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Gastrointestinal parasites of captive and free range non-human primates in savanna zone of Nigeria

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Abstract
Primates being reservoirs of parasites that cause major problems to humans, livestock and under stressful conditions to themselves is a major concern to wildlife conservation, our study compared the gastrointestinal parasites of some Non-human Primates at University of Ilorin Zoological Garden (Unilorin Zoo) and Kainji Lake National Park (KLNP), Nigeria in order to determine the level of their prevalence and possibility of infection on tourists visiting the sites. Species sampled were restricted to those common to both sites; *Papio anubis*, *Erythrocebus patas* and *Chlorocebus aethiops tantalus*. A total of 128 faecal samples were screened from apparent healthy individuals. Samples were processed using sedimentation, sugar floatation technique, Stoll's dilution technique, cultured and infective larvae were recovered using Baermann technique.110 (85.7%) samples were positive for infestation of at least one eggs. Among the positive samples were three species of nematodes; *Strongyloides* spp., *Ascaris* spp., *Ancyclostoma* spp. Primates on free-range had higher prevalence of infection. However, under both conditions, there is no significant difference in the prevalence and intensity of parasites harboured over seasonst (4) = 1.968, p =0.120).

Keywords: Non-human primates, important reservoirs, prevalence, zoonotic infections

Introduction
Gastrointestinal parasites are organisms including protozoans and worms that live within organs of the digestive system. The presence of these parasite in animals can affect host ecology, causing morbidity, host survival leading to mortality of the animals and in livestock it brings about low productivity by reducing reproduction rate mainly due to reduction in host appetite and inefficient gut functioning [1]. They are important and ubiquitous component of primates natural communities [2].

Non-human primates (NHPs) are the closest biological relatives are reservoir of many human pathogens since increasing anthropogenic activities results in altered interfaces between animals and people, this can enhance the transmissions of parasites from humans to primates and vice versa [3]. Primates are particularly vulnerable to the infections of directly transmitted parasites because they often live in close social groups that facilitate their transmission [4].

Parasites and infectious diseases have become a major concern in conservation of endangered species as they can lead to mortality, dramatic population decline, and even contribute to animal extinction [5] both in captive and wildlife primates. Infectious diseases play important roles in natural systems, from influencing host genetic diversity to altering species composition in ecological communities. Infectious diseases are also recognized as a source of threat to natural populations because they have been implicated as causing declines in previously thriving populations and in already imperiled species. Infectious diseases are the third most important driver of population decline of wildlife [6] after hunting and habitat degradation.

Different surveys have been carried out on captive and free ranging animals in their habitat but only few have been on the gastrointestinal parasite of the animals in both captivity and in the wild. Adeniyi, Morenikeji, & Emikpe, (2015), [7] worked on the prevalence and intensity of gastrointestinal parasites on carnivores in three zoological garden in South west Nigeria, Ola-Fadunsin, Ganiyu, Rabiu, Hussain., Sanda, Musa, *et al*., (2019) [8] determined the prevalence,

Infection burden, and risk factors associated with the occurrence of gastrointestinal parasites in different avian species in Ilorin, Nigeria, while Kolapo & Jegede (2017) [9] investigated the gastrointestinal parasites of animals at the University of Ilorin zoological garden. In KLNP, Ajibade, & Agbede, (2008) [10] studied Tse tse fly species diversity in Kainji Lake National Park, Nigeria, Adeola, Adeola, Fajobi, Babatunde, Akande, Ajayi, (2019) [11] compared the gastrointestinal parasites of captive and wild Olive Baboon (Papio anubis) in Federal College of Wildlife Management Mini Zoo and Kainji Lake National Park, Nigeria.

Since Wild animals plays important roles as sources and reservoirs of emerging human and livestock diseases [12]. It is important to research on diseases and parasites that are harbored by them. It is critical to have information on the helminths of these primates, these invariably will help in formulating preventative protocols in the management. Information on the diseases and parasites of wild animals in Nigeria is scanty compared to East and South Africa [13] especially when comparing two different microhabitat. Therefore, there is an urgent need to determine the gastrointestinal parasites of these wild animals for their survival and safety in captivity and in the face of increased anthropogenic activities that tends to increase pressure on them in the wild.

Materials and Methods

Study area

The study was carried out in Kainji Lake National Park and University of Ilorin Zoological Garden.

Kainji Lake National Park (KLNP) which was established in 1979 by the merging of the two former Game Reserves – Borgu Game Reserve (located in Niger and Kwara State) and Zugurma Game Reserve (located in Niger State), the two sections had been gazetted in 1962 and 1971 respectively as Game Reserves by the then Northern Regional Government. It is the first National Park and the second largest of all the seven National Parks in Nigeria. It is located between latitude 90 401 and 10 0 301N and longitude 3 501 E and has a total landmass of 5,370.82km2. It has a savanna climate, with mean annual rainfall which varies from 1100mm in the eastern part to 1150mm in the western part.

The University of Ilorin Zoo was established in 1985 and it is located on at the main gate of University, in Kwara state with a Guinea Savannah vegetation belt south of the Sahara. It is one of the largest wildlife sanctuaries in south western Nigeria. Ilorin is between latitudes 8° 05`N to 10° 05`N (8°30′N) and longitudes 2° 50`E to 6° 05` E (4°33′E). The annual rainfall range is 1000 mm to 1500 mm. The wet season begins at about the end of March and lasts until early September, while the dry season begins in early October and ends in early March. Temperature is uniformly high and ranges between 25oC and 30oC in the wet season throughout the season except in July – August when the clouding of the sky prevents direct insolation (heatstroke) while in the dry season it ranges between 33oC to 34oC. Relative humidity at Ilorin in the wet season is between 75 to 80% while in the dry season it is about 65% [14].

Sample collection and preservation

A total of 128 freshly voided feacal droppings were collected from the selected primate species in the wet (September-October 2016 and April, 2017) and dry season (February-March, 2017); primates inhabiting KLNP (Kali Camp, Roan Gate, Oli camp) and Unilorin Zoo. Non-invasive sampling techniques were employed, faecal samples were collected immediately after defecation to avoid contamination using opportunistic sampling. In general, a single troop was surveyed in one day, starting the collection at dawn and moving along with the troop to gather as many samples as possible, avoiding repeated sampling of the same individual. The topmost part of the faeces were scooped to prevent contamination, stored in a well labelled 30ml sample bottles indicating name, species and date of collection, and kept inside a cooler with ice Packed and transported to the laboratory of the Department of Animal Production and Health (APH) of Federal University of Technology, Akure, Ondo State for examination.

Laboratory examination

On the field and in the Laboratory, faecal samples were immediately examined using microscope for the presence and evidence of consistency, presence of blood, mucus, of tapeworm proglottids and laval nematodes. After which they were processed using sugar floatation, sedimentation techniques as described by [3] whereas Baermann technique was used to isolate infective larvae from faecal cultures. The entire 22- by 22-mm coverslip were systematically examined with the low power objective (10x) and low light intensity; any suspicious objects was then examined with the high dry objective (40x).

Statistical analysis

Descriptive Statistics such as Percentage, or Mean ± Standard deviations were used to summarize the data obtained and the differences between means were determined using t-test at 95% level of significance with SPSS version [20].

Results

Identified gastro-intestinal parasites of some primates in captivity and free-range

Out of the 128 feacal samples screened, 14 (10.9%) of the samples were collected from captive, 114 (89.1%) were from free-range. A total of 110 (85.7%) were positive for at least one egg while 18 (14.3%) were negative. Out of the 12 (9.5%) feacal samples collected from captive primates 4 (28.6%) were positive while 10 (71.4%) were negative whereas in Free-range 106 (93%) were positive and 8 (7%) were negative (Table 1). Also, all of the positive samples were helmiths, no protozoans. All of the positive samples were three genera of Nematodes and they includes; Strongyloides sp., Ascaris sp., Ancyclostoma sp.

Prevalence of identified gastrointestinal parasites

The prevalence as well as the various species of GIT parasite in both captive and free ranging primates are presented in Table 2 and Table 3 respectively. The prevalence of infection in captive wild primates during the dry and wet seasons shows the mean percentage prevalence of infection (22.23± 38.50) during the dry is not significantly different (Table 2) than the mean of percentage prevalence of infection in captive primate in wet season (22.20±19.22) since t (4)=0.001, p=0.999. In both seasons, E. patas had no prevalence of infection, 33.3% is the Prevalence of Strongyloides Sp. infection in the dry season while its 66.7% in the wet season Ch. aethiops tantalus while in P. anubis there were no infection in the dry season but with 33.3% of Strongloides infection in the wet season (Table 2). The percentage prevalence of identified parasites in

primates on free-range during the wet and dry seasons is shown in Table 3. Strongyloides sp. was the most prevalent parasite, occurring in all primates irrespective of the species. Strongloides sp. had the highest prevalence of infection in all 3 species of primates in KLNP. Papio anubis of the 3 Species of primates had the highest diversity of infection (Strongyloides sp. Ascaris sp. Ancylostoma sp.) (Table 3). The comparative percentage prevalence of parasitic infections between captive and free-range primates (Table 4) shows a significantly higher mean percentage prevalence of parasitic infection (87.00 ± 17.80) in free-range than the mean percentage prevalence of parasitic infections in primates in captivity (22.23 ± 25.46) as t [4] =-3.611, p=0.023. Therefore primates on free-range habour higher load of infections than primates in captivity.

Table 1: Percentage of identified parasites

Study Sites	No. Of Samples Collected (%)	No. Of Samples positive (%)	No. Of Samples negative (%)
Unilorin Zoo	14 (10.9)	4 (28.6)	10 (71.4)
KLNP	114 (89.1)	106 (93)	8 (7)
Total	128	110 (85.9)	18 (14.1)

***Source*:** Olarewaju, Field Survey, 2017

Table 2: Prevalence of Identified GIT Parasites in Captive Wild Primates during the Dry and Wet Season.

	Dry Season				Wet Season			
Primates Species	**No. Collected**	**No. infected**	**Parasites Observed**	**Percentage Prevalence (%)**	**No. Collected**	**No. infected**	**Parasites Observed**	**Percentage Prevalence (%)**
P. anubis	3	0	-	0	3	1	*Strongyliodes spp.*	33.3
Ch. aethiops tantalus	3	2	*Ascaris spp.*	66.7	3	1	*Ascaris spp.*	33.3
E. patas	1	0	-	0	1	0	-	0
Total	7	2		28.6	7	2		28.6
Mean ± SD				22.23± 38.50				22.20 ±19.22

***Source*:** Olarewaju, Field Survey, 2017

Table 3: Prevalence of Identified GIT Parasites in primates on Free-range during the Dry and Wet Season

	Dry Season				Wet Season				
Primates Species	**No. of samples Collected**	**No. Infected**	**Identified Parasites**	**% Prevalence of Parasite**	**No. samples Collected**	**of**	**No. Infected**	**Identified Parasites**	**% Prevalence of Parasite**
P. anubis	79	73	*Strongyloides spp.*	92.4	28		28	*Strongyliodes spp.*	100
		15	*Ancylostoma spp.*	19					
		6	*Ascarids spp.*	7.5					
Ch. aethiops	6	4	*Strongyloides spp.*	66.7	0		0	-	0
tantalus									
E. Patas	1	1	*Strongyloides spp.*	100	0		0	-	0
Total	86	78		28	28				
Mean of % prevalence of primate Species ± SD			86.3±17.5		33.3 ± 57.5				

***Source*:** Olarewaju, Field Survey, 2017

Table 4: Comparative prevalence of identified GIT parasites in captive (Unilorin Zoo) and free ranging (KLNP) primates

	Captive			Free- range		
Primates Species	**No. Collected**	**No. Infected**	**Percentage Prevalence (%)**	**No. Collected**	**No. Infected**	**Percentage Prevalence (%)**
P. Anubis	6	1	16.7	107	101	94.4
Ch.aethiops tantalus	6	3	50	6	4	66.7
E. patas	2	-	0	1	1	100
Total	14	4	28.6	114	106	93
Mean ± SD			22.23± 25.46			87.0±17.8

***Source*:** Olarewaju, Field Survey, 2017

Intensity of infection in captive wild Primates in Unilorin Zoo and KLNP

Intensity of different parasites in the primates in both Unilorin Zoo and KLNP are presented in Table 5 & 6respectively. The Mean Egg Per Gram (EPG) were calculated for each of the primates. The highest mean EPG (eggs per gram) in captivity was found in Ch. aethiops tantalus in both wet (300±0.00) and dry season (250±70.70), followed by P. anubis (200±0) and E. patas with no infection at all (Table, 5). The intensity of infection in primates in captivity in the wet season (83.33 ± 144.34) was not significantly different from intensity of infection in primates during the dry season (166.67 ± 152.75), since t (4) =-0.687, p=0.530

In captive, Strongyloides spp had the highest mean EPG irrespective of species of Primate (Table, 6) followed by Ascaris spp (450 ± 83.7) then Ancyclostoma spp. P. anubis habours higher intensity of Strongyloides in the wet season (750 ± 323.8) than in the dry season (524.05 ± 322.73) (Table, 6). In free-ranging primates (KLNP), Ch.aethiops tantalus (875 ± 170.8) had the highest mean EPG followed by P. anubis (635.35 ± 32.92) then E. patas had the least mean EPG (Table, 7).

The comparative intensity of parasitic infections between captive and free- range primates were not singnificantly different as t (4) =-1.968, p=0.120 with intensity of parasitic infection in captivity being a mean EPG of 152.23 ± 134.852 whereas the intensity of parasitic infection in free-range had a mean EPG of 570.12 ± 342.20 (Table, 7).

Table 5: Intensity of Identified GIT Parasites in Captive wild Primates during the Dry and Wet Seasons

← Dry Season →			← Wet Season →	
Primates Species	**Intensity Infection**	**of**	**Mean EPG±SD**	**Intensity of Infection**	**Mean EPG±SD**
P. Anubis	-		0±0	+	200±0
Ch.aethiops tantalus	+		250±70.7	+	300±0
E. patas	-		0±0	-	0±0
Mean±SD			83.33±144.34		166.67±152.73

***Source*:** Olarewaju, Field Survey, 2017
- Nil
+ (below 300) Low intensity
++ (300-600) Moderate
+++ (above 600) Heavy Intensity EPG = Eggs per grams

Table 6: Intensity of Identified GIT Parasites in primates on Free-range Wild Primates during the Dry and Wet Seasons

← Dry Season →				← Wet Season →		
Primates Species	**Types of Infection**	**Intensity of Infection**	**Mean Egg/gram EPG ± SD**	**Intensity of Infection**	**Mean Egg/gram EPG ± SD**	**Type of Infection**
P. anubis	***Strongyloides spp.***	+++		+++		***Strongyloides sp.***
			524.05±322.73	750 ±323.8		
	Ancylostoma spp.	++	306.7±231.4			
	Ascarid spp.	+	450 ± 83.7			
Ch. aethiops tantalus	*Strongyloides spp.*	++	875 ± 170.8	-	0±0	-
E. patas	*Strongyloides spp.*	+	100±0	-	0±0	-
Mean±SD			524.05 ± 322.73		750 ± 323.8	

***Source*:** Olarewaju, Field Survey, 2017
- Nil
+ (below 300) Low intensity
++ (300-600) Moderate
+++ (above 600) Heavy Intensity EPG= Eggs per grams

Table 7: Comparative Intensity of Identified GIT Parasites between Captive and Free Ranging Primate

		Captive				**Free- range**			
Primates Species	**No. Collected**	**No. Infected**	**Intensity of Infection**	**Mean EPG ± SD**	**No. Collected**	**No. Infected**	**Intensity Infection**	**of**	**Mean EPG ± SD**
Papio anubis	6	1	+	200±0	107	101	+++		635.35 ± 32.92
Ch. aethiops tantalus	6	3	+	256.7±57.7	6	4	+++		875 ± 170.8
Erythrocebus patas	2	-	+	0±0	1	1	+		200±0
Mean ± SD				152.23±134.85					570.13±342.20

***Source*:** Olarewaju, Field Survey, 2017
- Nil
+ (below 300) Low intensity
++ (300-600) Moderate
+++ (above 600) Heavy Intensity EPG= Eggs per grams

Discussion

Identified gastrointestinal parasites of primates in the study area

Types, prevalence and intensity of gastrointestinal parasites in captive and free-ranging primates was revealed in this study. The gastrointestinal (GIT) parasites observed among these primates includes; Strongyloides spp. and Ancyclostoma spp. Ascaris spp. The GIT parasites observed in this study have also been earlier reported by other researchers in other primates (15; 7). All the parasites recorded in this study are nematodes and agreeswith Rossanigo, C.E. & Gruner, L. (1995) [16] who reported that nematodes are responsible for most of the helminthes diseases of veterinary importance.

Prevalence and Intensity of gastrointestinal parasites of the study area

The overall prevalence of GIT parasites in the captive primates was 10.9% while on free-range was 89.1%, with free-range having the higher prevalence. The high prevalence encountered in this study could be explained by the existence of favourable climatic conditions [17], and this support prolonged survival of infective nematode larvae in the environment, while low prevalence in P. anubis and no prevalence in E. patas in captivity could be attributed to regular cleaning and vaccination of primates in captivity.

Bezjian M, Gillespie T. R, Chapman C. A, & Greiner E. C. (2008) [18] had reported that months with a total rainfall not less than 51mm mean maximum and minimum temperature of not less than 25 °C and 11 °C, respectively had a greater potential to support prolonged survival of infective nematode larvae on pasture with subsequent transmission to livestock, of which the study areas is within these rainfall and temperature limits.

Nematodes are known to have direct life cycle that do not involve any intermediate host and are transmitted by feco-oral route through contaminated feed, water, and soil, and have the ability to survive in harsh environment, which could be the major reason why they are found during the study irrespective of the season. Also there is a high possibility of environmental contamination as the reason for their

occurrence, higher prevalence and intensity [19] Higher Prevalence and intensity in free-ranging primates could be attributed to more exposure to a higher degree of environmental contamination from the larger environment been explored by theses group of primates including farms around the KLNP. Since NHPs especially P. anubis have become notorious crop-raiders, supplementing their diets [20] with varieties of cultivated food crops grown in farms near their habitat. Whereas the captive animals have a smaller environment in conjunction with [21] whose contamination can be from contaminated water, or zoo workers acting as vectors of parasites through their shoes, clothes, hands, food, or with working tools.

Overtime, it has been observed that confinement of wild animals in zoo makes them more prone to different parasitic infections despite proper attention to feeding, water, and maintenance of hygiene in captivity [22], this is in contrast to this study in which even though the wild animals in captivity are susceptible to parasitic infections they habours lower parasites both in diversity, intensity of infection and prevalence as compared with primates in the wild.

Since non-human primates are known to be a major hosts of Strongyloides spp. [3] this is evident in this study in which Strongyloides spp were found to be the most prevalent parasites occurs in all primates studied, except in C. sabeaus in captivity which habour only Ascaris spp. Higher prevalence of Strongyloides spp in this study could be due to more conducive environment for the development of the pre-parasites stages in the hot and humid environmental conditions [23], and primates become infected with Strongyloides spp. by eating eggs or via skin penetration by third-stage infective larvae (L3). The skin penetration is made more possible because of frequency of the NHPs with contact with the ground in which Strongyloides spp. can be in the ground.

In this study, 28.6% of the samples collected from P. anubis at Roan gate of KLNP were found to contain varying degrees of intensity of the eggs of an ascarid and so also is C. aethiops tantalus at Unilorin. The occurrence of Ascaris spp. human parasite but unusual to be found among primates except for NHPs with very close contacts with human this was also observed in other primates such as chimpanzees and gorillas by Dupain, Nell, Petrzelkova, Garcia, Modrý, & Ponce-Gordo, (2009); Ramos, Giannelli,, Dantas-Torres, Brianti, & Otranto,(2013) (24;25). During the study, P. anubis at the were sighted on dumpsite close to the rangers camp for leftover at the roan gates, and this could be the reason for the presence of Ascaris spp. in 28.6% of the samples collected from Roan gate.

Influence of season on prevalence and Intensity

Nematodes are known to have direct life cycle and have the ability to survive in harsh environmental conditions. According to Ramos *et al*., (2013) [26], environmental parasite stages usually survive better in moist and warm conditions. This is in contrast to this study in which there is no significant difference in the prevalence of gastro intestinal helminths in both wet and dry season in both the captive or free-ranging primates. The ability to survive in harsh environmental conditions could be the major reason why they are found during the study irrespective of the seasons.

The similarities in the presence of parasites found in the different species of the primate could be as a result of the primates been of same subfamily Cercopithecinae but which was not established in this study.

Conclusion

Among different helminthic infections, primates both in captive and on free-range harbors only nematodes. The prevalence of gastrointestinal parasites in captive wild animals was 7% whereas the prevalence in free-range primates was 93% of which it was relatively high prevalence in free-range than in captivity. Lower intensity occurs in captive primates as compared with primates on free range. To have recorded a lower prevalence in primates in captive than on free-range, it shows a overall proper management of the primates in the Zoo in the areas of nutrition, sanitation, and deworming practices followed.

With respect to season, there was no effect of season on the prevalence and intensity of gastrointestinal parasites of both primates in captivity and on free-range. Only coprological analyses based on morphological examinations were performed and identifications were made at the genus level. The parasites found in this study are zoonotic, also the presence of Ascaris sp. indicates the effect of close contact of man with primates.

In the face of habitat destruction and degradation, encroachment of herdsmen, and continuous tourists visitations to free- range, which increase pressure on wildlife and can predispose them to stress. Routine contact with people, and the refuse dumped, sharing of food and feacal deposit by tourists or rangers may introduce new diseases to animals.

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