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Cross-transmission of gastrointestinal helminths and protozoan parasites between habituated chimpanzees *(Pan troglodytes schweinfurthii*) with humans in Budongo Forest Reserve, Uganda

L., Mugisha.[1*], Bwangamoi-Okot.[2], M.R. Cranfield[3,4], T.K. Graczyk[5], C. Dranzoa [1], L. Gaffikin[6].

[1]Department of Wildlife and Animal Resources Management, Faculty of Veterinary Medicine, Makerere University, P.O.Box 7062, Kampala, Uganda.
[2]Department of Pathology, Faculty of Medicine, Gulu University, P.O.Box 166 Gulu Uganda.
[3]Medical Department, The Maryland Zoo In Baltimore, Druid Hill Park, Baltimore, MD 21217,
[4]Mountain Gorilla Veterinary Project (MGVP, Inc.), Druid Hill Park Baltimore MD 21217
[5]Department of Comparative Medicine, Johns Hopkins School of Medicine, Johns Hopkins University, Baltimore MD 21205.
[6]Department of Molecular Microbiology and Immunology, School of Hygiene and Public health, Johns Hopkins University, Baltimore, Maryland 21205.
*Corresponding author.E-mail: lmugisha@vetmed.mak.ac.ug,

Abstract

A survey of gastrointestinal helminths and protozoa of free ranging chimpanzees and humans living in Budongo Forest Reserve (BFR) was conducted in the years 2002/2003 by examination of feacal samples using floatation, sedimentation and immunofluorescent antiboby techniques. *Cryptosporidium*, *Giardia* and Microsporidia were found in feacal samples of known free ranging chimpanzees and humans in BFR. In addition, both chimpanzees and humans were infected with the following helmiths: *Ascaris, Enterobius*, hookworm, *Strongyloides* and *Trichuris. Taenia* tapeworm and the fluke *Echinostoma* were only found in humans. The occurrence of potential zoonotic parasites both in chimpanzees and in humans living in close proximity suggests the possibility of cross-transmission of these parasites. This presents a public health challenge given the zoonotic potential of some of these parasites and potential risks of increased mortality in the already endangered chimpanzees from cross transmission.

Key words: Chimpanzees, *Cryptosporidium*, *Giardia*, *Microsporidia*, *Echinostoma*, Budongo Forest Reserve

Introduction

Budongo Forest Reserve (BFR) situated in Western Uganda is home to approximately 650 chimpanzees (1). The reserve is also inhabited by four species of diurnal primates: black-and-white colobus monkey (*Colobus guereza occidentalis*), blue monkeys (*Cercopithecus mitis stuhlmanni*), red-tailed monkeys (*Cercopithecus ascanius schmidti)* and olive baboons (*Papio cynocephalus anubis*) and one nocturnal primate, Bosman's potto (*Perodicticus potto*).

For the purpose of conservation, research and eco-tourism, some groups of chimpanzees have been habituated to humans by park management and researchers (1, 2) However, there is evidence that close proximity increases the risk of disease transmission between great apes and humans especially from researchers, guards, poachers and field assistants, due to close genetic relationship between the two species (3,4,5,6). The habituation process coupled with encroachment of chimpanzees' habitat by humans enhances the transmission of parasites (5 ,7, 8). Common intestinal parasites of human have been reported in primates suggesting a high potential for interspecies transmission (6, 9, 10, 11)
Cryptosporidium and *Giardia* are cyst-forming protozoa capable of infecting vertebrates (12,13) Previous studies reported *Cryptosporidium, Giardia and* microsporidia with similar PCR sequences in domestic animals, gorillas and humans in Bwindi Impenetrable Forest National Park in Uganda (14,15). Giardiasis is one of the most common enteric zoonotic diseases worldwide, and its reservoir is sustained by a variety of wild and domestic animals (12). *Giardia lamblia* is common in children and young primates (16, 17). At least 10 species of Cryptosporidia affect humans but the source of infection is unknown (18) This study was undertaken to get

more information on prevalence of helminths and protozoan parasites in habituated free ranging chimpanzees and humans.

Materials and Methods

One hundred feacal samples were collected from known individual habituated chimpanzees of Soso community in BFR soon after defaecation and stored in 10% formalin. Sixty five human feacal samples were collected randomly from individuals residing in Nyakafunzo close to the BFR (n=65) and also from employees of Budongo Forest Project (BFP) (n=18) within a period of three months in 2002. Two individuals: an adult male and a child were randomly selected from each household and instructed to collect their feces using applicator sticks and to put approximately one third of a thumb-sized feacal sample into a universal bottle containing 10% formalin, seal the bottle with adhesive tape to avoid spillage and shake it to make a thorough mixture. Each sample was labelled with a number, the date of collection, the individual's age and sex.

The samples were transported (utilizing appropriate permits) to laboratories: Department of Parasitology, Faculty of Veterinary Medicine, Makerere University, Uganda and Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, John Hopkins University, Baltimore, Maryland, USA for analysis.

The identification of oocysts of *Cryptosporidium* species and *Giardia* species in the feaces was done using immunofluorescent antiboby technique (IFAT-MERIFLUOR test kit, Meridian Diagnostic, Inc., Cincinnati, Oh). Microsporidium spores were detected by light microscopy in Chromotrope-2 stained slides.

The feacal samples were subjected to flotation and sedimentation and techniques for identification of helminth eggs. Formol-ether concentration technique was used as it concentrates a wide range of parasites with minimum damage to their morphology. The samples were emulsified in formol water and the suspension strained to remove large feacal particles. Ether was added to the suspension and mixed by centrifuging at 750-1000g for 1 minute. The tube was then inverted to discard the ether, feacal debris, and formal water leaving the sediment at the bottom. The sediment was then transferred to the slide and covered with a cover slip and microscopically examined at 400 x magnification.

Saturated sodium chloride floatation technique was used to identify helminth eggs that float in solutions of high specific gravity. Feacal samples were emulsified in saturated sodium chloride solution at specific gravity (relative density of 1.200) and the suspension left undisturbed for 30-45 minutes to give time for eggs to float. The cover glass from the tube was carefully lifted by a straight pull upwards and placed face downwards on a slide. The slide was then examined microscopically using the 10X and 40X objective lenses.

Identification of helminth eggs was based on size, colour (colourless, pale yellow, brown) and morphological features as previously described (19). Statistical analysis was carried out using Statistix 4.1 (Analytical Software, St. Paul, Minnesota, USA).

Results

Oocysts of *Cryptosporidium* and *Giardia* species and spores of microsporidia were present in feacal samples of both chimpanzees and humans (Table I). The proportion of feacal samples positive for *Cryptosporidium* and *Giardia* was highest in humans from the village community (Nyakafunzo), 10.6% and 8.5% respectively.

There was significance difference ($P< 0.05$) in the proportion of feacal samples positive for *Cryptosporidium* infections between chimpanzees (2%) and overall human population under the study (7.7%). However, there was no significant difference in the rate of *Cryptosporidium* and *Giardia* infections between chimpanzees and human employees of BFP. The proportion of positive samples with microsporidia was higher (5.6%) in employees of BFP than in humans from the community (2.1%), though there was no significant difference ($P>0.05$) between chimpanzees and humans.

Both human and chimpanzees were found to harbour the eggs of nematodes namely *Strongyloides, Ascaris, Enterobius* and *Trichuris. Ascaris* was the most frequent worm of chimpanzees and human (20% and 9.2% respectively). There was a highly significant difference ($P<0.01$, $X^2=7.5$) in the proportion of samples that tested positive for *Ascaris* between chimpanzees (20%) and humans (6.4%) from the surrounding community. The proportion of positive feacal samples for *Strongyloides* in humans and chimpanzees in BFR was 8% and 3.1% respectively. *Echinostoma* eggs were only found in employees of BFP. The proportion of feacal samples positive for any helminth in chimpanzees and humans were summarized as shown in Table 1.

Table 1. Proportion of feacal samples positive for helminths and protozoa among humans and chimpanzees tested in Budongo Forest Reserve, Uganda

Type of parasite	Hosts			
	Chimpanzee	Humans		
		Overall	Community	Employees
a) Helminth				
Strongyloides species	8	3.1	2.1	5.6
Ascaris species	20	9.2	6.4	16.7
Trichuris species	5	6.2	6.4	5.6
Hookworm species	8	6.2	8.5	0
Enterobius species	3	3.1	3.8	0
Echinostoma species	0	1.5	0	5.6
Taenia species	0	6.2	6.4	5.6
b) Protozoa				
Cryptosporidium species	2	7.7	10.6	0
Giardia species	7	7.7	8.5	5.6
Microsporidia species	2	3.1	2.1	5.6

Discussion

Different protozoan parasites as *Cryptosporidium, Giardia* and microsporidia were identified in habituated free-ranging chimpanzees and humans living in close proximity in BFR in Uganda. In addition, both the chimpanzees and humans were infected with *Strongyloides*, hookworms, *Ascaris, Trichuris* and *Enterobius.* This study revealed that chimpanzees in BFR and humans in close proximity were infected with a variety of helminths and protozoa with potential of cross-transmission. This study shares similar findings with previous studies which had reported *Cryptosporidium* and *Giardia* infection in domestic animals, gorillas and humans in Bwindi Impenetrable National Park in Uganda (14,20) The occurrence of *Cryptosporidium* in habituated free-ranging chimpanzees (2%) and humans (employees) working on a regular basis in the forest (5.6%) presents a potential risk of cross-transmission of these parasites. The risk of cross-transmission is likely to be high with increased levels of human (local communities and employees) interaction with the habituated free-ranging chimpanzees. No *Cryptosporidium* oocysts were found in feaces of employees probably due to the on-going hygiene implementation measures of packing boiled drinking water, wearing of personal protective gear (gumboots and overalls) and sensitization extended to employees compared to neighbouring communities accessing the forest illegally.

Both employees and local communities were infected with microsporidia and all of them frequent the forest for various activities related to their livelihoods. In this study a questionnaire (data not presented here) revealed that more than 60% of the households do not have latrines and relieve themselves wherever convenient at farming plots and while in the forest. In addition, 65% of the employees had defecated in the forest more

than once per week. This study revealed that simple regulations regarding burying of feaces while in the forest were not being followed even by the employees. The studied community was at the edge of BFR with the furthest household being about 800m from the forest edge. This close proximity has increased the levels of chimpanzee-human interactions as a result of increasing pressure to the forested area for agriculture and settlement. The increasing forest edge accessibility by humans for crop production was further reducing the habitat for the wild animals which were in turn invading the human settlements (21). The resultant impact of this increased interaction was the increase in potential risk of disease transmission.

No attempt was made to characterize the genotype of microsporidian spores and *Cryptosporidium* oocysts found in this study due to limitations of locally available technologies as it is the established method for determining the zoonotic potential of the organism (20). However, our results shade more information on the existence of the potential zoonotic pathogens: *Cryptosporidium, Giardia* and microsporidia species in chimpanzees and humans with a possibility of cross-transmission given the prevailing close proximity and or unhygienic conditions.

Ascaris was the most frequent worm genus found in 20% of all samples from chimpanzees. It was also the most frequent worm identified from employees of BFP (16.7%). *Ascaris* has been previously identified from other apes such as the mountain gorillas in the Virungas Parc des Volcanos and lowland gorillas (22). Feacal samples from humans and chimpanzees of BFR were positive for *Strongyloides* eggs. This agrees with earlier reports that strongyloidosis was a common infection of wild non-human primates (23). It is known that *S. fulleborni* and *S. stercoralis* of primates occur in human populations in tropical Africa (16, 17).

Both chimpanzees and humans in BFR were infected with *Trichuris* species with a higher prevalence in humans (6.2%) than in chimpanzees (5%). *Trichuris* species was a common parasite in humans especially where sanitation was poor, as found in the Nyakafunzo village. This parasite has been reported from several primates, including baboons (16, 24). The presence of *Trichuris* species in chimpanzees in BFR suggests a possible transmission from humans to chimpanzees.

The eggs of *Enterobius* species were found in feacal samples of humans (3.1%) and chimpanzees (3%) with no significant difference ($P>0.05$) between the species. The eggs of *Echinostoma* species were only found in employees of BFR. *Echinostoma* species are members of the family Echinostomatidae which are known zoonotic flukes that infect birds and mammals, including humans. The findings of *Echinostoma* species only in humans could be explained by the eating habits and diets with more chances of humans ingesting an infected intermediate host as compared to chimpanzees.

It is hoped that the findings of this study would castigate more studies on parasite dynamics and ecology in chimpanzee habitat and surrounding human communities. The study would also aid in designing proper conservation projects that integrate wildlife health research on interspecies transmission. A preventive health program for those working with free-ranging chimpanzees should be developed, and community education programmes instituted.

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