COMMUNICATION

PREVALENCE AND MORPHOTYPE DIVERSITY OF Trichuris species AND OTHER SOIL-TRANSMITTED HELMINTHS IN CAPTIVE NON-HUMAN PRIMATES IN NORTHERN NIGERIA

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

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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.
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 helmith 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 Corden et al. 2008). Several studies have been carried out on the prevalence of helmith 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 helmith 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 helmiths 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 *Cerceboes* sp. were sampled during this study (Table 1).

Prevalence of helminths in NHPs from northern Nigeria

Helmint 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 *Cerceboes* 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 | Patas Monkey *Erythrocebus patas* | Tantalus Monkey *Chlorocebus tantalus* | Mona Monkey *Cercopithecus mona* | Vervet Monkey *Chlorocebus pygerythrus* | Mangabey Monkey *Cerceboes* sp. | Baboon *Papio* sp. | Chimpanzee *Pan troglodytes* | Total (%) |
|----------------|---------|----------------------------------|---------------------------------------|-----------------------------------|---------------------------------------|---------------------------------|---------------------|---------------------------|----------|
| Jos Zoo        | 15      | 0/4                              | 0/2                                  | 1/2                               | 0/3                                  | 0/1                             | 1/4                  | 6/15 (40)                |
| Jos Wildlife Park | 2/3   | 1/1                              | 1/1                                  | 0/0                               | 2/2                                  | 0/1                             | 2/2                  | 6/8 (75)                 |
| Gombe Zoo      | 9       | 5/5                              | 0/0                                  | 0/0                               | 1/2                                  | 0/1                             | 1/2                  | 8/9 (89)                 |
| Maiduguri Zoo  | 0/3     | 0/1                              | 0/0                                  | 0/0                               | 1/1                                  | 0/1                             | 1/1                  | 1/3 (33)                 |
| Abuja Park/Zoo | 1/1    | 1/1                              | 1/1                                  | 0/0                               | 0/0                                  | 0/0                             | 0/0                  | 4/4 (100)                |
| Abuja National Park | 0/1 | 4/5                              | 0/1                                  | 0/0                               | 1/3                                  | 0/0                             | 0/0                  | 5/10 (50)               |
| Kano Zoo       | 0/2     | 1/1                              | 1/1                                  | 0/1                               | 1/2                                  | 2/2                             | 4/8                  | 35/58 (60)               |
| 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 *Erythrocebus patas* | 17               | 8 (47)          | 7                | 1             | 0               |
| Tantalus Monkey *Chlorocebus tantalus* | 9               | 7 (78)          | 5                | 2             | 0               |
| Mona Monkeys *Cercopithecus mona* | 7               | 5 (71)          | 2                | 2             | 1               |
| Vervet Monkey *Chlorocebus pygerythrus* | 2               | 1 (50)          | 1                | 0             | 0               |
| Baboon *Papio* sp.             | 14               | 10 (71)         | 1                | 3             | 0               |
| Chimpanzee *Pan troglodytes*   | 8                | 4 (50)          | 10               | 0             | 0               |
| Mangabey Monkey *Cerceboes* sp. | 1                | 0 (0)           | 0                | 0             | 0               |
| Total                         | 58               | 35             | 26               | 8             | 1               |
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

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

| Helminth genera | Morphotype | Egg size (µm ± SD) | Egg shape | Egg shell appearance and color |
|-----------------|------------|--------------------|-----------|-------------------------------|
|                 |            | Length             | Width     |                               |
| *Trichuris* sp. | T1         | 43.1 ± 5           | 25.1 ± 1  | Ellipsoidal                    | Thick with prominent bipolar plugs, dark brown |
|                 | T2         | 50.8 ± 2.5         | 30.5 ± 1.5| Rounded lemon                  | Thick less prominent bipolar plugs, light brown |
|                 | T3         | 54.7 ± 0.154.2    | 22.9 ± 5.7| Barrel-like                    | Thick prominent bipolar plugs, dark brown |
|                 | T4         | ± 1.0              | 22.4 ± 3.2| Lemon                         | Thick, flat bipolar transparent plugs, dark brown |
| *Ancylostoma* sp| A          | 73.5 ± 5.3         | 31.5 ± 2.3| Ellipsoidal                    | Thin transparent light color |
| *Strongyloides* sp | S     | 48.3 ± 8.8        | 34.3 ± 4.7| Ellipsoidal                    | Thin transparent folded motile larva light color |
| *Enterobius* sp | E          | 54.5 ± 5.3         | 30.5 ± 2.3| Irregular                      | Thin with folded larva light color |
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 *Trichurus* 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 *Trichurus* sp. infection amongst NHPs. Therefore, a comprehensive study to elucidate the genetic diversity of Trichuris 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 disease 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 *Trichurus* 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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Editorial

Pakshirajan Lakshminarasimhan: a plant taxonomist who loved plants and people alike
— Mandar N. Datar, Pp. 16195–16203

Communications

The worrisome conservation status of ecosystems within the distribution range of the Spectacled Bear Tremarctos ornatus (Mammalia: Carnivora: Ursidae) in Ecuador
— José Guerrero-Casado & Ramón H. Zambrano, Pp. 16204–16209

Living with Leopard Panthera pardus fusca (Mammalia: Carnivora: Felidae): livestock depredation and community perception in Kalakad-Mundanthurai Tiger Reserve, southern Western Ghats
— Bawa Mohitil Krishnakumar, Rajarathinavelu Nagarajan & Kanagaraj Muthamish Selvan, Pp. 16210–16218

An updated checklist of mammals of Odisha, India
— Subrat Debata & Himanshu Shekhar Palei, Pp. 16219–16229

Negative human-wildlife interactions in traditional agroforestry systems in Assam, India
— Yashmita-Ulman, Manoj Singh, Awadhesh Kumar & Madhubala Sharma, Pp. 16230–16238

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, Pp. 16239–16244

Detection of hemoparasites in bats, Bangladesh
— Shahnur Islam, Rakib Uddin Ahmed, Md. Kaisar Rahman, Jinnat Ferdous, Md. Helal Uddin, Sazed Akter, Abdullah Al Faruq, Mohammad Mahmudul Hasson, Afarful Islam & Ariful Islam, Pp. 16245–16250

Ecology of the Critically Endangered Singidia Tilapia (Teleostei: Cichlidae: Oligoplites) of Lake Kayanja, Uganda and its conservation implications
— Richard Olve, Herbert Nakiyende, Elias Muhumza, Samuel Bassa, Anthony Taabu, Munyalo & Winnie Nkalubo, Pp. 16251–16256

Length-weight relationships of two conservation-concern mahseers (Teleostei: Cyprinidae: Tor) of the river Cauvery, Karnataka, India
— Adrian C. Pinder, Rajeev Raghavan, Shannon D. Bower & J. Robert Britton, Pp. 16257–16261

The identity and distribution of Bhavania annandalei Hora, 1920 (Cypriniformes: Balitoridae), a hillstream loach endemic to the Western Ghats of India
— R.S. Ganesh, M. Rameshwaran, Naveen A. Joseph, Ahamed M. Jerith & Sushil K. Dutta, Pp. 16262–16271

Records of two toads Duttaphrynus scaber and D. stomaticus (Amphibia: Anura: Bufonidae) from southeastern India
— S.R. Ganesh, M. Rameshwaran, Naveen A. Joseph, Ahamed M. Jerith & Sushil K. Dutta, Pp. 16272–16278

Some rare damselflies and dragonflies (Odonata: Zygoptera and Anisoptera) in India: a preliminary study from Kerala, India
— Kuttanelloor Roshni, Chelapurath Radhakrishnan Renjithkumar, Rajeev Raghavan, Neelesh Dahanukar & Kutty Ranjeet, Pp. 16380–16384

First report of the assassin bug Epidius wangi (Heteroptera: Reduviidae: Harpactorinae) from India
— Swapnil S. Boyane & Hemant V. Ghathe, Pp. 16385–16391

Observations of the damselfly Platyplestes cf. platystylus Rambur, 1842 (Insecta: Odonata: Zygoptera: Lestidae) from peninsular India
— K.J. Rison & A. Vivek Chandran, Pp. 16392–16395

Floristic diversity of Anjaneri Hills, Maharashtra, India
— Sanjay Gajanan Auti, Sharad Suresh Kamble, Kumar Vinod Chhotupuri Gosavi & Arun Nivrutti Chandre, Pp. 16295–16313

A checklist of macrofungi (mushroom) diversity and distribution in the forests of Tripura, India
— Sanjit Debnath, Ramesh Chandra Upadhyay, Rahul Saha, Koushik Majumdar, Panna Das & Ajay Krishna Saha, Pp. 16314–16346

Notes

First photographic record of the Dhole Cuon alpinus (Mammalia: Carnivora: Canidae) from the Sirumalai Hills in Tamil Nadu, India
— B.M. Krishnakumar & M. Eric Ramanujam, Pp. 16373–16376

Tracing heavy metals in urban ecosystems through the study of bat guano
— Jithin Johnson & Morcey Vincent, Pp. 16377–16379

Population dynamics and management strategies for the invasive African Catfish Clarias gariepinus (Burchell, 1822) in the Western Ghats hotspot
— Kuttanelloor Roshni, Chelapurath Radhakrishnan Renjithkumar, Rajeev Raghavan, Neelesh Dahanukar & Kutty Ranjeet, Pp. 16380–16384

First observations of the damselfly Platyplestes cf. platystylus Rambur, 1842 (Insecta: Odonata: Zygoptera: Lestidae) from peninsular India
— K.J. Rison & A. Vivek Chandran, Pp. 16392–16395

Hermium longilobatum (Orchidaceae), a new record for Bhutan
— Ugyen Dechen, Tandin Wangchuk & Lam Norbu, Pp. 16396–16398

Recent record of a threatened holoparasitic plant Sopria himayalayana Griff. in Mehao Wildlife Sanctuary, Arunchal Pradesh, India
— Arif Ahmad, Amit Kumar, Gopal Singh Rawat & G.V. Gopi, Pp. 16399–16401

Eleven new records of lichens to the state of Kerala, India
— Sonia Anna Zachariah, Sanjeeva Nayaka, Siljo Joseph, Pooja Gupta & Scaria Kadookunnel Varghese, Pp. 16402–16406

Short Communications

A threat assessment of Three-striped Palm Squirrel Funambulus palmarum (Mammalia: Rodentia: Sciuridae) from roadkills in Sigur Plateau, Mudumalai Tiger Reserve, Tamil Nadu, India
— Arockianathan Samson, Balasundaram Ramakrishnan & Jabamalainathan Leonaapriy, Pp. 16347–16351

Impact of vehicular traffic on birds in Tiruchirapalli District, Tamil Nadu, India
— T. Siva & P. Neelanarayanan, Pp. 16352–16356

Ichthyofaunal diversity of Manjeera Reservoir, Manjeera Wildlife Sanctuary, Telangana, India
— Kante Krishna Prasad, Mohammad Younus & Chelmala Srinivasulu, Pp. 16357–16367

New distribution record of the endemic and critically endangered Giant Staghorn Fern Platycerium grande (Fée) Kunze (Polypodiaceae) in central Mindanao
— Cerie Cano-Mangoaang & Charissa Joy Aroyo Gumban, Pp. 16368–16372

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