40]. This discrepancy may be attributed to a nearly two-year lapse in deworming at the CDP due to limited resources. This high prevalence highlights the increased susceptibility of NHPs to gastrointestinal parasites and remains consistent with the report, which reports a prevalence ranging from 22 to 100% in NHPs [15].

Our coprological analyses identified a total of 16 parasitic taxa infecting NHPs. The diversity of parasites observed varied across studies, largely due to the significant influence of environmental conditions on the dynamics of gastrointestinal parasites, as previously documented in the literature [15,41–43]. Since environmental factors differ from one location to another, they can substantially affect parasite diversity. Among the taxa identified in this study, some species belonging to Oesophagostomum, Enterobius, Ascaris, Trichostrongylus, Strongyloides, and hookworms are particularly known for their high zoonotic potential. Species of these genera have been implicated in bidirectional transmission in environments shared by NHPs and humans [44-46]. However, a reliance on microscopic analysis alone limits the ability to ensure accurate identification down to the species level. A notable instance is Enterobius anthropopitheci, the primary parasite of the Enterobius genus found in the chimpanzees [47]. This parasite is closely related to Enterobius vermicularis with which it shares similar morphological characteristics [48]. Documented instances of co-infection between these two species have been observed in environments where humans and primates coexist [49]. In light of this complexity, the utilization of molecular tools is imperative. These advanced techniques are of great importance for resolving the identification ambiguities associated with cryptic species and for gaining deeper insights into the dynamics of infection in areas of human-primate interaction. This finding underscores the need for rigorous feeding protocols to minimize pathogen exchange risks. Additionally, NHPs may act as reservoirs for human-infecting parasites, indicating that primatology centers could be sources of parasitic infections that pose significant health risks, particularly to young children [50].

The most prevalent parasitic genera identified were *Oesophagostomum* (83.5%), *Strongyloides* (52.58%), *Trichostrongylus* (50.52%), *Balantioides coli/Buxtonella* (38.14%), Hookworm (29. 03%), and *Trichuris* (16.49%). These findings are consistent with studies in sub-Saharan Africa, which often reported helminth infections as the most common among the NHP population [33,51,52]. The high prevalence of *Oesophagostomum* may be due to its low immunogenicity, allowing the effective colonization of host environments. Additionally, the promiscuity and reuse of the same soil in captive and semi-captive settings can facilitate the spread of intestinal parasites [12,18]. Nematodes from the *Oesophagostomum* genus are known to cause severe diseases in primates, including granulomas, caseous lesions, and abscesses in the intestinal wall, with some NHPs potentially acting as reservoirs for human oesophagostomosis [53].

The highest diversity and load of intestinal parasites were observed in mandrills, chimpanzees, and solatus, likely due to their direct contact with natural soil, which harbors a wider variety of parasites. Natural soil serves as a reservoir for infectious forms of intestinal parasites, promoting multi-parasitism in these animals [22,53,54]. Our study found similar infection rates in males and females, regardless of habitat or species, consistent with the findings of Eke et al. [55]. The absence of significant differences between sexes may be due to the communal living conditions of NHPs, where both males and females experience the same level of parasite exposure, as shown in other studies [55,56]. Differences in parasite infestation rates between sexes in NHPs are often associated with differences in home range and foraging behaviors. At our center, both sexes are housed together and receive equal food from caretakers [57,58]. Our results also show that individuals living in aviaries (cemented floor) had a much higher parasite burden than those living in pens (natural floor). Our observations are similar to those of Opeyemi et al. [59]. on helminth infections in captive birds, confirming the risks associated with aviary habitats. This finding may be explained by the fact that natural floors, in contrast to cemented ones, possess a capacity for self-regulation of parasite populations [59].

The potential for cross-species transmission, especially in captive settings with close contact between humans and NHPs, is a serious concern [18,60–63]. This issue would not only jeopardize the health of both primates and humans but also impacts the conservation of NHP populations. It is imperative to consider animal, human, and environmental factors to improve illness prevention at this interface. Effective measures should include improved hygiene, sanitation, and veterinary care.

The One Health approach is crucial to effectively addressing concerns related to intestinal parasites. This framework integrates efforts across human, animal, and environmental sectors, enhancing surveillance, improving environmental management, and fostering collaboration among stakeholders [6]. By recognizing the interconnectedness of these health domains, the One Health approach is essential for improving health outcomes and promoting sustainable coexistence between humans and NHPs. This holistic perspective is essential for combating intestinal parasites and advancing public health and conservation efforts.

5. Conclusions

The findings of this study highlight a significant risk of zoonotic disease transmission associated with the presence of gastrointestinal parasites in primatology centers in Gabon. The high prevalence and diversity of these parasites among non-human primates (NHPs) highlight the urgent need for enhanced surveillance and robust biosecurity measures. Our findings identified several genera of parasites, including *Strongyloides, Oesophagostomum*, Hookworm, and *Enterobius*, which harbor species with zoonotic potential that could present a risk to public health in cohabitation scenarios. Furthermore, these parasites may have a significant impact on the health of NHPs, potentially leading to increased morbidity and susceptibility to other infections. To gain a full understanding of the implications of these findings, further molecular analysis is essential to trace the origins and transmission pathways of these parasites. The close contact between humans and NHPs presents significant

health risks for both groups, reinforcing the necessity of a One Health approach to ensure the safety of all parties. This integrative framework acknowledges the interconnectivity between human, animal, and environmental health, which is essential for the effective management of zoonotic risks. To mitigate these threats, it is essential to implement a systematic monitoring program for potential parasitic infections and to restrict unauthorized contact between visitors and staff with NHP food and water sources. By implementing these preventive measures and adopting a One Health perspective, we can protect the health of both primates and humans, fostering a safer coexistence in shared environments while enhancing our collective capacity to address zoonotic threats comprehensively.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jzbg5040048/s1, Figure S1: Various forms of larvae of observed gastrointestinal parasites.

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Institutional Review Board Statement: This study was approved by the scientific committee of our institute, the Centre Interdisciplinaire de Recherches Medicales de Franceville (CIRMF), in accordance with the ethical principles of animal research. All samples were collected with due regard to animal welfare, and, in this study, all samples were collected with the consent of the animal owners. In addition, animal sampling procedures were evaluated by the Institutional Committee for Animal Use and Care of the National CIRMF.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

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