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Gastrointestinal Parasites of Captive and Free-roaming Primates at the Afi mountain Primate Conservation Area in Calabar, Nigeria and their Zoonotic Implications

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Abstract: A study on the gastrointestinal parasites among free-living and captive primates at the Afi Mountain, Primate Conservation Area in Calabar, Nigeria was undertaken for the first time to ascertain their zoonotic implications. Faecal samples were subjected to direct smear, floatation, quantitative estimation of helminth eggs (epg) and oocysts (opg), larval isolation and identification by modified Baerman's technique and oocyst sporulation for specie identification. Out of the 108 primates examined, 75(69.44%) were found to be shedding the ova and oocysts of several gastrointestinal parasites of which, the mona monkeys (*Cercopethicus mona*) 16(80%) followed by the white collared mangabey (*Cercocebus torquatus*) 7(77.78) had the highest ($p<0.05$) prevalence of infection. Meanwhile, the chimpanzees (*Pan troglodytes*) had the highest ova or oocyst counts and variety of gastrointestinal parasites such as *Ascaris lumbricoides, Trichuris trichiura, Balantidium coli, Enterobius vermicularis, Entamoeba histolytica, Strongyloides stercoralis, Blastocystis hominis, Hymenolepis nana, Schistosoma mansoni, Ancylostosoma duodenale and Cryptosporidium* species. Similarly, the drill (*Mandrillus leucophaeus*), Sclater's white-nosed monkey (*Cercopethicus erythrotis sclateri*), white-collared mangabey (*Cercocebus torquatus*) and others, had *Ascaris lumbricoides or Ancylostoma duodenale*. All captive primates were more infected than those under free-roam. The young (<12 months) and females had higher infection rates ($p<0.05$) than their counterparts. In conclusion, the primates harboured several parasites of zoonotic importance.

Key words: Afi-mountain primate sanctuary, calabar, captive primates, free-roaming primates, gastrointestinal parasites, zoonosis

INTRODUCTION

Wild primate population, as members of biologically diverse arboreal or terrestrial habitats are regarded as major source of emerging infectious diseases and may hold valuable clues to the origins and evolutions of some important zoonosis (Wolfe *et al*., 1998). Primates over the years have been identified as reservoirs of human gastrointestinal parasites (Soulsby, 1982). The anthropoid primates and to a lesser degree simian primates share broadly similar physiologic and genetic characteristics and thus susceptible to gastrointestinal parasites (Wolfe *et al*., 1998). The ability of parasitic infections to cross primate-species boundary to affect man has been documented (Brack, 1987; Jin *et al*., 1994). Since primates live in game reserves or National parks in tropical rain forests, the degree of interactions between primates and humans occur in this high-risk interface (Meslin, 1992; Turner, 1996; Wolfe *et al*., 1998). Similarly, handling of primates in zoological gardens or through pet trade in new world monkeys could lead to the transfer of parasitic zoonosis. Although primates act as reservoir of common gastrointestinal parasites of man without manifesting obvert clinical signs (Nwosu, 1995; Mbaya *et al*., 2006b), severe outbreaks of clinical amoebiasis, trichurosis and balantidosis have been reported in a colony of captive chimpanzees (*Pan troglodytes*) in Sanda Kyarimi Park, Maiduguri Nigeria (Mbaya and Nwosu, 2006; Mbaya *et al*., 2006a). In view of the prospects of emerging infectious diseases from primates to man or vice-versa, the search for answers is usually turned to the non-human primate as a sentinel. This study was therefore, designed for the first time in Nigeria, to investigate the gastrointestinal parasitic infections of primates at the Afi Mountain Primate Conservation Area in Calabar, Nigeria and their possible zoonotic implication on one hand and the conservation of the primates on the other hand.

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MATERIALS AND METHODS

Study area: The primate preserve, where this study was conducted is located in the Afi mountain Wildlife Conservation Area in Calabar, Nigeria, occupying 1.6 km square of the about 100 km square of the wildlife sanctuary. The area lies between Latitude 5' N and longitude 8' E in Boki local Government Area, North of Calabar, Nigeria. It contains a number of spatially separate captive or free-roaming primate colonies. Each colony consist of multiple primate species such as the chimpanzee (*Pan troglodytes*), mandrill (*Mandrillus sphinx*), drill (*Mandrillus leucophaeus*), mona monkeys (*Cercopithecus mona*), spot-nosed guenons (*Cercopethicus nictitans*), white-collared mangabey (*Cercocebus torquatus*) and Sclater's white-nosed monkey (*Cercopethicus erythrotis sclateri*) in a very rich biodiversity of undisturbed ecosystem. The primates in captivity were fed on fruits and vegetables and water was provided *ad-libitum* while their free-roaming counterparts fed on twigs, fruits, vegetables, root barks and termites at specific periods of the year and drink water from rivers within the preserve. Non-governmental organizations (Cercopan) and (Pandrillus) were actively involved in the conservations of orphaned primates that were victims of habitat loss and the ever-increasing bush meat trade in Nigeria. They provide captive environment that mimic the primates natural habitat with a view of re-introducing them into the wild.

Sample collection and examination: The study involved 108 primates, with 49 under captivity and 59 under free-roam. No form of capture whatsoever was involved during the study. Similarly, the Ethical and Animal Welfare Committee for the use of animals for Biomedical Research of the Faculty of Veterinary Medicine University of Maiduguri, Nigeria and The Nigerian National Parks Service approved the research. The various captive primate groups were monitored daily and freshly passed faecal samples collected from their cages. Meanwhile, faecal samples from the free-roaming primates were randomly collected opportunistically as they were dropped. The samples were labeled, according to captive or free-roam status, species, sex and age. The samples were subjected to direct smear to establish the presence of gastrointestinal parasitic infections and transported to the Parasitology laboratory of the University of Maiduguri, Nigeria and subjected to concentration by floatation technique (Soulsby, 1982). Egg counts per gram of feces (epg) and oocyst counts per gram of faeces (opg) were determined by the modified McMaster technique using saturated sodium chloride solution as floating medium (Soulsby, 1982). Identification of helminth ova and oocysts were done by standard parasitological criteria (Soulsby, 1982; Smyth, 1994; Urquhart *et al*., 1996; Lynne and David, 2005). Modified Baerman's technique for larval isolation and identification of helminthes and sporulation of oocysts of protozoa was carried out for identification of the species using standard parasitological criteria (Soulsby, 1982; Smyth, 1994; Urquhart *et al*., 1996; Lynne and David, 2005).

Statistical analysis: Data obtained were either summarized as Means±Standard deviation or percentages and the difference between means were determined using the t-test at the 5% level of significance (Mead and Curnow, 1983).

RESULTS

The prevalence of gastrointestinal parasites as well as the various species of parasites with their respective worm burdens in the various captive and free roaming primate colonies examined at the Afi Mountain Wildlife Sanctuary in Calabar, Nigeria, is presented in Table 1. Out of 108 primates examined, 75 (69.44%) were found to be shedding the ova and oocysts of at least one specie of gastrointestinal parasite in their faeces. Although the prevalence of infection was generally high irrespective of species, the mona monkeys (*Cercopethicus mona*) 16(80%) followed by the white-collared mangabey (*Cercocebus torquatus*) 7 (77.78%) had the highest prevalence of infection ($p<0.05$) but with few species of parasites among other primate groups. Meanwhile, the chimpanzees (*Pan troglodytes*) had the highest variety of gastrointestinal parasites such as *Ascaris lumbricoides* 5 (21.74%), *Trichuris trichiura* 6 (26.17%), *Balantidium coli* 2 (8.70%), *Enterobius vermicularis* 2 (8.70), *Entamoeba histolytica* 2 (8.70%), *Strongyloides stercoralis* 3 (13.04%), *Blastocystis hominis* 3 (13.04%), *Hymenolepis nana* 5 21.74%), *Schistosoma mansoni* 10 (43.48%), *Ancylostoma duodenale* 2 (8.70%) and *Cryptosporidium* species 5 (21.74%). This was followed by the drill (*Mandrillus leucophaeus*) which mainly had *Ascaris lumbricoides* 5 (55.56%), *Ancylostoma duodenale* 2 (22.22%) and *Strongyloides stercoralis* 2 (22.22%). The mandrill (*Mandrillus sphinx*) had *Ascaris lumbricoides* 6 (50%), *Ancylostoma duodenale* 6 (50%) so also did the spot-nosed guenons (*Cercopethicus nictitans*) which had *Ascaris lumbricoides* 5 (100%) and *Ancylostoma* duodenale 5 (100%) while the Sclater's white-nosed monkey (*Cercopethicus erythrotis sclateri*) and the white-collared mangabey (*Cercocebus torquatus*) had only *Ascaris lumbricoides* with 3 (100%) and 7 (100%) prevalence rates, respectively. Similarly, egg or oocyst

Table 1: Various species of gastrointestinal parasites of captive and semi-captive primates and their associated worm burden at the Afi Mountain Primate Conservation Area in Calabar, Nigeria

Species of primates	No.Exam.	No. Infected (%)	Parasites encountered (%)	Mean epg/opg±S.D.
Chimpanzee (*Pan troglodytes*)	28	23 (57.14)[a]	(i) Ascaris lumbricoides 5 (21.74)[a]	450.0±0.15[a]
			(ii) Trichuris trichiura 6 (26.17)[a]	360.2±0.14[b]
			(iii) Balantidium coli 2 (8.70)[b]	350.6±0.13[b]
			(iv) Enterobius vermicularis 2 (8.70)[b]	300.8±0.12[b]
			(v) Entamoeba histolytica 2 (8.70)[b]	300.4±0.12[b]
			(vi) Strongyloides stercoralis 3 (13.04)[c]	340.7±0.13[b]
			(vii) Blastocystis hominis 3 (13.04)[c]	330.6±0.13[b]
			(viii) Hymenolepis nana 5 (21.74)[a]	374.0±0.14[b]
			(ix) Schistosoma mansoni 10 (43.48)[d]	380.2±0.14[b]
			(x) Ancylostoma duodenale 2 (8.70)[b]	500.0±0.16[a]
			(xi) Cryptosporidium parvum 5 (21.74)[a]	360.6±0.14[b]
Drill (*Mandrills leucophaeus*)	20	9 (45.0)[b]	(i) Ascaris lumbricoides 5 (55.56)[d]	105.4±0.12[c]
			(ii) Ancylostoma duodenale 2 (22.22)[a]	100.0±0.12[c]
			(iii) Strongyloides stercoralis 2 (22.22)[a]	150.2±0.15[c]
Mandrill (*Mandrillus sphinx*)	17	12 (70.59)[c]	(i) Ascaris lumbricoides 6 (50)[d]	120.2±0.17[c]
			(ii) Ancylostoma duodenale 6 (50)[d]	125.0±0.13[c]
Mona monkey (*Cercopethicus mona*)	20	16 (80)[c]	(i) Ascaris lumbricoides 10 (62.5)[d]	165.0±0.16[c]
			(ii) Ancylostoma duodenale 3 (18.75)[a]	140.0±0.14[c]
			(iii) Trichuris trichiura 3 (18.75)[a]	45.0±0.15[c]
Spot-nosed guenon (*Cercopethicus nictitans*)	7	5 (71.43)[c]	(i) Ascaris lumbricoides 5 (100)[e]	56.0±0.85[c]
			(ii) Ancylostoma duodenale 5 (100)[e]	120.0±0.75[c]
White-collared mangabey (*Cercocebus torquatus*)	9	7 (77.78)[c]	(i) Ascaris lumbricoides 7 (100)[e]	175.0±0.58[c]
Sclater's white nosed monkey (*Cercopethicus erythrotis sclateri*)	7	3 (42.86)[b]	(i) Ascaris lumbricoides 3 (100)[e]	125.6±0.76[c]

Values with different superscripted in columns differed significantly ($p<0.05$)

Table 2: Comparative prevalence of gastro intestinal parasitic infections between captive and free-roaming primates examined at the Afi Mountain Primate Conservation Area Calabar, Nigeria

	Captive		Free-roaming	
Species of primates	No. of examined	No. of infected (%)	No. of examined	No. of infected (%)
Chimpanzees (*Pan troglodytes*)	16	16(100) [a]	12	7(58.33) [b]
Drills (*Mandrillus leucophaeus*)	3	3(100) [a]	17	6(35.29) [b]
Mandrills (*Mandrillus sphinx*)	7	7(100) [a]	10	5(50.0) [b]
Mona monkeys (*Cercopethicus mona*)	15	15(100) [a]	5	1(20.0) [b]
Spot-nosed guenons (*Cercopethicus nictitans*)	3	3(100) [a]	4	2(50.0) [b]
White-collared mangabey (*Cercocebus torquatus*)	3	3(100) [a]	6	4(66.67) [b]
Sclater's white-nosed monkey (*Cercopethicus erythrotis sclateri*)	2	2(100) [a]	5	1(20.0) [b]
Total	49	49(100) [a]	59	26(44.07) [b]

Values with different superscripted in rows differed significantly ($p<0.05$)

Table 3: Prevalence of gastrointestinal parasitic infections of primates examined at the Afi Mountain Primate Conservation Area in Calabar, Nigeria according to sex

	Sex			
	Male		Female	
Species of primates	No. of examined	No. of infected (%)	No. of examined	No. of infected (%)
Chimpanzees (*Pan troglodytes*)	18	13(72.22) [a]	10	10(100) [b]
Drill (*Mandrillus leucophaeus*)	9	1(11.11) [a]	11	8(72.73) [b]
Mandrill (*Mandrillus sphinx*)	9	5(55.56) [a]	8	7(87.5) [b]
Mona monkeys (*Cercopethicus mona*)	13	9(69.23) [a]	7	7(100) [b]
Spot-nosed guenons (*Cercopethicus nictitans*)	2	1(50.0) [a]	5	4(80.0) [b]
White-collared mangabey (*Cercocebus torquatus*)	4	2(50.0) [a]	5	5(100) [b]
Sclater's white nosed monkey (*Cercopethicus erythrotis sclateri*)	4	1(25.0) [a]	3	2(66.67) [b]
Total	59	32(54.24)[a]	49	43(87.76)[b]

Values with different superscripted in rows differed significantly ($p<0.05$)

counts were significantly high ($p<0.05$) and ranged between 300.0±0.25 to 500.0±0.16 among the chimpanzees (*Pan troglodytes*) as compared to the other primate groups.

The comparative prevalence of infection between the captive and free-roaming primates is presented in Table 2. All captive primates irrespective of species had significantly ($p<0.05$) higher prevalence of infection as compared to those roaming freely in the sanctuary. The prevalence of infection between sex and age among the primates are presented in Table 3 and 4. The females irrespective of species had higher prevalence ($p<0.05$)

Table 4: Prevalence of gastro intestinal parasitic infections of primates examined at the Afi Mountain Primate Conservation Area Calabar, Nigeria according to age

	Age			
	Young (<12 months)		Adults (> 12 months)	
Species of primates	No. of examined	No. of infected (%)	No. of examined	No. of infected (%)
Chimpanzees (*Pan troglodytes*)	8	8(100) [a]	20	15(75.0) [b]
Drill (*Mandrillus leucophaeus*)	7	3(42.86) [a]	13	4(30.77) [b]
Mandrill (*Mandrillus sphinx*)	6	5(83.33) [a]	11	7(63.64) [b]
Mona monkeys (*Cercopethicus mona*)	8	7(87.50) [a]	12	9(75.0) [b]
Spot-nosed guenons (*Cercopethicus nictitans*)	2	2(100) [a]	5	3(60.0) [b]
White-collared mangabey (*Cercocebus torquatus*)	2	2(100) [a]	7	5(71.43) [b]
Sclater's white nosed monkey (*Cercopethicus erythrotis sclateri*)	2	2(100) [a]	5	1(20.0) [b]
Total	35	29(82.86)[a]	73	44(60.27)[b]

Values with different superscripted in rows differed significantly ($p<0.05$)

of infection than the males, similarly, the young primates (<12 months) of age had significantly ($p<0.05$) higher prevalence of infection than the adults (>12 months) of age.

DISCUSSION

The results of the study revealed that primates at the Afi Mountain Primate Conservation area, located in the rain forest region of eastern Nigeria, harboured a variety of potentially pathogenic gastrointestinal parasites of zoonotic importance (Soulsby, 1982; Lynne and David, 2005). Some of the parasites encountered in this study have been reported among primates elsewhere in the world (Looms and Wright, 1986; Brock-Utne *et al*., 1988). They have also been reported in a reservoir status among captive primates in the arid zone of northeastern, Nigeria (Nwosu, 1995; Mbaya *et al*., 2006b) or in the southwestern, Nigeria (Okon and Dipeolou, 1975; Bamidele and Ogunrinade, 1980) and in few fatal cases (Emikpe *et al*., 2002). However, *Blastocystis hominis, Enterobius vermicularis, Schistosoma mansoni* and *Strongloides stercoralis* encountered during this study are being reported for the first time among chimpanzees (*Pan troglodytes*) in Nigeria. *Ascaris lumbricoides* and *Ancylostoma duodenale* were however, the most common parasites encountered among the various primate species. Some of the parasites such as *Balantidium coli, Entamoeba histolytica and Trichuris trichiura* have been associated with severe outbreaks in a colony of captive chimpanzees (*Pan troglodytes*) in Sanda Kyarimi Park in the arid zone of northeastern Nigeria (Mbaya and Nwosu, 2006; Mbaya *et al*., 2006a, b). Similarly, *Balantidium coli* were reported concurrently with *Ancylostoma* species, *Enterobius* species and *Strongyloides* in a red pattas monkey (*Erythrocebus pattas*) in the University of Ibadan Zoological Garden in Western, Nigeria (Adedokun *et al*., 2002). The outbreak reported among the captive chimpanzees (*Pan troglodytes*) in Sanda Kyarimi Park, Maiduguri, Nigeria was however, traced to a human attendant who was found to be shedding trophozoites and oocyst of *Entamoeba histolytica, Balantidium coli* and ova of *Trichuris trichiura* in his faeces (Mbaya and Nwosu, 2006; Mbaya *et al*., 2006a). In this present study, however, human faecal samples could not be obtained from game wardens or attendants due to cultural reasons. It is therefore, clear from the findings of this study that the parasites encountered in this primate preserve are zoonotic and may be potential sources of human infections or of future outbreak among the primate colonies in the sanctuary. The public health importance of *Trichuris* species was extensively reviewed (Okon and Dipeolu, 1975; Chang and McClure, 1975). However, that the primates living in absolute captivity had higher prevalence of infection than their counterparts on free-roam may be a reflection of captivity on the host-parasite relationship (Soulsby, 1994). Many gastrointestinal parasites of primates occur sporadically and appear to exist in balance with the host. When this equilibrium is disrupted by diverse stress factors as obtained in captivity, clinical disease and mortality ensue. Previous studies have shown that animals ordinarily presumed to have specific resistance to parasites while on free-roam in the wild usually harbour higher prevalence and burdens of the same parasite when subjected to captivity (Nwosu, 1995; Mbaya *et al*., 2006b). Confinement predisposes to the build up of high concentrations of infective stages of gastrointestinal parasites within primate housing in contrast, large expanse of land available for roaming in conservation area often ensues a wider dispersal of infective parasite stages and hence a reduction of the primates roaming freely to infection (Nwosu, 1995). This may therefore, account for the lower prevalence among the free roaming primates than among their captive counterparts. Captive form of conservation (*ex-situ*) in wild animals is generally, accompanied with severe stress with resultant increased corticosteroid out put, which in turn compromises their innate resistance through immunosuppression (Mbaya, 2007). This may therefore, further explain why *Cryptosporidium* oocyst was encountered among the captive primates. Cryptosporidiosis has been reported to occur in immunosupressed or immuno deficient individuals (Lynne and David, 2005). This agrees with the occurrence of cryptosporidiosis among captive wild animals and birds in the arid region of northeastern Nigeria (Ibrahim *et al*., 2007). Similarly, it will be expected that those primates in free-roam, may have lower prevalence of infection because recent evidences have suggested that wild primates in their natural habitat, often self medicate themselves with medicinal plants in their environment (Clayton and Wolf, 1993; Robles *et al*., 1995). The effect of age on the prevalence of infection among the primates in the Afi Mountain Primate Conservation area, however, showed that the young primates (<12 months) had higher ($p<0.05$) prevalence of infection as compared to the adults (>12 months) of age in the conservation area. This could be due to age susceptibility and lack of premunity in the young (Soulsby, 1982). Comparison of the prevalence of gastrointestinal infection among different sex groups, also showed a significant statistical variation ($p<0.05$) with a higher prevalence of infection among the females as compared to the males. This might be associated with the fact that primates generally live in social groups, where the bond of social interaction and 'social grooming' exist more commonly between the young and females thereby facilitating cross transmission of infection between them as compared to the males which usually assumes a more solitary defensive role in the colony. In conclusion, therefore, this study is important in being the first to be undertaken among primates in the Afi Mountain Primate Conservation Area in Calabar, Nigeria. The results also showed that the primates harboured various gastrointestinal parasitic infections of zoonotic importance. However, *Blastocystis hominis, Enterobius vermicularis, Schistosoma mansoni* and *Strongloides stercoralis* encountered during this study are being reported for the first time among chimpanzees (*Pan troglodytes*) in Nigeria. This may therefore, provide valuable clues to the origins and evolutions of some important zoonosis in the area. Similarly, captive primates, females and the young were significantly more infected than their counterparts were. These may provide useful information towards more effective primate conservation goals. It was therefore, advised that strategic and sustained broad-spectrum anthelminthic therapy be routinely carried out among the captive primates before re-introduction in to the wild.

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