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DISTRIBUTION PATTERNS OF GASTROINTESTINAL PARASITES IN VERVET MONKEYS (*Chlorocebus pygerythrus*) AT GOMBE NATIONAL PARK, TANZANIA

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ABSTRACT

Vervet monkeys at Gombe National Park in western Tanzania constitute a key component of the park's ecosystem through interactions with other animals and people in the area. However, the parasite fauna of these animals has not been investigated. Thus, 21 faecal samples obtained from the animals in September 2010 were examined for parasites using formol-ethyl technique. The parasites observed included Trichuris spp., Physaloptera spp., hookworms and unidentified nematodes. While egg counts for unidentified nematodes did not vary with vervet monkey communities (z = 0.759, p = 0.448), other parasites were significantly higher among vervets at north of the park near Mwamgongo village compared to those at southern park border close to Mtanga village (Trichuris spp.: z = 2.443, p = 0.0146; Hookworms: z = 2.084, p = 0.0371). This is the first baseline study on vervet monkey parasites at Gombe and it broadens our understanding of the animals' ecology and health. The observed parasites namely Trichuris and hookworms are also common in human populations in the area, and this suggests a potential health risk given the existing animal-human interactions. This stresses the need to better understand how these findings may relate to wildlife conservation and public health in the area.

Key words: Vervet monkeys, Gombe ecosystem, parasites

INTRODUCTION

Vervet monkeys (*C. pygerythrus*) are susceptible to a variety of gastrointestinal infections including water-borne, soil-transmitted and food-borne parasites. Studies in Ethiopia (Legesse and Erko 2004, Amenu et al. 2015), Sudan (Sulaiman et al. 1986) and Uganda (Gillespie et al. 2004) have indicated that parasite infection in vervet monkeys varies with local environmental factors such as temperature, levels of rainfall and availability of intermediate hosts such as snails. Like other ground-dwelling savannah primates such as baboons, vervet monkeys are likely to contract water-borne infections like schistosomiasis because of their habitual contact with water bodies (Cheever et al. 1970, Else and Satzger 1982, Macpherson and Craig 1991, Legesse and Erko 2004). The animals are known to defecate promiscuously in water bodies hence enabling schistosome eggs to hatch and invade suitable intermediate host snails. This in turn serves to maintain the parasite's life cycle. It has been noted in particular that helminth parasites from vervet monkeys in Ethiopia can pose a high risk to public health in areas where the animals interact closely with humans (Legesse and Erko 2004, Amenu et al. 2015).

Although vervet monkeys at Gombe do not form a major part of the chimpanzee diet (Goodall 1986), they are eaten from time to time as opportunistic prey enough to transmit infection to these endangered apes. This emphasizes the need for continued

understanding and monitoring of the parasite fauna of vervet monkeys in their habitats particularly in areas such as Gombe National Park where they intermingle easily with other animals, partly due to the small size of the park. In this area, humans and vervet monkeys live in close proximity to each. Quite often the monkeys leave the park and raid nearby villages, visiting farms, dumping sites and residential areas in search of food (Bakuza 2012), which puts them at risk of sharing parasites with humans.

The status of the parasite fauna in vervet monkeys in Tanzania is not clearly understood. The only known study on the parasites of these animals in the country was conducted in the early 1970s by Cheever *et al*. (1970) who found 11 out of 47 (23.04%) vervet monkeys from northeastern Tanzania infected with *S. mansoni* parasites. The authors inferred that much as the findings suggested a possible natural infection in wild monkeys, the parasites could have been a result of contamination as the animals had been kept in the laboratory for three days prior to examination. It was however concluded cautiously that the monkeys were probably infected before capture, indicating their role as permissive hosts for *S. mansoni.* The monkeys had originated from some areas in Musoma District around Lake Victoria (Cheever et al. 1970). No other study has investigated the status of parasitic infection in vervet monkeys in Tanzania. The present study was thus initiated to determine the types and infection levels of parasites harboured by vervet monkeys in Gombe National Park, and ascertain whether their infection patterns are related to locality.

METHODS

Study area

This study was conducted in Gombe National Park located along the shores of Lake Tanganyika in western Tanzania (Fig. 1). A detailed description of the park and its neighbourhood has been given elsewhere (Goodall 1986, Lombardozzi 2003, Bakuza 2012). The park has a tropical climate with very humid wet season especially in the forests (Goodall 1986). Vegetation in the area ranges from evergreen forest along river valleys, deciduous forest and thicket on the upper slopes, to grassland on the ridges. Rainfall averages about 1500 mm per year (Collins and McGrew 1988), with the wet season spreading over eight months (October-May) and the dry season experienced from June to September (Goodall 1986).

Although Gombe is globally famous for chimpanzees (*Pan troglodytes schweinfurthii*), there are also other primates such as the olive baboons (*Papio anubis*) and vervet monkeys (*C. pygerythrus*), which are equally important in terms of ecological significance in the park (Lyogello 1991, Bakuza 2012). There are two small communities of vervet monkeys, which are confined to the northern and southern boundaries of Gombe National Park, respectively (Fig. 1). There are no records on the number of individuals in the Gombe vervet communities but it is thought to be within the general range of 10 to 70 individuals, with an estimate average of 35 individuals per social group, (*Personal observation*), which is anapproximate to documented records (Borgeaud et al. 2016). The monkeys constitute a key component of the Gombe's ecosystem through their interactions with other animals and humans (Goodall 1986).



Figure 1: A sketch map of Gombe National Park and the neighbouring villages of Mwamgongo and Mtanga to the north and south of the park, respectively.

Study design

The permission to conduct this study in Gombe National Park (No. TNP/HQ/E.20/08B) was granted by Tanzania National Parks (TANAPA) through Tanzania Wildlife Research Institute (TAWIRI). Samples were obtained from the animals in accordance with accepted animal welfare and guidelines for working with wildlife subjects for research purposes. All samples (faeces) were obtained from the vervet monkeys non-invasively without

immobilising or touching the animals as detailed in Gillespie et al (2006, 2008) and Leendertz et al. (2004, 2006). The monkeys were sampled from two sites, at the park boundaries near the villages of Mwamgongo (to the north) and Mtanga (to the south). As recommended elsewhere (Lilly et al. 2002, Bakuza 2012), a total of 30 samples was targeted from each community of vervet monkeys with one independent sample obtained from each individual monkey.

Stool collection

In total, 21 stool samples were collected from the monkeys from June to September 2010. Vervet monkeys at Gombe National Park are not habituated to humans so it was not possible to collect multiple samples from the same individual, such as for instance on consecutive days. Thus sampling was conducted once in the dry season. Stool samples were collected following published guidelines (Lilly et al. 2002). Each vervet monkey community was visited at dusk a day before sampling and a site where the animals slept recorded. The nests were visited at dawn on the next day, and to minimize duplication of individual samples, each individual animal was watched as they woke up. The monkeys defecated as each of them left its nest and a portion of their stool (approximately 5g) was picked up immediately and preserved in 20ml vials (tubes) containing buffered 10% formalin (with isotonic salt solution buffer to maintain parasite morphology). Although formalin is known to have effect on samples after prolonged storage, it has reasonable good preservation of morphology for most helminth eggs and larvae and to some extent protozoan cysts (Cheesbrough, 1998).

Microscopical examination of stools

After being preserved in 10% formalin for between 4-6 months, the stool samples were examined for parasites using the formol-ethyl concentration technique (Allen and Ridley 1970, WHO 1991). To obtain 1g of the sample, each vial containing about 5g of vervet monkey faeces was vigorously shaken by hand to disperse the sample into solution. The slurry was then passed through a domestic strainer to filter out large particles as recommended in Cheesbrough (1998) and WHO (1991). Retained materials on the strainer were examined for any adult parasites under a dissecting (stereo) microscope and discarded. The filtrate was then transferred into a 15 ml centrifuge tube and the solution centrifuged at 3000 rpm for 1 minute to get rid of formalin preservative. The supernatant was discarded, while the debris at the bottom of the centrifuge was retained. Approximately one gram of the debris was picked up and weighed using a digital scale (Sartorius, Göttingen Germany). The sample (1g) was mixed with 6ml of 10% formalin, followed by an addition of 3ml ethyl acetate. The formalin-ethyl-faeces mixture was then re-centrifuged at 3000 rpm for one minute to isolate parasite eggs. The supernatant was discarded while the debris at the bottom of the centrifuge tube was retained. The whole debris was then spread onto a microscope slide and examined for parasites under a 10x objective of the compound light microscope. Complete examination of the field of view was achieved by moving the slide forward and backward in a zigzag fashion using the microscope's stage height adjustment (stage control) knob as recommended (WHO 1991). Since each examined stool sample weighed was one gram, observed parasite eggs or larvae were counted and reported as eggs per gram (epg) or larvae per gram (lpg) of stool, respectively (WHO 1991). The samples were examined in the Zoology laboratory at the University of Dar es Salaam. Parasites were identified based on morphology, size and appearance of eggs or larvae by using identification guidelines, keys and photographs in the literature (Cheesbrough 1998, WHO 1991). The size of each parasite stage observed (eggs or larvae) was measured using an eyepiece

micrometer and the measurements used to further confirm parasite identities.

Data analysis

Parasite prevalence and intensity (egg counts) were estimated based on a single stool sample per individual. Prevalence was calculated as the percentage of infected individuals out of all examined animals (Bush et al. 1997). Parasite intensity was expressed as the number of parasite eggs or larvae counted in 1gm of stool for each sampled animal (WHO 1991). Mean egg counts of parasites was calculated as suggested elsewhere (Montresor et al. 1998) using the formula: arithmetic mean = Σ (epg)/n, where: epg is the direct egg counts for each infected individual, and n is the number of subjects investigated. Data analysis was performed using Generalized Linear Models (GLM) in the R programming environment with parasite intensity (egg or larva counts) and prevalence as dependent variables and vervet monkey community (group) as an independent variable. The R code for parasite intensity was: zeroinfl(formula = Parasite_egg counts ~ Group, dist = "negbin", link = "logit") or zeroinfl(formula = Parasite_larva counts ~ Group, dist = "negbin", link = "logit") while for prevalence of eggs or larvae, the code was: glm(Parasite_prevalence~Group,family=bin omial). Checking for ZINB distribution was conducted as suggested elsewhere (Loeys et al. 2012) by plotting the frequency distribution of egg count data. The graph plot indicated an over dispersed pattern with excess zero counts, which is consistent with a zero-inflated negative binomial distribution, with expected large differences in egg counts (intensity) between individual hosts (Loeys et al. 2012). The frequency distribution of parasite egg counts in the vervet monkeys showed a Negative Binomial (NB) distribution with excess zero counts (overdispersion) for all parasites. Hence variation in parasite egg counts between the two vervet monkey communities was performed using Zero-Inflated Negative Binomial (ZINB) models as these can accommodate the overdispersion and excess zeros parts of the data. The statistical significance level was taken at $p < 0.05$.

RESULTS

Parasite species identified from vervet monkeys at Gombe

Twelve of the samples collected were obtained from the northern park boundary near Mwamgongo village while nine of them came from the southern park boundary close to Mtanga village. Four parasite types were detected in the Gombe monkeys, with two of them identified to genus level as *Physaloptera* spp. and *Trichuris* spp., the third identified as hookworms while the fourth was recognized as unidentified nematode due to the inherent difficult in identifying unknown eggs and larvae morphologically.

Patterns of parasite infection in vervet monkeys at Gombe

Analysis of variation of the prevalence of parasite eggs and larvae (dependent variable) against vervet monkey community (Fig. 2A) indicated that the differences were not statistically significant (*Trichuris* spp.: z = -0.005, p = 0.996; hookworms: z = 0.923, p = 0.356; Nematode eggs: z = 0.759, p = 0.448; Nematode larvae: z = 0.148, p = 0.882). On the other hand, the egg counts for all parasites were higher among the Mwamgongo vervet community than that of Mtanga (Fig. 2B). Results from Zeroinflated (ZINB) analysis of parasite egg and larva counts against vervet monkey community (independent variable) have shown that, except for unidentified nematode larva counts, the differences were statistically significant (*Trichuris* spp.: z = 2.443, p = 0.0146; Hookworms: z = 2.084, p = 0.0371; Nematode eggs: z = 2.647, p = 0.00811; Nematode larvae: z = -1.322, p = 0.18622).

DISCUSSION

The four parasite types identified in vervet monkeys at Gombe in the present study have previously been reported in vervet populations from other sites, namely the Rift Valley of Ethiopia (Legesse and Erko, 2004, Amenu et al. 2015), Blue Nile Province in Sudan (Sulaiman et al. 1986) and Lake Saka in Uganda (Gillespie et al. 2004). An earlier investigation on the parasite fauna of vervet monkeys in Barbados, which had been imported into the country from West Africa (Mutani et al. 2003), reported the presence of the same types of parasite species as those found in the Gombe monkeys. In addition, *Strongyloides* sp., *Oesophagostomum* sp., *Trichostrongylus* sp. and *Ascaris sp.* were also found in the Barbados monkeys. The small sample size of the vervet monkeys examined at Gombe in the present study might be the reason for the few parasite species found compared to those reported from other sites. Other studies e.g. Gillespie et al. (2004) also reported only three nematodes among vervet monkeys in Uganda and linked that to the small sample size studied. Results obtained from lower sample sizes may not reflect the real picture of parasitic infections in the host populations (Gregory and Blackburn 1991). Factors impacting on the parasites of vervet monkeys in such areas can be effectively understood by incorporating a relatively larger sample size over a longer sampling period. Although only 21 out of the estimated 30 individual monkeys in Gombe were sampled, the exact population size of the animals in the park is still unknown. Therefore, for comprehensive analysis of the factors influencing parasitic infections in the Gombe vervet monkeys, future studies may optimize their findings by including all possible individuals in the sampling.

The present analysis of parasitic infections among the Gombe monkeys has indicated however that the infections were generally higher among animals in the northern park boundary near Mwamgongo village than in the southern community close to Mtanga village (Fig. 2). This was the case for all parasites except for the prevalence of *Trichuris* spp. and unidentified nematodes. The analysis has also indicated that variation of larva counts (intensity) for unidentified nematodes did not vary significantly between the two vervet monkey communities, which could have resulted from the fewer number of larvae counted compared to eggs.

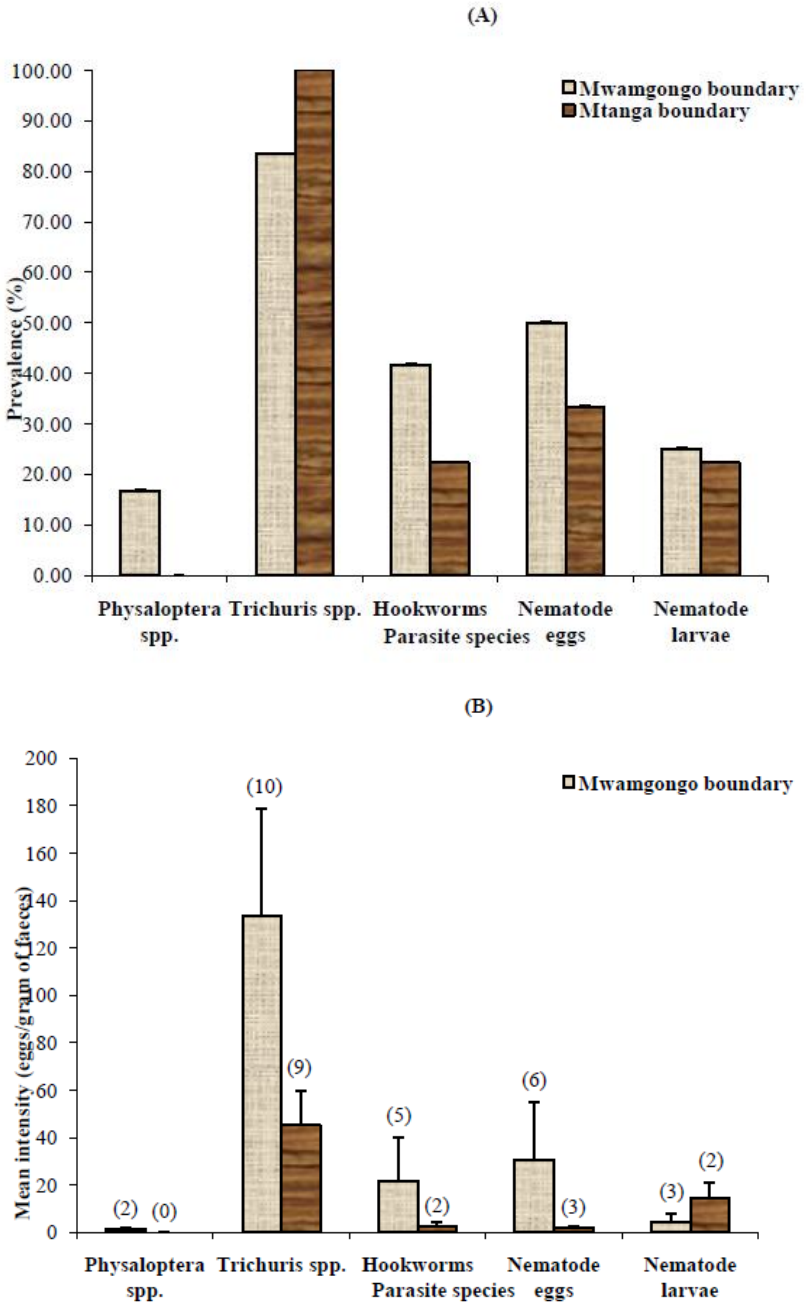


Figure 2: The influence of locality on the prevalence (A) and egg counts (B) of various gastrointestinal parasites infecting vervet monkeys at Gombe. Bars indicate standard errors while numbers on top of each bar indicate the number of individual monkeys that were found infected by each parasite taxon in each vervet community. The sample size (n) = 12 for Mwamgongo while n = 9 for Mtanga

These observations are of major local importance because of the presence of the same types of parasites among humans in Mwamgongo village and the existing close interactions between humans and animals in the area (Muller-Graf et al. 1997, Wallis and Lee 1999, Mung'ong'o 1999, Bakuza and Gamba 2009, Bakuza 2012). In a recent study, many people at Mwamgongo village were found to have heavy infections of *Schistosoma mansoni* (Bakuza 2012), the causative agent of intestinal schistosomiasis. Although *S. mansoni* was not detected in vervet monkeys in the present study, it has been reported in the animals elsewhere (Else and Satzger 1982, Gillespie et al. 2004, Legesse and Erko 2004). Since the Gombe monkeys quite often leave the park and go into the village residential areas where they come in contact with water bodies such as streams and pools that are used by humans (Bakuza 2012), there is a possibility that cross transmission of the infections may occur between people and animals. In such areas where humans and closely related animals (evolutionarily) such as the vervet monkeys interact regularly and share helminthic diseases, repeated treatments can lead to the selection and evolution of drug resistance in human populations that could spread to untreated animal reservoir hosts (Anderson et al. 1993). If humans are treated, alleles conferring drug resistance can then be transmitted back into the human populations from the animals, thereby compromising chemotherapy programmes (Anderson et al. 1993). In such settings, it is important to understand the genetic and epidemiological relationships between parasites infecting humans and those of animal reservoir hosts (Anderson et al. 1993). Such information could be useful in designing effective management of parasite's anthelminthic resistance (Anderson et al. 1993).

Furthermore, except for *Physaloptera* spp., all of the parasites observed in the vervet monkeys in the present study can also infect humans (Cheesbrough 1998). Given the close contacts between people and animals in Gombe, it is possible that non-human primate populations in the park may be at risk of acquiring human-related infections, which could have significant conservation implications. These observations are also of concern over the possible source and spread of parasitic infections in the park. Humans and vervet monkeys in these areas live in close proximity to each other while interacting in many ways, including sharing resources such as dumpsites (Wallis and Lee 1999, Bakuza 2012). The local people in Mwamgongo and Mtanga villages may enter and leave the park on daily basis with potential risk of bringing into the park parasitic infections and taking others back to the villages. The present study is thus useful in gauging the extent to which vervet monkeys in the area harbour intestinal worms and the potential risks this could have on public health in the area. Despite the small sample size involved, the present findings corroborate reports on parasitism as reported in other monkey populations. For instance, vervet monkeys living close to human settlements in Ethiopia were found to harbour protozoan and helminth parasites that were similar to those of humans (Legesse and Erko 2004) and thought to pose potential risk to public health (Amenu et al. 2015). Elsewhere, it has been reported that vervet monkeys in shared habitats with humans were infected with parasite species that also affected humans and concluded that such primates posed a significant health risk to public health (Mutani et al. 2003). However, without genetic evidence, it might be difficult to assume that parasites such as *Trichuris* or hookworm found in the Gombe vervet monkeys are of human origin, since they are commonly found in primates with and without contact with humans (Ash and

Orihel 2007). Future comprehensive studies using molecular techniques may draw more reliable conclusions regarding the interrelationships of human and vervet monkey parasites in areas such as Gombe where the two groups happen to interact regularly.

To conclude, the present study provides the first baseline assessment of the gastrointestinal helminths of vervet monkeys in Gombe National Park. Four parasite taxa were identified in the Gombe monkeys namely; *Physaloptera* spp., *Trichuris* spp., hookworms, and unidentified nematodes. Although the prevalence of the parasites did not differ among vervet communities, the parasite egg counts were significantly higher among monkeys at the northern Gombe boundary near Mwamgongo village, which possibly, is a result of differences in vervet monkey group size and other local environmental factors, although influence from nearby human populations cannot be ruled out. As most of the parasites observed in the present study are also capable of infecting humans, vervet monkeys in Gombe could potentially cause a health risk to nearby human populations. Future studies should apply modern techniques to identify the genetic relationships between parasite strains from humans and non-human primates in the area. Such studies should also explore the extent of parasite passage through carnivorous habits of the chimpanzees on vervets and other monkeys in Gombe National Park such as the colobus monkeys (*Colobus badius*), which are often eaten by the apes in the park.

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