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COMMUNICATION

PREVALENCE AND MORPHOTYPE DIVERSITY OF *TRICHURIS* SPECIES AND OTHER SOIL-TRANSMITTED HELMINTHS IN CAPTIVE NON-HUMAN PRIMATES IN NORTHERN NIGERIA

Joshua Kamani, James P. Yidawi, Aliyu Sada, Emmanuel G. Msheliza & Usman A. Turaki

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Prevalence and morphotype diversity of *Trichuris* species and other soil-transmitted helminths in captive non-human primates in northern Nigeria

Joshua Kamani¹, James P. Yidawi², Aliyu Sada³, Emmanuel G. Msheliza⁴ & Usman A. Turaki⁵

^{1,4}Parasitology Division, National Veterinary Research Institute, PMB 01 Vom, Plateau State, Nigeria.

^{2,3}Mohamet Lawan College of Agriculture, PMB 1427 Maiduguri, Borno State, Nigeria.

⁵Department of Animal Science Federal University Kashere, Gombe State, Nigeria.

¹mshelizakj@gmail.com (corresponding author), ²jamesyidawi198@gmail.com, ³aliyuvet@yahoo.co.uk, ⁴egmshel@yahoo.com, ⁵usmanturakialiyu@gmail.com

Abstract: A study to determine the prevalence and morphotype diversity of soil-transmitted helminths in captive non-human primates (NHPs) in northern Nigeria was conducted. Simple flotation and sedimentation methods were used to examine fecal samples. A Morphometric analysis was done on *Trichuris* spp. eggs to determine the diversity of whipworm circulating in NHPs in the study area. High prevalence (60%) of infection was recorded in captive NHPs; Patas Monkey (n=17), Tantalus Monkey (n=9), Mona Monkey (n=7), Vervet Monkey (n=2), Mangabey Monkey (n=1), Baboon (n=14), and Chimpanzee (n=8) from parks and zoological gardens located in four Nigerian states (Borno, Gombe, Kano, and Plateau) and the Federal Capital Territory (FCT), Abuja. Captive NHPs examined were infected with helminths either as single, double or triple infections. Four zoonotic soil transmitted helminth (STH) genera, *Trichuris*, *Strongyloides*, *Ancylostoma*, and *Enterobius* were detected in the examined animals. Eggs of *Trichuris* spp. were the most prevalent with four morphotypes suggesting several morphotypes of whipworm were circulating among the NHPs in this region. Further studies are required to elucidate the epidemiologic and public health implications of these findings.

Keywords: Helminths, morphotype, non-human primates, northern Nigeria, zoonosis.

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Author details: J. KAMANI—investigation of parasitic and vector-borne zoonoses. Parasitology Division, National Veterinary Research Institute, Vom Nigeria. J.P. YIDAWI—diagnosis and research of parasitic diseases of Aves. Mohamet Lawan College of Agriculture, Maiduguri, Borno state, Nigeria. A. SADA—epidemiology of parasitic livestock and wildlife diseases. National Veterinary Research Institute, Vom Nigeria. EMMANUEL G. MSHELIZA—investigation of ecto and endo parasite of animals. National Veterinary Research Institute, Vom Nigeria. USMAN A. TURAKI—research and teaching of parasitic diseases of animals. Federal University of Kashere Gombe state. NiNigeria h

Author contribution: JK—conceive and design study, Investigation and data analyses, writing of the manuscript. JPY—sample collection, data analyses, writing of manuscript. AS—sample collection, data analysis. EGM—Sample collection, investigation, writing of manuscript. UAT—sample collection, data analyses, writing of manuscript.

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INTRODUCTION

Non-human primates (NHPs) enclosures in zoological gardens or parks in Nigeria are among the most popular attractions to visitors, especially children; however, the maintenance of wild animals in captivity is fraught with numerous challenges particularly that of parasitic disease conditions due to high contamination of the environment (Rao & Acharjyo 1984; Vanitha et al. 2011). This is exacerbated by lowered immunity of the animals due to the stress of captivity, consequently diminishing their resistance to parasitic diseases (Gracenea et al. 2002; Perez Cordon et al. 2008). Therefore, gastrointestinal parasite infections are among the most common diseases found in non-human primates (Bezjian et al. 2008; Strait et al. 2012). Although the captive animals do not show alarming signs of parasitism, it has been reported that some of the helminth parasites they harbor have zoonotic potential and are, therefore, considered to be a threat to public health (Gillespie et al. 2008; Klaus et al. 2017). Soil-transmitted helminths (STHs) such as *Ascaris* spp., *Trichuris* spp., *Ancylostoma* spp., and *Strongyloides* spp. that can easily be transferred from NHPs to humans and vice versa through contaminated environments are a major concern (Ranglack & Yeager 1986; Bethony et al. 2006; Lynn 2010). Single or mixed infections of zoonotic STHs have often been recorded in NHPs from different countries: Bangladesh (Raja et al. 2014), Central African Republic (Hasegawa et al. 2014), Tanzania (Petrželkova et al. 2010), China (Li et al. 2017), India (Hussain et al. 2013), Sri Lanka (Aviruppola et al. 2016), Malaysia (Klaus et al. 2017), and Spain (Perez Cordon et al. 2008). Several studies have been carried out on the prevalence of helminth infection in NHPs in various zoological gardens and/or parks in the southern part of Nigeria: Oyo State (Adedokun et al. 2002; Emikpe et al. 2002; Adetunji 2014), Ondo State (Egbetade et al. 2014), Cross River State (Mbaya & Udendeye 2011), and Imo state (Opara et al. 2010). Comparatively, only a few studies have been conducted on the helminth infections of NHPs in northern Nigeria (Nwosu 1995; Mbaya & Nwosu 2006; Mbaya et al. 2006a,b; Dawet et al. 2013). The aim of this study, therefore, was to determine the prevalence and diversity of helminths in captive NHPs in northern Nigeria and to discuss the public health implications.

MATERIALS AND METHODS

Sampling sites

The study was conducted in Zoological Gardens and Parks in four northern states and Abuja (9.076°N, 7.398°E) the Federal Capital Territory (FCT) of Nigeria. The four states; Borno (11.831°N, 13.151°E), Gombe (10.279°N, 11.173°E), Kano (12.002°N, 8.592°E) and Plateau (9.896°N, 8.858°E) were selected based on convenience and accessibility of NHPs for sample collection.

Sample collection

Fecal samples were collected opportunistically from individual captive NHP with the help of the caretakers over a period of six months (November 2017 to April 2018). Fresh feces were collected from the ground under the nest of individual NHPs. Approximately 5g of feces was scooped from the surface of each fecal mass using a disposable hand glove and transferred into a screw capped bottle. Each sample was labeled appropriately and transported in a cold box to the laboratory for analysis.

Fecal analysis

Samples were processed and analyzed in the Helminthology Research Laboratory, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. First, each sample was examined macroscopically for the presence of helminths or taenid segments.

Microscopic analysis

Simple tube flotation and sedimentation: Fecal samples were individually processed by simple tube flotation in saturated sodium chloride solution (SG 1.20) and simple sedimentation techniques (Greiner & McIntosh 2009). The preparation was then examined using direct light microscope (100X and 400X magnifications) for the presence of parasite eggs. The identification of the parasites was based on egg morphology, shape, size and color according to standard keys (Samuel et al. 2001; Hasegawa et al. 2009).

Morphometry analysis

Helminth eggs were measured (length and width) by using a calibrated light microscope. Mean values of measurements were given in micrometers (μm) ± standard deviations (SD).

RESULTS

Fifty-eight captive NHPs in five zoological gardens located in Jos (n=15), Kano (n=9), Maiduguri (n=3), Gombe (n=9) & FCT Abuja (n=4) and two Parks located in Jos (n=8) & FCT Abuja (n=10) were examined. Seven NHP species including 17 Patas Monkey *Erythrocebus patas*, 14 Baboons *Papio* sp., nine Tantalus Monkey *Chlorocebus tantalus*, eight Chimpanzees *Pan troglodytes*, seven Mona Monkeys *Cercopithecus mona*, two Vervet Monkeys *Chlorocebus pygerythrus*, and one Mangabey Monkey *Cercocebus* sp. were sampled during this study (Table 1).

Prevalence of helminths in NHPs from northern Nigeria

Helminth eggs were detected in fecal samples of NHPs from zoos or parks in all the states studied. Overall, 60% of the animals studied had helminth eggs in their feces. The highest prevalence (100%, n=4/4) was recorded in samples from the Abuja Children Park

and Zoo, followed by (89%, n=8/9) in samples from the Gombe Zoo and the lowest prevalence (33%, n=1/3) was recorded in samples from the Maiduguri Zoological Garden (Table 1). Prevalence according to NHP species was highest (78%) for Tantalus Monkeys *Chlorocebus tantalus*. The lowest prevalence (47%, n=8/17) was observed in Patas Monkeys while the fecal sample of the only Mangabey Monkey *Cercocebus* sp. screened in this study was negative for helminth eggs (Table 1).

Diversity of helminths

Four helminth genera: *Trichuris*, *Ancylostoma*, *Strongyloides*, and *Enterobius* and one unidentified parasite egg were detected from the feces of NHPs from northern Nigeria. *Trichuris* spp. eggs were detected with a high prevalence (31/44) across all the NHP species screened in the study. This was followed by *Strongyloides* spp. (4/44) and *Ancylostoma* spp. (3/44) both affecting three NHP species each. An unidentified egg that resembled an egg of *Opisthorchis* sp. was detected in

Table 1. Prevalence of helminths in captive non-human primates in zoological gardens and parks in northern Nigeria.

Study location	Habitat	Number of animal positive/ no. screened according to NHP species							Total (%)
		Patas Monkey <i>Erythrocebus patas</i>	Tantalus Monkey <i>Chlorocebus tantalus</i>	Mona Monkey <i>Cercopithecus mona</i>	Vervet Monkey <i>Chlorocebus pygerythrus</i>	Mangabey Monkey <i>Cercocebus</i> sp.	Baboon <i>Papio</i> sp.	Chimpanzee <i>Pan troglodytes</i>	
Jos	Zoo	0/4	0	2/3	1/2	0	3/3	0/3	6/15 (40)
Jos	Wildlife Park	2/3	1/1	1/1	0	0	2/2	0/1	6/8 (75)
Gombe	Zoo	5/5	0	0	0	0	1/2	2/2	8/9 (89)
Maiduguri	Zoo	0/1	0/1	0	0	0	1/1	0	1/3 (33)
Abuja	Park/ Zoo	1/1	1/1	1/1	0	0	1/1	0	4/4 (100)
Abuja	National Park	0/1	4/5	0/1	0	0	1/3	0	5/10 (50)
Kano	Zoo	0/2	1/1	1/1	0	0/1	1/2	2/2	5/9 (56)
Total		8/17	7/9	5/7	1/2	0/1	10/14	4/8	35/58 (60)

Table 2. Prevalence of soil transmitted helminths in different species of non-human primates in northern Nigeria.

Host species	No of NHP tested	No positive (%)	Single infection	Dual infection	Triple infection
Patas Monkey <i>Erythrocebus patas</i>	17	8 (47)	7	1	0
Tantalus Monkey <i>Chlorocebus tantalus</i>	9	7 (78)	5	2	0
Mona Monkeys <i>Cercopithecus mona</i>	7	5 (71)	2	2	1
Vervet Monkey <i>Chlorocebus pygerythrus</i>	2	1 (50)	1	0	0
Baboon <i>Papio</i> sp.	14	10 (71)	1	3	0
Chimpanzee <i>Pan troglodytes</i>	8	4 (50)	10	0	0
Mangabey Monkey <i>Cercocebus</i> sp.	1	0 (0)	0	0	0
Total	58	35	26	8	1

Table 3. Morphologic and morphometric analyses of helminth eggs detected in non-human primates in northern Nigeria

Helminth genera	Morphology and morphometric characteristics of helminth eggs				
	Morphotype	Egg size (μm + SD)		Egg shape	Egg shell appearance and color
		Length	Width		
<i>Trichuris</i> sp.	T1	43.1 \pm 5	25.1 \pm 1	Ellipsoidal	Thick with prominent bipolar plugs, dark brown
	T2	50.8 \pm 2.5	30.5 \pm 1.5	Rounded lemon	Thick less prominent bipolar plugs, light brown
	T3	54.7 \pm 0.1	22.9 \pm 5.7	Barrel-like	Thick prominent bipolar plugs, dark brown
	T4	58.2 \pm 1.0	22.4 \pm 3.2	Lemon	Thick, flat bipolar transparent plugs, dark brown
<i>Ancylostoma</i> sp.	A	73.5 \pm 5.3	31.5 \pm 2.3	Ellipsoidal	Thin transparent light color
<i>Strongyloides</i> sp.	S	48.3 \pm 8.8	34.3 \pm 4.7	Ellipsoidal	Thin transparent folded motile larva light color
<i>Enterobius</i> sp.	E	54.5 \pm 5.3	30.5 \pm 2.3	Irregular	Thin with folded larva light color

the fecal sediments of a female baboon from Abuja. Parasite order richness (POR) ranged from 0 to 3 per fecal sample. Most of the positive samples harboured eggs of a single parasite genus (45%), followed by dual infections (12%) and triple infections in only 2% of the samples (Table 2).

Egg morphotypes detected in NHP examined

Four morphotypes of *Trichuris* spp. eggs (T1–T4) were detected in this study. All the trichurid egg morphotypes were thick-shelled, with prominent bipolar plugs but varied in shape, size, and colour. Egg morphotypes T1 and T2 appear to be more common in the various species of monkeys examined and occur as mixed infections, while T3 and T4 were commonly associated with baboons and chimpanzees. The eggs of other helminth genera, *Ancylostoma*, *Strongyloides*, and *Enterobius* detected in this study occurred as single morphotypes (Table 3).

DISCUSSION

Captive NHPs in Nigerian zoological gardens and parks attract attention due to their agility and playfulness. Thus, their wellbeing and survival is paramount to conservationists, veterinarians and zoo administrators. Parasitic diseases, particularly helminth infections have been reported to constitute a challenge to the health of NHPs (Samuel et al. 2001; Vanitha et al. 2011; Wren et al. 2015). The results from this survey showed that more than half (60%) of the examined NHPs in northern Nigeria were infected with helminth parasites. Four helminth genera of zoonotic importance, viz., *Trichuris*, *Ancylostoma*, *Strongyloides*, and *Enterobius* were detected in the NHPs examined in this study. These helminths have a high potential for transmission to humans because of their simple life cycles (Li et al. 2017). Thus, they are listed among the major cause of

soil-transmitted helminth infections globally (Bethony et al. 2006). The differences in prevalence of these helminths observed in the study locations could be attributed to differences in host species susceptibility to helminth infections and the variations in climatic conditions between the study sites. Such observation on the effects of climate on parasite prevalence have been reported (Cordon et al. 2008; Wren et al. 2015).

Trichuris eggs were the most prevalent (70.5%) in the infected NHPs in this study. This finding is in accord with an earlier survey of NHPs in northern (Dawet et al. 2013) and southern (Mbaya & Udendeye, 2011; Adetunji 2014) parts of Nigeria. High prevalence of *Trichuris* sp. was also observed in NHPs in Côte d'Ivoire (Kouassi et al. 2015), Sri Lanka (Aviruppola et al. 2016), Peru (Kimberley et al. 2004), Malaysia (Lim et al. 2008; Klaus et al. 2017), China (Li et al. 2017), India (Singh et al. 2009), and Spain (Perez Cordon et al. 2008) signifying its global distribution among NHP population. This study provides additional information on the metric details of the trichurid eggs present in the NHPs in northern Nigeria suggesting the diversity of this parasite in the region. It appears that NHPs in northern Nigeria are infected with various *Trichuris* spp. based on the morphology and dimensions of the eggs detected during this study. A similar observation was earlier made in a study in south west Nigeria but the authors did not provide any metric details of the *Trichuris* eggs detected (Egbetade et al. 2014). Therefore, our study is the first to provide morphometric analyses of *Trichuris* eggs infecting NHPs in Nigeria. Four morphotypes (T1–T4) of *Trichuris* eggs, with mean size ranging from 43–53 x 22–30 μm (length x width) were detected in this study suggesting a diversity of this parasite in the NHPs examined. Indeed, even among the NHP species there is variability in the morphology of *Trichuris* eggs detected. Similar observations have been reported (Petrzelkova et al. 2010; Klaus et al. 2017). Thus, our findings agree with several reports on morphometric and molecular



studies of trichurid eggs in NHPs conducted in areas with different climatic conditions from Nigeria (Hasegawa et al. 1983; Dupain et al. 2009; Ghai et al. 2014; Raja et al. 2014; Cavallero et al. 2015; Klaus et al. 2017; Li et al. 2017).

It is noteworthy that the dimensions of some of the eggs of *Trichuris* spp. detected in this study are similar to those of human *T. trichiura*, suggesting zoonotic or reverse zoonotic transmissions. This finding has implications for veterinarians, public health workers, and wildlife managers, in terms of the epidemiology of the disease and the choice of treatment and control measures to be adopted (Melfi & Poyser 2007). Therefore, the assumption hitherto, among wildlife parasitologists that all the *Trichuris* infecting NHPs are the same as *T. trichiura* of humans should be reconsidered, however, the other three zoonotic helminths genera; *Ancylostoma*, *Strongyloides* and *Enterobius* detected in this study occurred as monotypes each with dimensions similar to those of the species infecting human, suggesting possible circulation of these worms between humans and NHPs in the study area.

Taken together, our results demonstrate the presence of zoonotic helminths and a diversity of *Trichuris* sp. infection amongst NHPs. Therefore, a comprehensive study to elucidate the genetic diversity of Trichurids infection NHPs in Nigeria is desirable. This will assist to distinguish the species and genotypes of this parasite in NHPs in northern Nigeria and to determine their pathogenicity. Therefore, molecular studies on pinworm diversity in Nigerian NHPs are needed in order to elucidate the species and morphotypes circulating in the country.

CONCLUSION

Captive NHPs in zoological gardens and parks in northern Nigeria are infected with helminths of public health significance. High prevalence of *Trichuris* spp. coupled with a diversity of their egg morphotypes were observed in the NHPs examined. Further investigation using modern tools like molecular phylogenetics in order to fully understand their epidemiology and zoonotic potentials is warranted.

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