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**Epidemiology of *Schistosoma mansoni* infection in
sympatric humans and non-human primates in the
Gombe ecosystem Tanzania**

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College of Medical, Veterinary and Life Sciences

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Abstract

Increased interactions between humans and wild animals in and around protected areas have raised the risks for sharing diseases and parasites among them. Regular surveillance with intervention for these infections in such areas is therefore necessary for improving animal health and for controlling any spill-over of animal diseases into nearby human populations. Although both humans and non-human primates in the Gombe area in western Tanzania are infected with schistosomiasis, it is not known whether strains of their schistosomes are epidemiologically and genetically distinct. The distribution and transmission risk factors for the disease in these areas are also not well known. This study investigated the infection patterns of schistosomiasis in humans and non-human primates in Gombe National Park and surrounding villages of Mwamgongo, Bugamba, Kiziba and Mtanga and related the infection prevalence and intensity to locality and other demographic characteristics such as age and sex in humans. It also examined the dosage and number of praziquantel tablets administered to school children based on their weight and compared this to the dosage they would receive based on their height. The parasite fauna of baboons and vervet monkeys in Gombe was also examined to determine the parasite species assess whether their infection levels have changed over time. Snails were also sampled so as to gain a clear understanding of the species present in an area, their local distribution and infection status. The results showed a significant variation of *S. mansoni* prevalence between age groups in humans, which also depended on site. The parasite egg counts (intensity) also varied significantly between age groups and across study sites. The dosage range of praziquantel in mg per kg of bodyweight predicted by height was 23-43 (average: 35.2) while the dosage range given to children based on their weight was 29-78 (average: 45.2) and this variation was statistically significant ($p > 0.0001$). Overall, six children (5.3%) received a praziquantel dosage below the recommended range (30-60 mg/kg) based on their weight while two children (2.6%) would have received the drugs above the optimum range based on their height. The parasites

identified in baboons and vervets included *Trichuris* spp., *Physaloptera* spp., hookworms and unidentified nematodes, while *Paragonimus* spp., *Streptopharagus* spp. and *Schistosoma mansoni* were exclusively detected in baboons. Molecular analysis of baboon schistosome eggs confirmed them to be *S. mansoni*. A GLM analysis indicated that the interaction between season and baboon troop was a significant predictor of parasite prevalence and intensity in baboons. Snails obtained from all streams except at Mtanga were identified as *Biomphalaria pfeifferi* based on morphology and DNA analysis. These findings indicate a high infection of intestinal schistosomiasis in these areas of western Tanzania, suggesting that the distribution of the disease in the country could be more widespread than previously thought. The results also confirm that the infection of intestinal schistosomiasis in the area is focal, with marked variations between adjacent villages. The study shows that while both weight and height estimate the amount of praziquantel dosage that is within an acceptable range, weight tends to underestimate the drug while height slightly overestimates it. It is therefore essential to conduct further field studies to test the usefulness of the dose pole in praziquantel distribution and evaluate the extent to which the method could be wasting the drug by giving more than the necessary dosage. As most parasites diagnosed in baboons and vervets are capable of infecting humans, these animals can potentially serve as reservoirs of human helminths given the regular human-wildlife interactions in the area. The implication of these observations to wildlife conservation and public health issues in the area has been explored.

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Author's Declaration

I, Jared Sylvester Bakuza, declare that the work reported in this thesis is entirely my own, except where otherwise stated, and that it has not been submitted as part of a degree elsewhere. After working for several years on primate research with the Gombe Stream Research Centre in Gombe National Park and also having worked for my master's degree on chimpanzee parasites at the University of Dar es Salaam, Tanzania in 2007, I developed more interest in primate parasites. I was particularly interested in the relationships between human and non-human parasites. The motivation to that was based on previous research reports on primate parasites at Gombe such as that by McGrew and colleagues (1989), Müller-Graf and others (1997) and Murray and colleagues (2000). The report by Müller-Graf and colleagues (1997) was particularly important to me in this context as it formed the basis of my research ideas that were to follow. The report suggested that the use of more powerful techniques such as DNA analysis would possibly provide answers as to whether or not humans and baboons in Gombe area are infected by the same strains of schistosomes. I therefore started on from there and expanded the idea to cover snails and other potential definitive hosts such as vervet monkeys. I then reorganized the idea for using PCR and microsatellite analysis to uncover the factors driving schistosome infections in Gombe and the neighbouring villages. This thesis, which is the product of that work, has been produced in collaboration with others, and my personal contribution to individual chapters is explained below.

All of the concepts and ideas in Chapter 1 entitled "General Introduction" are my own excepted where information was drawn from other people's work as an emphasis on something or to give it as an example and full acknowledgement and citations were made in that regard. The work on general introduction involved integrating my general understanding of the field of biology and parasitology in particular.

Chapter 2, which is entitled “Epidemiological patterns of intestinal schistosomiasis among communities along the shores of lake Tanganyika, Tanzania” was my own idea. I developed the initial concept and proposed field work design and sampling strategies which were refined by Barbara K. Mable (BKM). I also conducted the whole of fieldwork sampling and also did all of the lab work and analysis of parasitological data. This work also involved extensive molecular lab work, which covered DNA extractions from schistosome eggs (performed by myself), PCR and preliminary analysis of microsatellite genotypes conducted by Aileen Adam (AA) and analysis of microsatellite genotype data (performed by BKM).

Chapter 3 entitled “Tablet pole efficiency in estimating praziquantel dose for treating intestinal schistosomiasis among school children in Tanzania” was based entirely on my own idea. I developed the initial concept and proposed field work design and sampling strategies. I then conducted the whole of fieldwork sampling and also did all of the lab work and data analysis and discussion of the results.

Chapter 4 entitled “Trends in parasitic infections in baboons and vervet monkeys at Gombe National Park, Tanzania” was based on ideas proposed by Müller-Graf and colleagues (1997). I refined the idea to make it broader and proposed the field work design and sampling strategies which were substantially refined by BKM. I also conducted the whole of data collection and all of the lab work and analysis of parasitological data. This work also involved extensive molecular lab work including DNA extractions from schistosome eggs (performed by myself), PCR and preliminary analysis of microsatellite results conducted by Aileen Adam (AA) and analysis of microsatellite genotype data (performed by BKM).

Chapter 5 entitled “Comparison of PCR and microscopy-based assessment of prevalence of *Schistosoma mansoni* from snails in the Gombe ecosystem in Tanzania” originated from me. I developed the initial concept and proposed field work design and sampling strategies which was originally planned to look at the molecular ecology and dispersal patterns of snails in the Gombe ecosystem. The idea was extensively modified by BKM due to limited resources that were available to me such as time, money and personnel. I then conducted the whole of fieldwork sampling for data collection for this project and also examined the snails for parasite infection under a microscope. I also did part of the lab work for DNA extractions and PCR amplifications from snail tissues while the rest of this work was done by an honours student Robert Gillespie under my supervision with BKM and AA overseeing the overall running of the work. The final part of the molecular work on snails, which involved DNA sequencing and data analysis, was performed by BKM.

Chapter 6 entitled “General discussion, conclusion and recommendations” is based on my own concepts excepted where citations are made. This work involved the integration of my ideas around the findings of the current study.

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Chapter 1: General Introduction

1.1 An overview of schistosomiasis and its public health significance

Schistosomiasis is an infectious tropical disease caused by parasitic worms of the genus *Schistosoma*, which can infect humans and some domestic and wild animals. To keep within the scope of this thesis, only schistosomes that predominantly infect humans will be comprehensively reviewed. There are two forms of schistosomiasis categorized based on the organ in the definitive hosts where adult schistosomes reside and lay eggs (Engels, 1997). First is urinary schistosomiasis, which is exclusively caused by *Schistosoma haematobium* that inhabit the blood venules around the urinary bladder. The parasites lay eggs that pass through the blood capillaries into the lumen of the bladder and are voided with urine. As the eggs pass through the tissues, the eggs rupture the blood vessels, causing bleeding and an appearance of blood in urine. The presence of blood in urine, which is known as haematuria, is a useful diagnostic feature of urinary schistosomiasis infection (Fenwick, 2011).

Another form is the intestinal schistosomiasis, which is caused by the other four schistosome species that infect humans, namely *S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum*. *S. mansoni* was the focus of the present study and the rationale for choosing this particular species is discussed in the ensuing sections. The adult forms of schistosomes causing intestinal schistosomiasis live in the mesentery of the intestine, where they lay eggs (Fenwick, 2011; Engels, 1997). To complete the parasite's life cycle, the eggs then pass through the capillary tubes into the lumen of the intestine and are washed down with the faeces. As they rupture through the blood vessels, the eggs cause lesions and subsequently bleeding in the intestinal tissues. This leads to diarrhoeal stools, which are useful as a symptom of intestinal schistosomiasis infection (Engels, 1997).

Schistosomiasis infection constitutes a major public health problem, particularly in countries where the disease is endemic. Worldwide, approximately 20,000 people die of schistosomiasis annually, while about 200 million are infected with the disease, most of them in Sub-Saharan Africa (van der Werf *et al.*, 2003; Fenwick, 2011). It is also estimated that globally, more than 700 million people are at the risk of contracting schistosomiasis (Chitsulo *et al.*, 2000; van der Werf *et al.*, 2003). The acute or short-term consequences of schistosomiasis infection in humans include skin rashes, fever and fatigue, while chronic or long-term effects of the disease involve damage to internal organs such as the liver, spleen and gall bladder (van der Werf *et al.*, 2003; Fenwick, 2011). Later stages of schistosomiasis can also cause abdominal pain, diarrhoea, undernutrition, stunted growth and impaired cognitive abilities in children (McGarvey, 2000; King *et al.*, 2005).

Other effects of schistosomiasis infection include the risks for developing cancer of the bladder due to urinary schistosomiasis (Mostafa *et al.*, 1999; Botelho *et al.*, 2010). There are also reports that *S. mansoni* eggs can be lodged in the scrotum in humans, leading to severe scrotal swelling and pain that can require surgical intervention (Rambau *et al.*, 2011). In some areas in Africa, *S. mansoni* infection causes severe anaemia in pregnant women, leading to maternal-ill health and mortality (Ajanga *et al.*, 2006). Several other schistosome species that predominantly infect birds and mammals can cause a skin rash infection in humans known as swimmer's itch (Rollinson and Southgate, 1987; CDC: <http://www.cdc.gov/parasites/schistosomiasis/biology.html>).

Schistosomiasis affects mostly poor people living in rural areas of developing countries (Fenwick, 2011). These are people whose livelihoods such as fishing, farming and animal husbandry, are directly linked to water bodies. Inadvertently, these people become infected with the disease as they work in their farms or while doing other water-related activities to meet their daily needs (Fig. 1.1).



Figure 1.1: A pond believed to be filled with fish and schistosome-transmitting snails apparently serving as a breeding site for schistosomiasis transmission as it is thronged by swimmers, fishermen and women doing the washing up and other domestic chores in Mali, West Africa (Courtesy of Prof. Alan Fenwick: Schistosomiasis Control Initiative, Imperial College, UK).

1.2 Schistosomiasis as a neglected disease

Like many other helminth infections, schistosomiasis is generally a chronic disease that often does not kill the patients, although it can debilitate and weaken them severely (van der Werf *et al.*, 2003). Infection with the disease is also asymptomatic, particularly intestinal schistosomiasis, which can remain undetected for a long time (Rugemalila, 1991; Meltzer *et al.*, 2006). The lack of early and acute symptoms in infected people leads to complacency and lack of urgency by both the patient and the community. Hence, until recently schistosomiasis was not considered a threat to public health in most endemic countries. It is not surprising therefore that the disease is recognized by the World Health Organization as a neglected tropical disease (WHO, 2010). Schistosomiasis and other neglected tropical diseases do not receive as much treatment or financial support compared to the three killer diseases of malaria, TB and HIV/AIDS (Hotez and Kamath, 2009). Schistosomiasis and other neglected tropical diseases are also ignored because they affect mainly poor people whose low incomes do not offer profitable market for drug-making companies (Oprea *et al.*, 2009).

As noted above, the public attitude towards schistosomiasis is changing and improving. As more people get infected and more scientific knowledge on the disease is produced, the magnitude of the disease has become more apparent. It is now widely recognised that the physical disabilities caused by schistosomiasis can halt economic development in low-income countries. This is because the few resources available in these countries are diverted for caring and treating people infected with schistosomiasis (Oprea *et al.*, 2009). It is also known that infection with schistosomiasis costs billions of dollars in lost productivity as infected people become too weak to work (Conteh *et al.*, 2010). This loss is estimated as the numbers of years of potential production lost due to premature death or as a result of inability to work caused to schistosomiasis infection also referred to in short as

DALYS (Disability Adjusted Life Years). The disease is now considered the second most prevalent and important parasitic infection after malaria, with devastating effects on the socio-economic wellbeing of mankind (WHO, 1985). Some pharmaceutical companies have therefore committed donations of drug therapies required for treating schistosomiasis. As a result, mass administration of drugs such as praziquantel has been successfully accomplished in several endemic countries (Reddy *et al.*, 2007; Fenwick, 2011). A considerable number of laboratory and field studies such as the present study are also underway in various endemic countries with a view to providing further understanding on the disease and its control in contemporary settings.

1.3 The transmission and life cycle of schistosomiasis

The life cycle of schistosomiasis has clearly been illustrated and elaborated in many published works (Engels, 1997; Fenwick, 2011; Gill and Beeching, 2009). A brief description on the transmission cycle of the disease is given below, supported by a representative illustration of the life cycle of *S. mansoni* (Figure 1.2). The life cycle starts when eggs released by adult schistosome worms into the human body (1) are discharged into the environment through urination or defaecation. Upon coming in contact with water, the eggs (2) hatch to release ciliated mobile larvae called miracidia (3), which within 6-8 hours have to penetrate suitable snail hosts (A). Once in the snail, the miracidia develop into mother or primary sporocysts, each of which asexually produces thousands of daughter sporocysts, which mature into larvae called cercariae. The larvae later leave the snail hosts (4) and enter into water. These larvae have a characteristic forked tail that aids them to swim in the water as they search for a suitable definitive host such as humans (4). When people wade into the water containing cercariae, the larvae pierce their skin and start burrowing into the body. The cercariae are able to enter human skin apparently through the action of proteolytic enzymes, which can digest body tissues (Wilson, 1987). The

larvae, which by this stage have lost their forked tails and are thus called schistosomulae, move into the blood circulation to the lungs, the heart and then to the liver. They then mature into adults at which stage a female (B) and a male (C) individual meet up and while joined, move to appropriate final resting tissues in the host (D), which depend on the type of the schistosome species (refer section 1.1 above; Fig. 1.2, No. 1). Urinary schistosomes go to the vesical plexus around the urinary bladder while intestinal schistosomes migrate to the mesentery in the small intestine. The worms stay paired for up to 20 years (Fenwick, 2011), although there is some disagreement on this duration. Until recently it was believed that the female and male schistosome worms remain united permanently (Erasmus, 1987), but recent observations suggest otherwise (Webster *et al.*, 1999; Pica-Mattoccia *et al.*, 2000). There is new evidence suggesting that the worms may get separated after 20 to 25 years and each one finds a new partner (Pica-Mattoccia *et al.*, 2000). Using labelled schistosomes in mice Pica-Mattoccia *et al.* (2000) showed that mate changes occurred among the adult worms of *Schistosoma mansoni* with the consequence of maximizing the species fitness. Nonetheless, on average, *S. mansoni* and other schistosome parasites can live up to 3 to 5 years although reports of these parasites surviving in the human body up to 30 years are also known (Brown, 1969; Gryseels *et al.*, 2006).

After reaching their destinations, the worms start to mate and the female partner lays schistosome eggs (up to 300 hundred daily; Fenwick, 2011). The eggs that are laid down in the mesentery end up in the lumen of the small intestine, where they are voided with faeces. Urinary schistosomiasis eggs in the vesical plexus find their way into the lumen of the bladder and get voided with urine. When urine or faeces containing schistosome eggs gets in contact with water, miracidia larvae are released and if there are suitable snail hosts, the life cycle starts again.

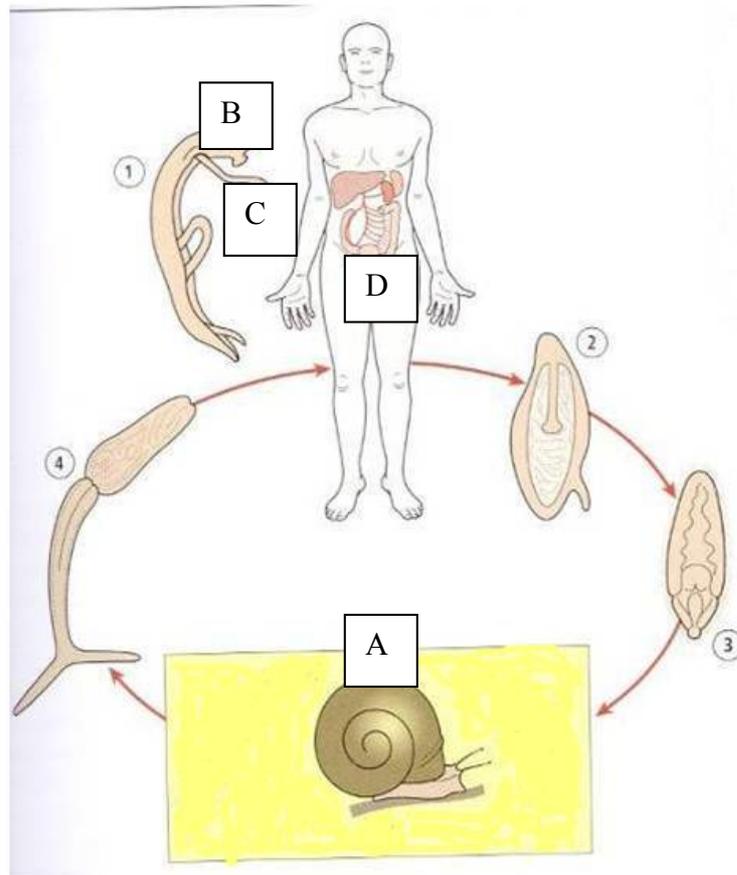


Figure 1.2: A simplified life cycle of *S. mansoni* indicating a pair of adult schistosomes (1) with a long and slender female (C) held in the “gynaecophoric canal” of a short and stout male adult (B) in the human host (D). The cycle starts when a schistosome egg (2) released in stool hatches to release an immature miracidium larva (3) upon contact with water. The larva (3) penetrates an intermediate snail host (A) in which it is transformed into a mature cercaria larva (4). After leaving the snail host, the larva (4) pierces the human skin and goes into the blood circulation, the lungs, heart, liver and finally settles in the mesentery vessels as joined male and female adults (1). After mating, the female lays eggs, which pass through the tissues into the lumen of the intestine. The eggs are then voided with stool and if they come into contact with water, the life cycle continues. Reproduced from Gill and Beeching (2009) with publisher’s permission (John Wiley and Sons Ltd.).

1.4 Snail vectors of schistosome parasites

The life cycle and transmission of *S. mansoni* and other schistosome species involve various species of snails (Fig. 1.2), which are species-specific to the schistosome parasites (Brown, 1994; Van den Enden, 2004). As detailed in Van den Enden (2004), each *Schistosoma* species has its own snail species as its vector. For instance *Biomphalaria* snails are vectors for *S. mansoni*, while *Bulinus* spp. transmit *S. haematobium* and *S. intercalatum*. The Oriental schistosome species *S. japonicum* and *S. mekongi*, use *Oncomelania* spp. and *Neotricula* spp., respectively, as their vectors (Ash and Orihel, 2007). Snails are critical to the life cycle of schistosomes as they serve as intermediate hosts in which young stages of the parasites multiply and mature before they can enter their definitive hosts (Fig. 1.2). Field studies have indicated that the transmission of schistosomiasis can only take place in areas where suitable snail hosts are present and where the human subjects have frequent contact with snail-infested water (Appleton, 1978). Hence, knowledge on the distribution and habitat preference of snails is a crucial tool in understanding the epidemiology and control of schistosomiasis (Utzing and Tanner, 2000).

1.5 Global distribution of schistosomiasis

The global distribution of schistosomiasis is limited by various physical and biological factors that affect the distribution of the snail intermediate hosts (Southgate and Rollinson, 1987). Temperature is one of the major determinants for the snails' existence and survival as it affects their larval development (Poulin, 2006), growth and consequently their rate of schistosomiasis transmission (Southgate and Rollinson, 1987). Hence, snails transmitting human schistosomiasis are not found in areas of extreme temperatures such as high altitudes or temperate zones (Southgate and Rollinson, 1987). They are instead confined to the warm tropical and subtropical regions particularly in Africa, Middle East, Asia,

Caribbean and South America (WHO, 1985). Only non-human schistosomes such as those infecting avian host species are known to have a worldwide distribution (Lindblade, 1998). Three of the five human schistosome species, namely *S. haematobium*, *S. mansoni*, and *S. japonicum*, are widely distributed, with *S. mansoni* as the most abundant (Morgan *et al.*, 2001). *S. haematobium*, with its characteristic terminally spined eggs (Fig. 1.3A), occurs in Africa, the Middle East and parts of India while *S. mansoni*, which is distinguished by its laterally spined eggs (Fig. 1.3B), is found in Africa, the Middle East, South America and the Caribbean (WHO, 1985). *S. mansoni* was the focus of the present study due to current lack of knowledge on its distribution and epidemiological significance in Tanzania, particularly in the country's western areas. Further discussion on this aspect is given in subsequent sections of chapters one and two of this thesis.

Another important human schistosome is *S. japonicum* (Fig. 1.3C), which is predominantly found in China, the Philippines and some parts of Indonesia (WHO, 1985). *S. japonicum* is particularly important epidemiologically because in addition to humans, it also infects several species of domestic and wild mammalian hosts (He *et al.*, 2001; Fenwick, 2011). Other schistosome species that are less widespread and therefore have minor epidemiological significance are *S. mekongi* (Fig. 1.3D) found in the Lao People's Democratic Republic, Cambodia and Thailand in the Mekong River Basin and *S. intercalatum* (Fig. 1.3E), whose distribution is limited to West and Central Africa (Cheesbrough, 1998).



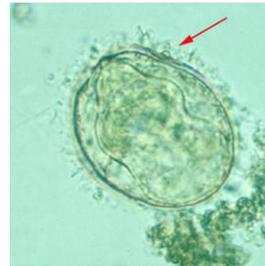
A



B



C



D



E

Figure 1.3: Eggs of the major schistosome species and their distinguishing features which are a terminal spine for *S. haematobium* (A) and a prominent lateral spine for *S. mansoni* (B). The eggs of *S. japonicum* (C), which are round in shape and usually smaller than those of *S. mansoni* and *S. haematobium*, have a characteristically inconspicuous terminal spine (see arrow). *S. mekongi* eggs (D) look like those of *S. japonicum*, with a small and inconspicuous terminal spine, but are largely smaller in size. Like *S. haematobium*, the eggs of *S. intercalatum* (E) have a terminal spine and slightly larger size and a characteristic bulging pattern at the centre. Reproduced from permitted source at: http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Schistosomiasis_il.htm

1.6 Occurrence of schistosomiasis in Tanzania

Both *S. mansoni* and *S. haematobium* species are endemic throughout Tanzania, with a prevalence of up to 80% in some areas (Doumenge *et al.*, 1987; Rugemalila, 1991; Lwambo *et al.* 1999; Outwater and Mpangala, 2005). Recent estimates indicate that *S. mansoni* infection is most prevalent around the Lake Victoria basin while *S. haematobium* infection is distributed along the coast of the Indian Ocean and also on the shores of Lake Victoria (Brooker *et al.* 2009). While *S. mansoni* is more focal and virtually absent in the coastal regions and on Zanzibar and Pemba islands, *S. haematobium* is widespread in the country (Sturrock, 1965; Stothard *et al.*, 2002; Brooker *et al.* 2009). Estimates in 1977 indicated that 19% of the people in Tanzania were at the risk of acquiring schistosomiasis but exposure risks and prevalence of the disease and snail vectors have been increasing (Rugemalila, 1991). However, current information on the distribution of schistosomiasis is not available for most parts of Tanzania, particularly in the southern and western areas of the country (Brooker *et al.* 2009). For instance, the western corner of the country near the Burundi border where the present study was conducted was once thought to have both *S. mansoni* and *S. haematobium* species based mainly on hospital reports (Rugemalila, 1991). A recent study report by Brooker *et al.* (2009) based on published data, suggested that the area may be free of schistosomiasis but this conclusion was possibly due to lack of sufficient and updated data for the area (Fig. 1.4). In their analysis, Brooker and colleagues (2009) excluded unsystematic data such as hospital or clinic surveys, non-geo-referenced data and surveys conducted on confined population groups such as refugees, prisoners and nomadic people. This deliberate exclusion may have affected the results obtained in the study. As a result, Brooker *et al.* (2009) did not make reference to previous studies that had reported high infection of *S. mansoni* in baboons in this area such as Müller-Graf (1994) and Müller-Graf *et al.* (1996; 1997). Müller-Graf *et al.* (1997) for instance had

alluded that the existence schistosomiasis mansoni was already common and a well known phenomenon among the local communities (C. Müller-Graf, *personal communication*).

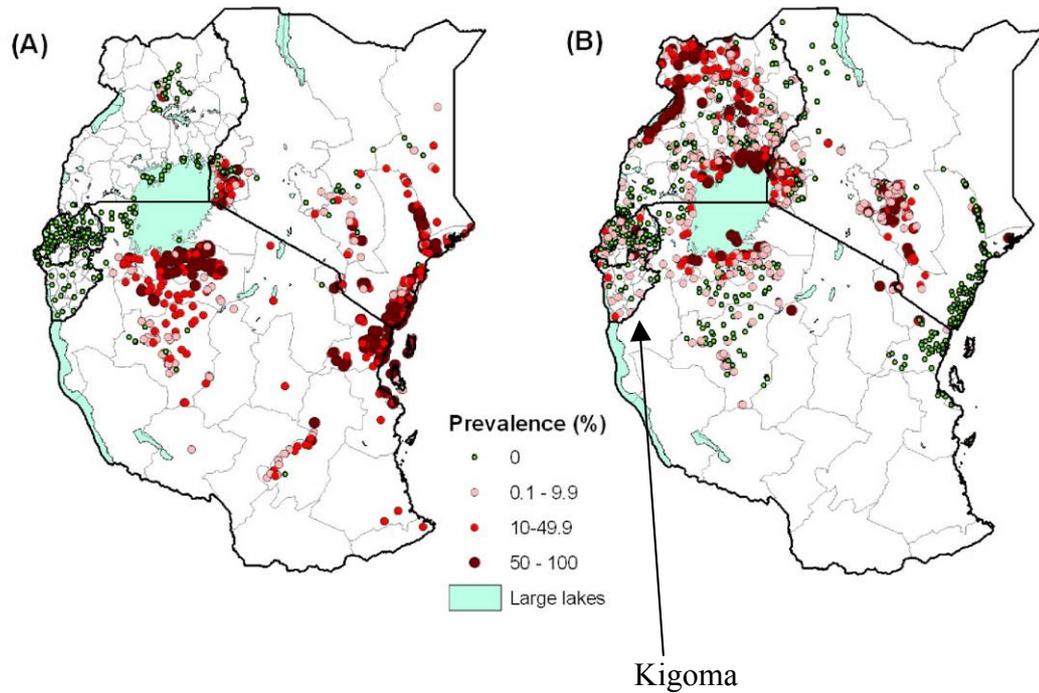


Figure 1.4: Currently known geographical distribution of *Schistosoma haematobium* (A) and *S. mansoni* (B) infection in Tanzania and other areas of East Africa, according to Brooker *et al.* (2009).

The data for this report were obtained from local libraries and archives, online bibliographic databases and researchers' consultation between 1980 and 2009, excluding information from hospital or clinic surveys, non-geo-referenced data and restricted population groups such as refugees, prisoners and migrating people. This exclusion could have affected the comprehensiveness of the report. An upward arrow on the right map (B) points to Kigoma region in which the present study was conducted apparently indicating that the area is free of schistosomiasis. (Reproduced from Open Access Source in Brooker *et al.* 2009: <http://www.ij-healthgeographics.com/content/8/1/42>).

1.7 Distribution of schistosome-transmitting snails in Tanzania

The snail species transmitting schistosomiasis in Tanzania belong to the bulinid and planorbid sub-types (Doumenge *et al.*, 1987). Urinary schistosomiasis in the country is transmitted by four snail species belonging to the genus *Bulinus*. They include *Bulinus nasatus* and *Bulinus globosus*, which are widely distributed in the country both on the mainland and on the Zanzibar and Pemba islands (Doumenge *et al.*, 1987). Other bulinids found in the country are *Bulinus africanus* and *Bulinus truncates*, which are mainly found on the mainland Tanzania. Planorbid snails of the genus *Biomphalaria* that transmit intestinal schistosomiasis in the country include *Biomphalaria pfeifferi*, which is thought to occur throughout the country except on the coastal belt and Zanzibar and Pemba Islands (Rugemalila, 1991). Other planorbid species include *Biomphalaria sudanica*, found in the northern part of the country and *Biomphalaria choanomphala*, which is confined to the shores of Lake Victoria (Doumenge *et al.*, 1987). Despite this knowledge, the identities and distribution of snail species that transmit schistosomiasis in Tanzania are poorly known (Rugemalila, 1991). This is complicated by the fact that most of the snail hosts belong to species complexes that have not been fully disentangled (Brown, 1994). The focal nature of snail distribution, which can significantly vary between adjacent sites, makes any generalization about their distribution in Tanzania impossible. For instance, while Müller-Graf *et al.* (1997) identified the snails collected from a site in Gombe National Park as *Biomphalaria pfeifferi*, their ability to transmit schistosomiasis was not established. The abundance and distribution of the snails in other sites in the area have not been systematically investigated, although schistosomiasis transmission occurs in the area (Müller-Graf *et al.*, 1997; Murray *et al.*, 2000). Lake Tanganyika has been regarded as schistosomiasis free and it has been thought that snails transmitting the disease may not exist in the lake because the rocky shores of Lake Tanganyika. The factors profiting the existence of snails along the shore of the lake are not well known although one possibility

could be the presence of constant heavy waves, which may kill the snails (Müller-Graf *et al.*, 1997).

1.8 The occurrence of schistosomiasis in wild baboons

Although *S. mansoni* generally infects humans, the parasite has also been reported in other mammals, including rodents and non-human primates (Fenwick, 1969; 2011). Natural infections of *S. mansoni* have been reported from some primates, particularly species that come into contact with water, such as baboons and vervet monkeys (Zahed *et al.*, 1996, Müller-Graf *et al.*, 1997; Munene *et al.*, 1998; Murray *et al.*, 2000; Legesse and Erko 2004). Baboons are probably able to maintain the cycle of *S. mansoni*, especially in areas where they share their habitats with humans (Muchemi, 1992). They are also thought to be capable of maintaining the infection on their own for a long time, even in the absence of humans (Zahed *et al.*, 1996; Legesse and Erko 2004). It is still unclear, however, whether schistosomiasis infection in animals such as baboons can affect humans and vice versa (Ouma and Fenwick, 1991; Müller-Graf *et al.*, 1997; Legesse and Erko 2004).

1.9 Previous observation of schistosomiasis in Gombe National Park

S. mansoni parasites have been reported in baboons at Gombe National Park, with the highest prevalence being in baboon groups that have had the most human interactions (McGrew *et al.*, 1989; Müller Graf *et al.* 1997; Murray *et al.*, 2000). These baboons spent most of their time around residential areas in the park and often entered houses in search of food (Müller-Graf *et al.*, 1997). This observation has raised concern over the possible source and spread of schistosomiasis in the park, given the close and regular interactions between people and animals in the area. Humans and primates at Gombe live in close proximity to each other while interacting in many ways, including sharing resources such as water and the forest (Wallis and Lee, 1999). The people interacting with animals at

Gombe are mostly researchers and National Park staff and their families. Some of these people, especially those hailing from the local communities around Gombe, would go in and out of the park regularly, with the risk of bringing into the park diseases such as schistosomiasis (Müller-Graf *et al.*, 1997). Since schistosomiasis is a water-borne disease that is often spread through poor sanitation and unhygienic conditions, the situation in the park has for a long time demanded a study to understand the relationship between schistosome strains from humans and baboons living there. The study would help to understand the extent to which the non-human primates such as baboons in this area harbour *S. mansoni* and could possibly serve as potential reservoirs for human schistosomiasis.

Another primate species that is likely to be of significance in the transmission dynamics of schistosomiasis in the Gombe area is the vervet monkey (*Cercopithecus aethiops*). There are two isolated communities of vervets in Gombe National Park that are confined to the northern and southern park boundaries. Since vervet monkeys can also be infected with schistosomiasis (Legesse and Erko 2004) it is worth investigating the role these might play in the transmission dynamics of schistosomiasis in the Gombe area. Moreover, the parasite fauna of vervet monkeys in Gombe National Park has not yet been studied, but data are available from other sites in Ethiopia (Legesse and Erko, 2004), Sudan (Sulaiman, 1986) and Uganda (Gillespie, *et al.* 2004). It would thus be important to find out about the parasites that are harboured by these animals, given their regular interactions with humans and other primate species in the area. Quite often the monkeys leave the park to go into the villages, visiting dumping sites and residential areas in search of food (Figure 4.5). This behaviour puts them at risk of picking up human diseases or spreading their own infections into the human population. The current investigation will help to establish the types of parasites infecting these animals and ascertain whether their infection patterns are related to those of humans and baboons in the area.

1.10 Molecular epidemiology of human schistosomes

Molecular epidemiology, which is the use of DNA-based techniques for detecting and characterizing pathogens, is increasingly becoming a popular practice among disease ecologists and epidemiologists (Tibayrenc, 2007). Due to their sensitivity and specificity in parasite diagnosis and identification, molecular techniques are often preferred over conventional methods in disease and pathogen investigations (Morgan *et al.*, 1998; Tibayrenc, 2007). Several studies have applied these techniques to elucidate various aspects of schistosome epidemiology and genetics, including population size and structure (Rodrigues *et al.*, 2002; Silva *et al.*, 2006), gene flow (Rudge *et al.*, 2008), genetic diversity (Agola *et al.*, 2009) and mating systems (Prugnolle *et al.*, 2004). However, the biology of schistosomes can present unique challenges to researchers studying them and no genetic investigations have been conducted in the Gombe park region.

Adult schistosomes are located in the blood vessels of their vertebrate hosts and this makes it difficult to obtain adequate samples for molecular analysis (Steinauer *et al.*, 2008a). To overcome this, investigators have opted for the long route of hatching schistosome larvae (miracidia) from host stools, infecting snails and then passaging mature larvae (cercariae) through laboratory mice (Curtis *et al.*, 2001b; 2002; Rodrigues *et al.*, 2002; Stohler *et al.*, 2004; Agola *et al.*, 2006). Adult schistosomes would then be perfused from mice and subjected to molecular analysis. However, this route is rigorous, time consuming and ethically limiting due to the involvement of laboratory animals. In addition, sampling bias due to loss of genotypes or selection as the parasites pass through the snail and mice hosts has been reported (Agola *et al.*, 2006; Gower *et al.*, 2007). Hence, in recent years, scientists have chosen to work directly on the accessible stages in the life cycle of schistosomes, such as faecal eggs (Blank *et al.*, 2009) and larvae hatched from the eggs (Steinauer *et al.*, 2008a; Valentim *et al.*, 2009).

Faecal eggs, however, produce low quality and quantity of DNA due to various inhibitors that interfere with DNA extraction and amplification (Guy *et al.*, 2004). Thus, faecal eggs are not always suitable for studying the genotypes of schistosome populations (Blank *et al.*, 2009; Steinauer *et al.*, 2008a). The use of miracidia larvae for this purpose also presents some problems. Miracidia larvae are difficult to isolate from host stools due to their intermittent shedding and requirement for optimum shedding conditions such as appropriate temperature and light (Steinauer *et al.*, 2008a). In addition, the larvae are very small in size and this yields very low amounts of DNA (Steinauer *et al.*, 2008a). It is known that low quality DNA can result in genotyping errors (Gerllof *et al.*, 1995; Pompanon *et al.*, 2005; Gunn *et al.*, 2007). Through modification and improvement of these techniques, information has been obtained on schistosome population genetic structure and patterns of variation. For instance, Steinauer *et al.* (2008a) confirmed the use of microsatellites in genotyping the molecular epidemiology of *S. mansoni* using individual miracidia. Rudge *et al.* (2008) reported evidence of gene flow between *S. japonicum* infecting humans and dogs in the Philippines and China. The latter study corroborated the previously held views that *S. japonicum* can infect animals other than humans (Ouma and Fenwick, 1991; He *et al.*, 2001; Fernandez *et al.*, 2007). It has been confirmed that in addition to humans, *S. japonicum* can also infect cattle, water buffalo, pigs and domestic dogs (He *et al.*, 2001; Fenwick, 2011). In rare cases, *S. mansoni* has also been found to infect baboons and mice in nature with unconfirmed reports that the animal schistosomes may infect humans (Ouma and Fenwick, 1991).

1.11 Molecular studies on schistosome parasites in wild baboon populations

Although it is accepted that baboons and possibly vervet monkeys in the wild can be infected with schistosomiasis (Fenwick, 1969; Müller-Graf *et al.*, 1997; Erko *et al.*, 2001; Hahn *et al.*, 2003; Legesse and Erko, 2004; Howells *et al.*, 2011), very few studies have investigated how the infections are related to those of humans in sympatric or allopatric habitats (Ouma and Fenwick, 1991; Standley *et al.*, 2012). In their recent review on the studies of schistosomiasis in non-human primates, Standley *et al.* (2012) argued that previous investigations have only implicated baboons in the possibility of contributing to the transmission of the disease in humans. They thus observed that it is not known whether schistosomiasis transmission among baboons can be sustained among the animals and to what extent human schistosomiasis can cross over into nearby baboon populations.

It has been suggested that to determine the genetic relationships between schistosome strains from human and non-human primates, the use of modern techniques such as DNA analysis is essential (Ouma and Fenwick, 1991). Few studies have investigated schistosomiasis infections in wild baboons beyond the microscopy approach. For instance, Muchemi (1992) studied human and baboon *S. mansoni* in Kenya, through analysis of the parasite's protein enzymes, and found similar electromorphs of schistosomes from both hosts. However, the study's findings were said to be inconclusive, largely due to the inability of isozyme analysis to deduce the relationship between human and baboon schistosome strains (Muchemi, 1992). The author therefore called for further detailed studies that would use more sensitive techniques, such as DNA probes. This was later reiterated by a study by Müller-Graf *et al.* (1997) on the epidemiology of *S. mansoni* in wild baboons at Gombe National Park in Tanzania. Based on the unusually high prevalence of *S. mansoni* in baboons that had regular contact with humans, the authors recommended an investigation to determine whether baboon schistosomes were genetically

linked to those found in humans in the area. It is still unclear to what extent non-human primates such as baboons and vervet monkeys harbouring *S. mansoni* in sympatric areas with humans can serve as reservoirs for human schistosomiasis. This study was initiated to address these questions by utilizing molecular techniques to assess whether baboon schistosomes are genetically and epidemiologically associated with human schistosomiasis in Gombe area and the neighbouring villages.

1.12 Epidemiological processes for studying schistosomiasis and other vector-borne disease systems

Epidemiology is the study of the distribution of a disease and the determinants or factors influencing its distribution (Harrington, 2010). Like other vector-borne diseases, schistosomiasis transmission is determined by four major factors, including characteristics of the host, the parasite, the vector and the environment in which they live (Wilkins, 1987; Day, 2010). Host characteristics such as sex, age, immunity and economic status (e.g. access to clean water) can influence the rate of disease transmission in humans (Day, 2010; Harrington, 2010). For instance, because of undeveloped immunity, children are more susceptible to schistosomiasis infection than adults (Wilkins, 1987).

Vector survival, longevity and abundance are also critical in disease dynamics as, for instance, snails occurring in high numbers can lead to higher probability of cercariae shedding. A combination of high abundance of cercariae combined with human contact and contamination of water is likely to cause high rates of schistosomiasis transmission (Southgate and Rollinson, 1987). Environmental variables such as temperature, rainfall, humidity, landscape and land use can also influence the transmission of vector-borne infections (Harrington, 2010). For instance, land use changes such as dam construction and retention of water for irrigation can affect the rates of schistosomiasis transmission

(Fenwick, 2011). It is thus critical that any epidemiological investigation undertakes to include in the study all or most of the possible influencing factors in order to obtain a true picture on the patterns of disease infection.

1.13 Epidemiological techniques and measures

Various parameters are used to describe observed infection patterns of vector-borne diseases. The most commonly used measures are prevalence, incidence and intensity (Montresor *et al.* 1998). Prevalence, which is usually expressed as a percentage, is the proportion of individuals in the population that are infected with the disease during a particular period of time. On the other hand, the intensity of infection denotes a measure of the number or burden of worms or parasitic eggs and is obtained by counting the number of worms or eggs of a particular parasite in the host. It is generally impossible to count the number of worms in the field unless the subject under study has been sacrificed. So, in practice, the intensity of infection is determined by counting the number of parasite eggs voided in faeces or urine (Wilkins, 1987). On the other hand, incidence is the proportion of initially uninfected individuals who become infected during a given period of time. The measure is commonly expressed as the percentage of new cases of infection per year or as a proportion of new cases of infection per unit time in relation to those at risk during a period of time (Wilkins, 1987; Harrington, 2010). Incidence measurements are particularly important in the monitoring of control programmes, as reductions in the incidence would indicate drug effectiveness. Despite its usefulness, incidence is difficult to measure because not all cases would be reported, and some cases would not be recognized (Wilkins, 1987). While the failure to recognise all cases would also apply to prevalence and intensity, missed data would have more effect on incidence since its interpretation is based on new infections only. Because of that, incidence parameters are rare in many routine epidemiological investigations, as was the case in the present study

where this measure was deliberately excluded. Instead, prevalence and intensity were used as measures or indicators of infection for *S. mansoni* and other pathogens that were detected.

1.14 Expected outcomes of epidemiological surveys

As described by other workers (Day, 2010; Harrington, 2010; Fenwick, 2011), the general goals of any epidemiological investigation for an infectious disease like schistosomiasis is to confirm its presence, establish its distribution, and determine the risk factors for its transmission. Information on risk factors provides knowledge on the exposure patterns of infection, as well as enabling the planning of successful treatment and control strategies for the diseases. Epidemiological studies are useful for identifying susceptible individuals and for properly targeting control programmes (Harrington, 2010). The studies also generate information that can be used to determine the causes of diseases and for setting out procedures for conducting intervention and control strategies.

1.15 Study areas

I conducted my study in Gombe National Park and the neighbouring villages of Mwamgongo, Bugamba, Kiziba and Mtanga, which are located along the eastern shores of Lake Tanganyika (Fig 1.5). Each village is inhabited by approximately 5000 people, with most of them being engaged in fishing activities in Lake Tanganyika. The villages were chosen for inclusion in the present study because of their proximity to Gombe National Park and hence likely to influence the park. Description of the key features in Gombe is given below, while detailed and specific information for each village has been described elsewhere; Mtanga (Lwoga, 1997), Mwamgongo (Mung'ong'o, 1999) and very briefly about Bugamba and Kiziba (Lombardozi, 2003).

Gombe National Park (4°53' S, 29°38' E at its centre) is made up of unique geomorphologic features, including many hills and stream valleys. The park is a narrow strip of rugged terrain along the lakeshore, with the top of the hills forming its eastern boundary. To the south and north of the park lie Mtanga and Mwamgongo villages, respectively, and the lakeshore forms the park's western boundary (Fig. 1.5). The terrain in Gombe rises from 773 m above sea level at the lakeshore to over 1500 m at the top of the rift escarpment on the east (Goodall, 1986; Müller-Graf *et al.*, 1996). The Gombe vegetation ranges from evergreen forest along the river valleys, deciduous forest and thicket on the upper slopes, to grassland near and 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 (Fig. 1.6A-C; Goodall, 1986). In addition to olive baboons (*Papio cyanocephalus anubis*) and the vervet monkeys (*Cercopithecus aethiops*), both of which were the focus of this study, the park also harbours other primates, particularly the endangered chimpanzees (*Pan troglodytes schweinfurthii*). Baboons live mainly in the forested area of the park, although their home ranges extend to the shores of Lake Tanganyika and to the research camp where park and research staff and their families live. The vervets and some groups of baboons live on the park boundaries, and often raid human settlements and farms outside the park (Goodall, 1986).

Other non-human primates found in Gombe include red colobus monkeys (*Colobus badius*), blue monkeys (*Cercopithecus mitis*) and red tailed monkeys (*Cercopithecus ascanius*). There are also a few other mammal species such the bushpig (*Potamochoerus* sp.), bushbuck (*Tragelaphus* sp.), pangolin (*Manis* sp.), bushbaby (*Galago* sp.) and various reptiles, birds and invertebrates (Goodall, 1986; Collins and McGrew, 1988; Lyogello, 1991).

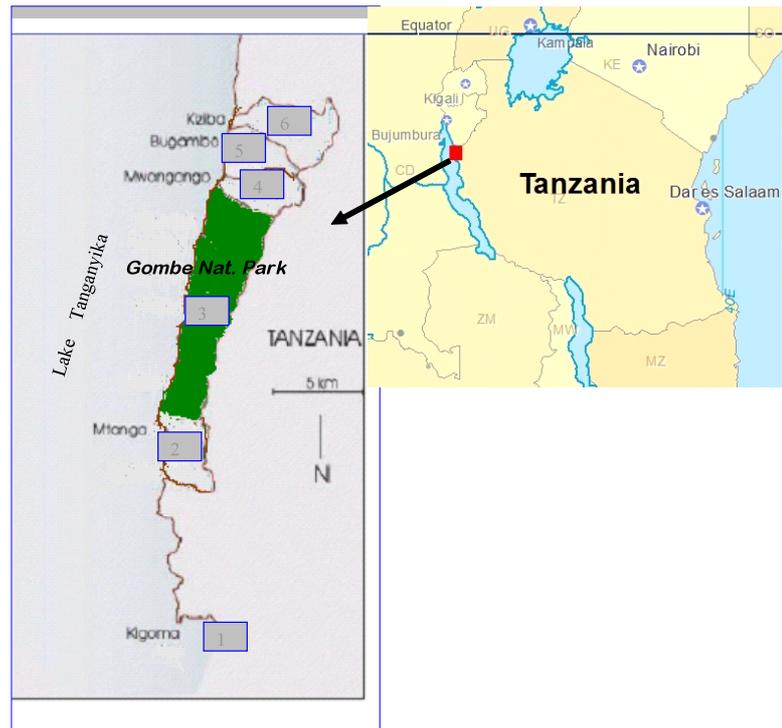


Figure 1.5: Study area with the study sites as shown by numbers in increasing order: Kigoma town (1); Mtanga village (2); Gombe National Park (3); Mwamgongo village (4); Bugamba village (5); and Kiziba village (6). An insert map indicates the geographical location of the study area in the northwest corner of Tanzania close to the Burundi border. Map of study area sites (1-5) was reproduced with permission from Lombardozzi (2003). Insert map, was a courtesy of Lilian Pintea, the Jane Goodall Institute.



A



B



C

Figure 1.6: Some impressive scenery from selected sites in the study area:

From top left to right: An area of Gombe National Park showing the condition of the vegetation during the wet season (A) and the same area in dry season (B). A distant view of the shore area of Lake Tanganyika as viewed from the top of a hill near Mwamgongo village (C).

1.16 Objectives of the thesis

The main objective of my study was to establish the endemism and distribution of *S. mansoni* in western Tanzania and understand the parasite's infection levels in humans and non-human primates in the Gombe ecosystem. The study also aimed to determine the risk factors for schistosomiasis transmission in humans, baboons and vervet monkeys in Gombe National Park. The study had the following specific goals:

- 1) To determine the prevalence and intensity of *S. mansoni* infection in humans in Gombe National Park and surrounding villages and establish how the infection measures vary with season, site and demographic characteristics such as age and gender. The study was also intended to assess the influence of human occupation on the prevalence and intensity of schistosomiasis and determine the perceptions and attitudes of local people on the disease.
- 2) To establish the effectiveness of height as a proxy for estimating the dosage of praziquantel drugs among school children, by comparing the number of tablets given to each child based on their weight and assess the dosage of the drug that would have been recommended based on their measured height. I was also interested in assessing the efficacy of praziquantel drugs in curing and reducing the prevalence and intensity of schistosomiasis among patients in a single treatment incidence.
- 3) To determine the intestinal parasites of baboons at Gombe National Park and evaluate how their infection is influenced by season, baboon troop membership and habituation. The current infection patterns of baboon parasites at Gombe were also reviewed in the context of previous infections in the area. The parasites of vervet monkeys in the area were also analysed, with a view to establishing the species present and their distribution patterns.

4) To assess the distribution and infection status of intermediate snail hosts in Gombe and the surrounding villages using microscopy and molecular techniques. I also aimed to evaluate the effectiveness of combined microscopy and molecular techniques in assessing the prevalence of schistosomiasis infection in snails.

5) In addition, the original goal of my study was to determine the population genetic structure and variation of *S. mansoni* parasites among humans and baboons sharing the same environment in Gombe. However, results from this part of the study were hindered by methodological problems due to difficulties with using DNA extractions from eggs obtained from faeces in humans and baboons. The preliminary findings are presented in Appendix 2

Although both *S. mansoni* and *S. haematobium* pose significant health problems in Tanzania, the present study has focused on the former. Studies indicate that *S. mansoni* can readily be transmitted and maintained in wild baboons while *S. haematobium* is not common among the animals (Ouma and Fenwick, 1991). It was thus reasonable to focus the study on *S. mansoni* so as to achieve the scope and goals of the study. This study is part of a larger epidemiological programme aimed at uncovering the factors influencing parasite and disease transmission among humans and wildlife at Gombe National Park. Results from this study will contribute to the understanding of the patterns of infection of not only schistosomiasis but also other diseases that could be shared between humans and non-human primates in Gombe. It will thus provide useful information for preventing disease transmission between humans and wild animals.

1.17 Outline and structure of the thesis

This thesis includes six chapters, four of which are data chapters, each covering a different inter-related research theme. Chapter one gives a general introduction and background to the research problem, highlighting on the study objectives and goals. This chapter finishes with a summary of the structure and outline of the thesis. Chapter two covers the human component of schistosomiasis infection in the Gombe ecosystem, while chapter three deals with praziquantel distribution for treating schistosomiasis among school children in the study area. Infection trends of schistosomiasis and other helminthiases in non-human primates (baboons and vervet monkeys) in the Tanzania's Gombe National park are covered in chapter four. Chapter five explores the use of both conventional microscopy and molecular techniques to study levels of schistosomiasis infection in snails. Chapter six gives a general overview on schistosomiasis infection in the Gombe ecosystem and finishes with concluding remarks and recommendations on how to manage the disease in the area. Appendix 2 discusses the approaches attempted to assess population genetic structure of schistosomes in humans, baboons and snails. Since the technique did not produce interpretable results (due to problems with mixed infections and poor quality of DNA from eggs), materials in the appendix are presented as a guide for other researchers.

**Chapter 2: Epidemiological Patterns of Intestinal Schistosomiasis among
Communities along the shores of Lake Tanganyika, Tanzania**

2.1 Abstract

Intestinal schistosomiasis due to *Schistosoma mansoni* poses a major public health problem in Tanzania. Despite that, its distribution and transmission risk factors are not well known, particularly in the country's western areas. This study was initiated to: 1) determine the current infection levels of *S. mansoni* in Gombe National Park and the neighbouring villages of Mwamgongo, Bugamba, Kiziba and Mtanga; and 2) relate the infection prevalence and intensity to locality, host sex and age. Between January and September 2010, stool samples were collected from 235 children and 171 adults and examined for parasite ova using the Kato-Katz technique. *S. mansoni* infection was recorded at an overall prevalence of 45% across study sites, ranging from 19% at Mtanga to 68% at Mwamgongo. The mean intensity was 18 eggs per Kato Katz slide, ranging from 1 to 225 eggs and a median of 8 eggs. Generalised Linear Models were used to analyse parasite data, applying binomial distribution models for prevalence and Zero-inflated negative binomial (ZINB) for intensity. A hierarchical analysis for the best fitting model indicated that host sex did not influence infection variation, while age and site interacted strongly. The analysis showed significant variation of *S. mansoni* prevalence between age groups, which also depended on site. ZINB analysis results showed also that egg counts significantly varied between age groups and across study sites. These findings indicate a high infection of intestinal schistosomiasis in the study area, suggesting that the distribution of the disease in the country could be more widespread than previously thought. They also confirm that the infection of intestinal schistosomiasis in the area is focal, with marked variations between adjacent villages. The wide implications of these findings and the observed variation in *S. mansoni* infection between age groups and study sites are discussed.

2.2 Introduction

S. mansoni infection constitutes a major public health problem in Tanzania as a causative agent of intestinal schistosomiasis and its associated morbidity (Malenganisho *et al.*, 2008). Moderate to severe levels of *S. mansoni* infections have been reported in most parts of the country except for the eastern coastal areas (Kardorff *et al.* 1997). The severity of this infection (that is the degree of its damage to the body) is revealed in the observed high prevalence of gastrointestinal symptoms, hepatosplenic problems and morbidity among adults in some areas along the shores of Lake Victoria (Malenganisho *et al.*, 2008). The impact of schistosomiasis is exacerbated by its co-infection with other pathogens and diseases due to synergistic effect (Sangweme *et al.*, 2010). Sangweme *et al.* (2010) found that the density of malaria gametocytes in blood tended to be higher among children with schistosome-malaria co-infection in Zimbabwe. They thus concluded that the co-infection of schistosome was likely to lead to more transmission of malaria than would be the case with malaria only infection. High rates of co-infection of *S. mansoni* with the malaria parasite, *Plasmodium falciparum* have also been reported, particularly among school children along the shores of Lake Victoria in north-western Tanzania (Mazigo *et al.*, 2010b). Lwambo *et al.* (1999) also reported a co-infection of *S. mansoni* with hookworms among school children in Magu District around Lake Victoria. The occurrence of schistosomiasis with other diseases is known to aggravate the impact that each of the infections will have on the patient (Shapiro *et al.*, 2005). Intestinal schistosomiasis therefore presents a formidable health challenge in Tanzania and the efforts aimed for its control are justified.

Currently, the geographical distribution of *S. mansoni* in Tanzania is not fully known (Rugemalila, 1991; Brooker *et al.*, 2009). Accurate and reliable information on the distribution of schistosomiasis in the country is mainly available for the northeast and Lake Victoria areas, which have been extensively studied due to their accessibility and improved

infrastructure (Brooker *et al.* 2009). According to Brooker *et al.* (2009) there is little knowledge on *S. mansoni* distribution in the southern and western areas of Tanzania such as the Kigoma region (Brooker *et al.*, 2009). Information on schistosomiasis infection in this area has been obtained predominantly from hospital records, which are likely to be inaccurate and unreliable. Hospital records are often subject to multiple sources of uncertainty such as poor recording (Gething *et al.*, 2006) and lack of random sampling and are therefore unreliable for estimating population health attributes such as prevalence (Wang *et al.*, 2010).

The goal of this chapter is to determine the prevalence and intensity of *S. mansoni* infection in humans in Gombe National Park and surrounding villages and establish how these infections vary with season, site and host demographic characteristics such as age and sex. Given that schistosome transmission is highly dependent on human activities such as fishing and farming, this chapter also assesses the influence of human occupation on the prevalence and intensity of schistosomiasis. In the context of schistosomiasis being a neglected disease, this chapter concludes with an investigation on the perceptions and attitudes of local people to the disease.

2.3 Materials and Methods

2.3.1 Ethical considerations and approvals

Ethical Clearance (Ref. No. NIMR/HQ/R.8a/Vol.IX/892) used during this study was issued by the Tanzania's National Institute for Medical Research (NIMR). The permission to conduct the survey in villages and schools was obtained from the Executive Director of Kigoma District (Ref. No. KDC/G1/6/70), based on an introductory letter/research clearance (Ref. No. AB3/3(B) issued by the University of Dar es Salaam on behalf of the Tanzania Commission for Science and Technology (COSTECH). The study involved working with human subjects, so it was conducted following standard procedures and ethical guidelines, as stipulated in the ethical clearance and according to WHO's guidelines (<http://www.ufrgs.br/bioetica/cioms2008.pdf>). Before collecting stool samples full consent was obtained from adults and guardians and teachers for children. A meeting was held with the health and school personnel and the village leaders to discuss the objectives of the study and its benefits to the communities involved. The village executive officers (VEOs) were then asked to inform people in their respective villages about the survey, asking them to volunteer in the exercise. Those wishing to participate in the study were asked to assemble at the village clinics or other designated locations for instruction on sample collection (Figure 2.1A). On the survey day, those who consented were registered, weighed and asked a few questions such as their age, occupation, health status and area of residence in the village (Figure 2.1A). This information was entered onto Annex 4 of the Helminthiasis and Schistosomiasis School Survey form that was slightly modified with permission from the World Health Organization (Appendix 1).

For children, an oral assent was obtained from each child on whether or not they were willing to take part in the study, even after their parents had agreed for them to do so. The children not wishing to be sampled were excluded from the study and the willing were registered, as for their parents above (Figure 2.1B). An extra parameter was measured for children, which involved taking their height in addition to weight. School children were registered at their respective schools in all study sites except at Mwamgongo, where the children came to the village health centre, as it is located close to the school. Non-schooling children were registered alongside accompanying adults but information collected about them was as for school children. For both adults and children, no reward or compensation was promised for providing samples but all people infected with schistosomes or other helminths were treated free of charge, in compliance with the study's research and ethical clearance requirement. Registration of study participants and subsequent treatment of infected people were conducted in collaboration with health and medical personnel from the local health centres or clinics in each village (Figure 2.1A and B). Confidentiality was observed during sample collection and analysis and participant identifiers such as names were removed from the database before analysis, rendering all of the samples anonymous.



A



B

Figure 2.1: Registration of participants for collection of stool samples: (A) adults and (B) school children

2.3.2 Sample selection and sampling strategy in humans

This was a cross-sectional study where the goal was to sample 540 participants consisting of 290 adults and 250 children. At least 120 individuals would be selected randomly from each of the 4 villages with equal numbers of adults and children. The number of children from Gombe National would be less than 60 targeted from each village. This is because the park's residents during the sampling period were fewer than the 120 sample size set for each site. The park's demography is different from the villages, with fewer children than adults at any time. There is no school in the Gombe so children living there have to be sent away for schooling in other areas. Thus, a target of at least half the village sample size was set i.e. 60 individuals, including all 10 children that were living in the park at the time who were aged one year and above. The overall sample size (i.e. 540 participants) exceeded the minimum range of 200-250 individuals recommended by the World Health Organization for establishing baseline data on schistosomiasis prevalence and intensity in homogeneous geographical areas (Montresor *et al.*, 1998). Age categories for participants were set according to the Tanzanian law, where anyone aged 18 years and above is considered an adult while a person aged 1-17 years old is a child. For administrative purposes, each

village is divided into sub-villages, hereinafter referred to as sub-divisions or *vitongoji* in Swahili, singular: *kitongoji*. Village sub-divisions or *vitongoji* were used as selection units for adults and non-school children individuals. A cut-off point of at most 10 individuals from each sub-division (*kitongoji*) for each village was set so as to have representation from all parts of the village.

For school children, one school was selected from each village and 60 children were selected from Standard three in the school. Standard three pupils were chosen because they represent the median age of primary school children in Tanzania (9-12 years). For schistosome survey in schools, the World Health Organization (WHO) also suggests sampling third year primary school classes, as results obtained are comparable with other studies and are also useful for control programmes (Montresor *et al.*, 1998). For cross-sectional surveys of schistosomiasis, WHO also recommends studying children aged or close to 9-10 years (Montresor *et al.*, 1998). Schools were selected based on proximity to the clinic laboratory where samples were processed or to strategic location in the village. For the latter, preference was given to schools enrolling children from across the village so as to obtain a representative sample of the village's sub-population of children. In schools where a class size was less than 60, all children in the class were sampled to achieve the target sample size. In cases where Standard three pupils were fewer than 60 children, additional subjects were recruited from Standard four. For villages where the number of people who turned up for sampling exceeded 120, participants were selected on first-come first-served basis. In the end the sample sizes obtained for each site were lower than planned (Table 2.1).

2.3.3 Collection of stools

A single fresh stool sample was collected from each participant per season; i.e., only one sample during the wet season (January to May consecutively) and another in the dry season (July to September consecutively). On the sampling day, sampling materials were distributed to each participant and instructions given on the collection protocol. The materials included a wooden spatula for picking up a stool sample, a plastic vial (120 ml) for depositing the stools and a polythene plastic bag for carrying the samples back to the laboratory. The sampling materials were distributed in the morning of day one and collected on the morning of the following day. The vials for depositing stools were labelled with the provider's name, sex, village and sub-village name, as well as collection date. For school children, age was taken from the school register and from medical clinic cards for non-enrolled children. Age for children who lacked this information in their register books was obtained by asking them to state their age or year of birth. The exact age of both adults and children was rounded up to the nearest year. The number of participants sampled in wet season is shown in Tables 2.1.

2.3.4 Microscopical examination of human stools

In the laboratory, stools were examined for *S. mansoni* and other helminths using the Kato-Katz kit (Bio-Manguinhos, Rio de Janeiro, Brazil), in addition to the guidelines in Ebrahim *et al.* (1997) and WHO (1991). The Kato-Katz technique, also known as the cellophane thick smear examination, is widely used for diagnosis in epidemiological studies of schistosomiasis (and soil-transmitted helminthiasis). The technique was chosen over other techniques because of its ability to produce both qualitative and quantitative measurements of infection. The technique enables the examination of a large quantity of faeces per slide (50-60 mg) without the need for concentration procedures required for other techniques (WHO, 1991; Ash and Orihel, 2007). In addition, the Kato-Katz technique is cheap and

easy to use and it was thus an appropriate method to use in resource-poor areas where the present study was conducted.

Procedures for the Kato-Katz method involved pressing each faecal material through a mesh screen filter to keep away large particles. A template with hole that takes a fixed amount of faecal material for quantifying the number of schistosome eggs in the faeces was placed on a microscope slide. The filtrate from above, was then filled into the template hole as suggested in WHO (1991) and Montresor *et al.*, (1998). The template was removed leaving the faecal material on the microscope slide. A hydrophilic cellophane previously soaked in glycerol-malachite green solution was then placed on the faecal material. Glycerine clears the faecal debris, rendering the parasite eggs visible, while malachite green provides a contrasting background for observing the eggs (WHO, 1991). The faeces, covered with a cellophane strip, were then pressed between two microscope slides to spread out the sample for easy observation of parasite eggs. The bottom slide was then stood upright and left to clear for 30-60 minutes. After clearing, the slide was placed under a compound microscope and the entire preparation examined using a 10 x objective. Each microscopical field on the slide was systematically and completely examined by moving the slide up and down in a zigzag fashion before moving to the next field (Figure 2.2; WHO, 1991). Observed parasite eggs or larvae were photographed by holding a digital camera onto one of the two eyepieces on the microscope. Due to limited time and personnel, a single Kato-Katz thick smear slide was examined for each stool and the number of parasite eggs reported as eggs per gram of faeces (epg). Given the technical and financial costs for using the Kato-Katz, it has been suggested that accurate epidemiological measures for schistosomiasis infection can be obtained using a single Kato-Katz smear supported with large sample size from the population and full examination of each slide (Figure 2.2; Ebrahim *et al.* 1997; Engels, 1997).

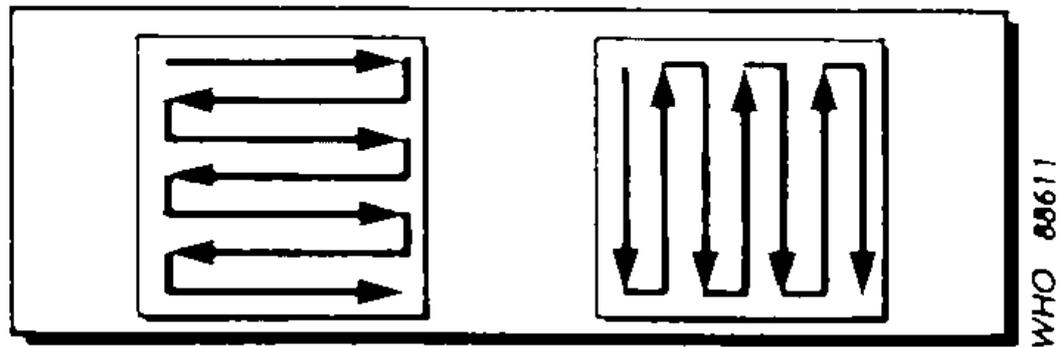


Figure 2.2: A zigzag pattern indicating the recommended up and down or side ways movement of a slide during parasite examination under a microscope.

Reproduced from WHO (1991) with permission from the World Health Organization

2.3.5 Parasite identification

All schistosomes and other helminth eggs observed on each slide were identified and counted based on standard guidelines (WHO, 1991; Cheesborough, 1998). The eggs of *S. mansoni* were easily identified based on their distinguishing lateral spines, while other helminth eggs were identified based on their morphology, size and appearance of eggs/larvae (WHO, 1991; Cheesbrough, 1998). Observed parasite eggs or larvae were photographed by holding a digital camera onto one of the two eyepieces on the microscope. The pictures would later be used in further confirmation of the parasite's identity. As a quality control strategy, 10% of randomly selected Kato Katz slide smears were sent for re-examination by an experienced laboratory technician at the Tanzania's National Institute for Medical Research in Mwanza. The technician did not have prior information on previous results for the slides.

2.3.6 Distribution of praziquantel drugs to schistosome infected participants

Most participants who were diagnosed with schistosome eggs were treated with praziquantel drugs and counselled about schistosomiasis transmission. The drugs were distributed to school-children at their respective schools, while treatment for adults and non-school children was provided through health centres in their village. Participants who did not receive praziquantel drugs in the wet season but were re-sampled in the dry season were used to assess the impact of season on *S. mansoni* infection. Participants who had received praziquantel treatment were excluded from the seasonal analysis so as to avoid the confounding impact of the drugs on season. A summary of participants sampled before and after praziquantel distribution is given in Table 2.2. A comprehensive analysis on the impact of praziquantel treatment on the prevalence and intensity of schistosomiasis and the usefulness of a tablet pole in estimating praziquantel dosage for school children is given in chapter 3.

2.3.7 Isolation of schistosome eggs for DNA extraction

Before praziquantel distribution, additional stool samples were collected from infected individuals for isolating schistosome eggs and larvae for molecular analysis. Stool samples for DNA extraction were collected from randomly selected individuals with 14 or more schistosome eggs per Kato-Katz slide as suggested in Sorensen *et al.* (2006). A total number of 41 individual samples were obtained, with representatives from each participating village except Mtanga and Gombe where the number of schistosome eggs per Kato-Katz slide was too low for this sampling to be conducted. The original goal was to identify the genotype of the human schistosome species present and compare it with schistosomes sampled from baboons. Among those re-sampled, 21 came from the most infected village (Mwamgongo) and was comprised of 19 school age children and two adults. Another 15 samples came from school children at Bugamba village, while five

others were obtained from school children at Kiziba. The samples were kept in dark containers immediately after collection to prevent hatching of parasite larvae (miracidia) before reaching the laboratory. Laboratory procedures described in Steinauer *et al.* (2008a) and Blank *et al.* (2009) were slightly modified to isolate schistosome larvae and eggs from pooled samples (that is all of the eggs in each stool sample put together). Approximately 5g of stools was mixed with filtered stream water and then the homogenate was passed through a series of graded sieves (800 and 212 μm) to remove large debris and then through a 38 μm sieve to collect eggs (which are approximately 100 μm x 70 μm in size; Cheesbrough, 1998; Ash and Orihel, 2007). The filtrate containing parasite eggs was washed into a 15ml centrifuge tube filled with distilled water to the rim. The mixture was then centrifuged at 3000 rpm and cleaned by getting rid of the supernatant and adding new water. Part of the pellet was observed under the microscope to confirm the presence of schistosome eggs. The tube containing the pellet was then fully filled with distilled water and the homogenate exposed to sunlight for 1-4 hours to hatch the parasite larvae. Possibly due to the low number of schistosome eggs in the stools and other factors, such as insufficient illumination, none of the faecal eggs hatched. The unhatched eggs were isolated by centrifuging the homogenate and discarding the supernatant. The pellet (approximately 1g) containing schistosome eggs and possibly eggs of other parasites was then mixed with 3ml RNA-later solution (QIAGEN GmbH, D-40724, Hilden, Germany) to preserve them. The samples were kept at room temperature until required for DNA extraction.

2.3.8 DNA extraction of trematodes from faecal schistosome eggs

DNA was extracted from the faecal pellets described above (in the molecular biology laboratory at the University of Dar es Salaam) following Verweij *et al.* (2001) and by modifying and combining some procedures in the Qiagen DNeasy Blood Spin-Column

Protocol (2006) and QIAamp DNA stool (2010) kits (QIAGEN, Hilden, Germany). The unweighed faecal samples (approximately 1g) preserved in 15 centrifuge tubes containing 3ml RNA-later solution were shaken vigorously to mix the suspension well, as suggested in Cheesbrough (1998). For samples that were still in solid form, a wooden spatula was used to break the stool into the solution, while a pipette with a cut tip was used to draw 200 μ l from watery stools. The mixture was then centrifuged at 3000 rpm for 1 minute to remove the preservative. The supernatant was discarded and 200mg of the sediment (containing schistosome eggs) measured using a digital scale (Sartorius, Göttingen, Germany). The sediment was transferred into a 2ml vial and mixed with 180 μ l Buffer ATL (instead of ASL recommended in QIAamp DNA stool kit). The suspension was then vortexed for five minutes to mix it thoroughly and one quarter of an InhibitEX tablet (QIAGEN, Hilden, Germany) added to remove DNA inhibitors in the stool. Then 200 μ l of distilled water was added to the solution to dissolve the InhibitEX tablet. Stool particles and inhibitors (bound to InhibitEX matrix) were removed as pellets after centrifuging the suspension at 14,000 rpm for 3 minutes. Then, 200 μ l of the supernatant was pipetted and transferred into a 1.5ml microcentrifuge tube. This solution was mixed with 20 μ l proteinase K and incubated in a water bath at 56°C for 3 hours to lyse the cells.

Following incubation, 200 μ l Buffer AL and 200 μ l Ethanol was added to the solution, which was then vortexed to mix it well. The mixture was then pipetted into DNAeasy spin columns placed into 2ml collection tubes. The solution was centrifuged at 8000 rpm for one minute and the DNAeasy spin column was retained while throwing away the collection tube containing the flow-through. The DNAeasy spin column was placed into a new 2ml collection tube, followed by an addition of 500 μ l of Buffer AW1 to wash the DNA. Washing was performed by centrifuging the DNAeasy spin column placed into a new 2ml collection tube at 800 rpm for one minute. The DNAeasy spin column was kept and the collection tube and flow through were discarded. This procedure was repeated

using buffer AW2 and the solution centrifuged at 14000 rpm for three minutes. The collection tube and flow-through were treated as above, while the DNAeasy spin column was placed into a clean 1.5 ml microcentrifuge tube. Finally, 150µl Buffer AE was added directly to the DNAeasy membrane in the DNAeasy spin column and the solution incubated for one minute at room temperature. This was then centrifuged at 8000 rpm for one minute to elute the DNA. The elution process was repeated by adding 50µl Buffer AE to increase the DNA concentration. DNA was then kept frozen at -20°C until required for PCR amplification. No controls were used during the DNA extraction process. Negative control during DNA extraction which is usually distilled water is essential to check for contamination in the reagents used. Thus the lack of control in this particular instance is may have affected the overall quality of the samples and subsequent results.

2.3.9 PCR and sequencing to confirm trematode species identity

PCR amplification of two regions of the ribosomal RNA array was used to assess the identity of parasites diagnosed as trematodes based on microscopy. PCR targeting a conserved region of the small subunit rRNA (18S gene) was first performed, as recommended for schistosome DNA in Melo *et al.* (2006). A nested PCR approach was taken, using the primers designed by Melo *et al.* (2006): Schfo11 (5' GTTACGATCAGGACCAGTGT 3'), which is specific to *S. mansoni*, was used with the conserved primer Unvre16 (5' CCGGACATCTAAGGGCATCA 3') in the first PCR round, while Schfo17 (5' TGCTGGTGGGTTGACGAGTTC 3') and Schre19 (5' CTAAACGAGCACAGAGGAC 3'), which are both specific to *S. mansoni*, were used as internal primers in the second round.

Secondly, the primers ETTS2 (5' TAACAAGGTTTC CGTAGGTGAA 3') and ETTS17 (5' CGAGCCGGATGATCCACCGC 3'), designed by Stothard *et al.* (1996), were used to

amplify a region of the internal transcribed spacer region (ITS1). These primers are in conserved regions, to allow simultaneous amplification of host DNA, as well as pathogens present in the extractions. However, the ITS region should evolve at a faster rate than the 18S gene, which should allow finer-scale resolution of sequence variation.

For both sets of primers, 20 μ l reactions were set up using 1 μ l of 10 mM solutions of each primer, 2 μ l of 10X buffer, 1 μ l of 50 mM MgCl₂, 2 μ l of 2 mM dNTPs, 0.2 μ l of 500 Units per μ l Taq (Invitrogen, Inc), and 2 μ l of DNA, made up to the full volume using double distilled H₂O. For the second PCR round, 2 μ l of the PCR products from the first round was used. The PCR profile for both rounds consisted of an initial denaturing step at 95°C for three minutes, followed by 34 cycles of denaturing at 95°C for 45 seconds, annealing at 55 °C (65°C for the second round of PCR using the nested primers) for 60 seconds, and extension at 72 °C for 60 seconds. The cycle was completed with a final extension at 72°C for 5 minutes and then holding at 10°C. For each set of reactions, a negative control (using distilled water) and positive control (using DNA from an identified *S. mansoni*) was run to check for contamination in the reagents used.

PCR products were run on 2% agarose gels prestained with 1.2 μ l of 10 μ M Ethidium Bromide and visualized using a Gel Doc system (Bio-Rad Inc., Hemel Hempstead, UK), with Quantity One Software. Amplified products were compared to a 100 bp DNA ladder (Promega, Madison, USA) to determine whether the DNA fragments were of the size expected for schistosomes (Melo *et al.*, 2006). Bands of the appropriate size were excised from the gel and purified using Qiagen Gel extraction kits, using the manufacturer's protocol (Qiagen, Inc) prior to sequencing. Purified gel bands were sequenced on an ABI 3730 automated sequencer at the Sequencing Service, University of Dundee. Chromatographs were visualised and base-calling errors corrected using Sequencer version 4.5 (Gene Codes, Inc.). Sequence identity was confirmed using NCBI BLAST to identify

the most similar sequences deposited to the Genbank database. The preliminary findings on the sequencing of humans and baboon schistosome DNA are presented in Appendix 2

2.3.10 Data analysis

2.3.10.1 Estimation of the prevalence, intensity and mean intensity of schistosomiasis infection

The use of the terms prevalence, intensity (egg count) and mean intensity as indicators of parasite infection followed Bush *et al.* (1997) and Montresor *et al.* (1998). Prevalence (expressed as a percentage) was obtained as the proportion of infected individuals out of all examined subjects at each site. The intensity of infection was taken as the number of *S. mansoni* eggs in each Kato Katz faecal smear in each infected individual; i.e., eggs per gram of faeces (epg). Other terms that would be synonymous to intensity here are worm burden, parasite load, and degree, level, or extent of infection but to avoid ambiguities, their use is uncommon (Bush *et al.*, 1997). At the population level, mean intensity of infection was expressed as an arithmetic mean obtained as the sum of each individual's intensity (egg counts) divided by the number of infected individuals (Bush *et al.*, 1997; Montresor *et al.*, 1998) and calculated as: arithmetic mean = $\Sigma \text{epg}/n$, where: epg is the egg count from each infected individual and n is the number of infected subjects (Montresor *et al.*, 1998). The arithmetic mean is more informative and has clearer biological interpretation than geometric mean. For instance, while Montresor *et al.* (1998) suggest the use of either arithmetic mean or geometric mean for monitoring infection levels in control programmes, Ro'zsa (2000) recommended the use of the former as it is more informative than the latter.

Studies on *S. mansoni* and confirmation of its existence in humans depend on the collection and examination of faecal stools for the presence of ova. It has been accepted that parasite eggs counts in faecal stools can indicate the intensity of schistosome infection

(Engels, 1997; Montresor *et al.*, 1998; Seivwright *et al.*, 2004). Several studies have also utilised parasite egg counts values (infection intensity) as indicators of worm burden (Seivwright *et al.*, 2004). Ideally, worm burden would be obtained by counting the number of worms or all of the eggs of a particular parasite in a host (Engels, 1997). It is impossible to obtain total worm count of parasites in an individual host without sacrificing them. Instead, egg counts are commonly used as a proxy for intensity, since a linear relationship has been found between them (Anderson and Schad, 1985; Sithithaworn *et al.*, 1991; Seivwright *et al.*, 2004). For example, Seivwright *et al.* (1991) concluded that, despite some limitations, faecal egg counts can reliably estimate the intensity of *Trichostrongylus tenuis* in red grouse (*Lagopus lagopus scoticus*). Other studies in humans have also established a relationship between egg counts and worm recovery after anthelmintics delivery (Forrester and Scott, 1990; Elkins *et al.*, 1991). Although egg counts may work with certain species of parasites, it is not necessary that the same would with schistosome eggs because of their different biology. This is therefore one of the limitations for the present study.

2.3.10.2 Statistical analyses

Paraziquantel drugs were provided to all infected participants in the wet season. As a result, very few of them turned up for sampling after treatment in the dry season (Table 2.2). Hence the data collected in the dry season were not sufficient for robust analysis compared to wet season samples. Hence, statistical analysis for the impact of site, age and sex on schistosome infection was based only on samples collected in the wet season. The analysis of only wet season samples also served to avoid the confounding influence of praziquantel treatment on parasite infection, which was applied in the wet season but should still have affected numbers in the dry season. Dry season samples were only analysed to assess seasonal effects on schistosome infection by comparing samples from individuals examined in the wet and dry season who but did not receive praziquantel drugs.

Age was used as a discrete (categorical) variable with two levels; i.e., adults and children because 92% of the latter sampled were school children aged between 9 and 17 years, while only 8% consisted of non-enrolled children aged between 2 and 8 years. Due to insufficient data, host occupation and health status as related to schistosomiasis infection were not subjected to statistical analysis. They were instead presented graphically to show their relationships with infection levels. Data analysis was performed using the Generalized Linear Models (GLM) approach which has been described elsewhere (Wilson and Grenfell, 1997). The analysis was implemented in the programming environment R version 2.12.0 (Venables *et al.*, 2008) and run as in Zuur *et al.* (2009). Plotting the frequency distribution of egg count data suggested an overdispersed pattern, with an excess of zero counts (Figure 2.3). This is consistent with a zero-inflated negative binomial distribution, where there are expected to be large differences in egg counts (intensity) between individual hosts (variance > mean) and the presence of many zero counts. Zero egg counts data were excluded when calculating the mean of infection and for drawing graphs but were included in the statistical analysis where they could be accommodated by the zero-inflated negative binomial models used.

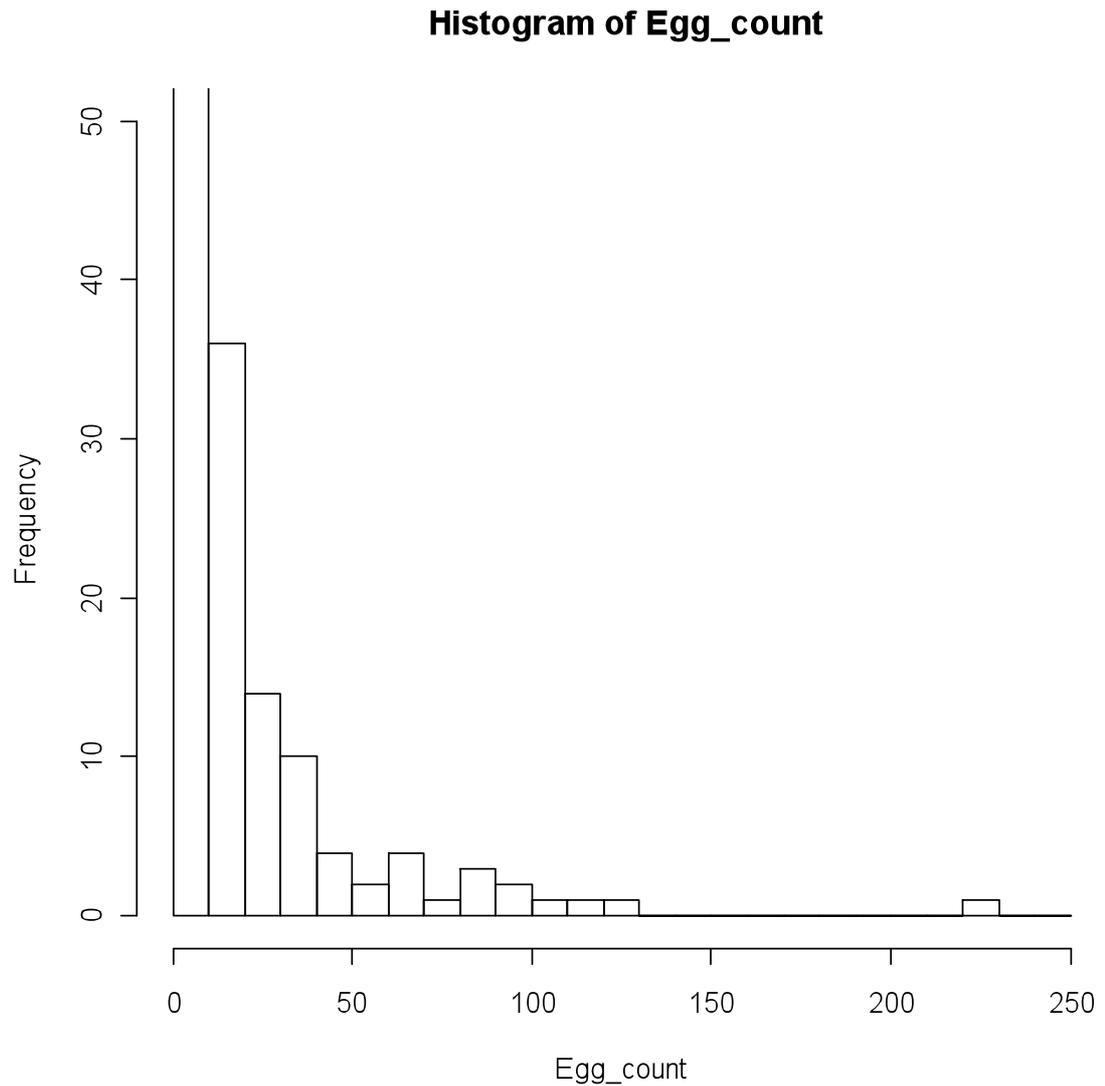


Figure 2.3: Frequency distribution of *S. mansoni* egg counts.

The distribution indicates an overdispersion feature of the parasite's egg counts i.e. variance (440) > mean (18) and a zero-inflation pattern shown by the presence of excess zeros in the first bar on the extreme left.

Prevalence data (i.e. presence or absence) were analysed using a GLM assuming a binomial distribution (Zuur *et al.*, 2009). The analysis of count data involved several steps as described below. Data were not transformed because as suggested in Ro'zsa (2000), altered data are less biologically informative compared to untransformed data. Instead,

two candidate distribution models that are often used for parasite count data as recommended in Zuur *et al.* (2009) were compared: 1) the zero-inflated poisson model (zip) and 2) the Zero-Inflated Negative Binomial models (zinb). As the ZIP and ZINB are nested models, the best fitting model for analysing parasite egg counts (intensity) was evaluated using likelihood ratio tests. The model testing was performed in the R package pscl, which is used for zero inflated models (Jackman, 2011; <http://CRAN.R-project.org/package=pscl>). A likelihood ratio test was applied to test the best-fitting model under the null hypothesis that they have equal variance. Results showed that variance between the two models was significantly different ($p < 2.2e-16$); the ZINB was the better distribution model for the data due its higher log Likelihood ratio (-881.1) compared to the ZIP model (-3848.0). Throughout the analysis, a statistical significance level was taken at <0.05 .

The use of a combined zero-inflated (ZI) and negative binomial (NB) distribution models adds value to the strength of the test and the two models complement each other (Nødtvedt *et al.*, 2002). This supports the review by Cameron and Trivedi (1998) that the same factors causing variance in egg counts (overdispersion) will also cause excess zeros. Using the Vuong Non-Nested Hypothesis Test-statistic (Ridout *et al.*, 1998), comparisons of the difference in likelihoods under a chi-square distribution indicated that the zero-inflated model is a significant improvement over a standard negative binomial model. The test-statistic results under the null hypothesis that the models are indistinguishable showed that model1 (ZI) $>$ model2 (NB), $df = 405$, $p = 8.877415e-06$. For both prevalence and count data, a hierarchical model selection approach was used, starting with the most complex model containing all explanatory variables i.e. host site, age (categorical) and sex and their interactions. A “drop-one” command in R was used to compare the relative goodness of best-fitting model by comparing the Akaike information criterion (AIC) values.

Interactions that increased the AIC score when removed were considered to be integral to the model and so were retained as shown in Table 2.3 for prevalence and Table 2.4 for egg counts (intensity).

2.4 Results

2.4.1 Occurrence of helminths in the population: General trends

In total, 540 people were enrolled in the study and had received sampling materials, but in the end only 406 of them (75% participation rate) returned the materials with valid and recordable samples during the first sampling period in the wet season (Tables 2.1 and 2.2). Out of these, 235 were children (<1-17 years old) and 171 were adults aged between 18 and 83 years (Table 2.1). The number of samples obtained from adults was lower than those of children due to low compliance by the former (Table 2.2). The samples examined indicated the presence of *S. mansoni* eggs, which were unambiguously identified based on their characteristic lateral spine (Figure 2.4). Sequencing of the small subunit rRNA region of the DNA extracted from eggs later confirmed the parasite to be *S. mansoni*. In addition to *S. mansoni*, a few geo-helminth species were diagnosed at very low prevalence including hookworms (Figure 2.5; 2.96%), *Trichuris trichiura* (Figure 2.6; 1.49%) and *Ascaris lumbricoides* (Figure 2.7; 0.99%). Due to low infection rates, these parasites were excluded from statistical analysis. Overall, *S. mansoni* prevalence was 45% across study sites, ranging from 19% in the least infected site to 68% in the most infected site. Schistosome eggs directly counted in individual participants ranged from 1 to 225 eggs per Kato Katz slide with a median of 8 eggs per Kato Katz slide, while the mean intensity of infection at the population level was 18 eggs per gram (epg) of faeces.

Table 2.1: The five study sites (Site column) covered in the present study indicating the number of participants from each site during the wet season, 2010.

Participants are categorized under girls and boys (<18 years old) and females and males (\geq 18 years old) to show the sample size for each category (N = 406). The sample size collected from each site is given in the Total column to the right where most participants came from Mwamgongo while the least number was from Gombe National Park.

Site	Girls (<18 years old)	Boys (<18 years old)	Females (\geq 18 years old)	Males (\geq 18 years old)	Total
Mwamgongo	37	23	19	30	109
Bugamba	30	26	14	13	83
Kiziba	22	40	14	3	79
Mtanga	26	22	21	15	84
Gombe Nat. Park	5	4	14	28	51

Table 2.2: Marked variation in compliance to sampling and treatment in children and adults.

The category of participants is shown as adults or children (Age category) and the number and percentage of those who cooperated in the initial sampling (Returned sampling materials) in relation to those who had been enrolled for the study (250 children and 290 adults), turned up for treatment (Treatment attendance) and participated in the post treatment sampling (Post-treatment). The table demonstrates clearly that children had more compliance than adults in all three stages of the study.

Age category	Returned sampling materials with samples (wet season)	Treatment attendance	Post-treatment (dry season)
Children	235(94%)	121(48.4%)	120(48%)
Adults	171(59%)	32(11%)	41(14.1%)



Figure 2.4: Schistosome eggs (x400) observed on a Kato-Katz thick smear slide and identified for its size and the presence of lateral spine.

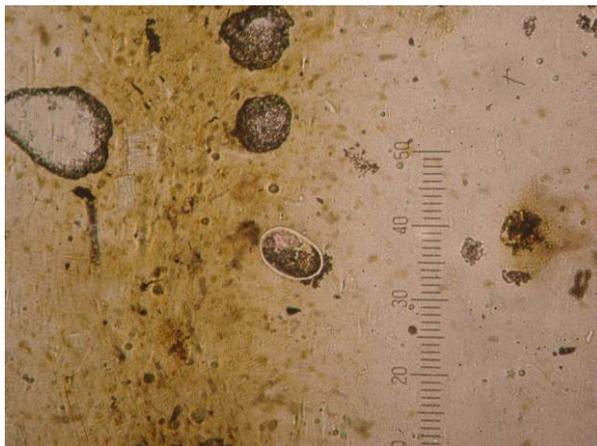


Figure 2.5: A hookworm egg (x400) isolated from a human stool in Mwamgongo village.

The egg is characterized by a blunt ended smooth shell surrounding segmented contents of an early cleavage stage.

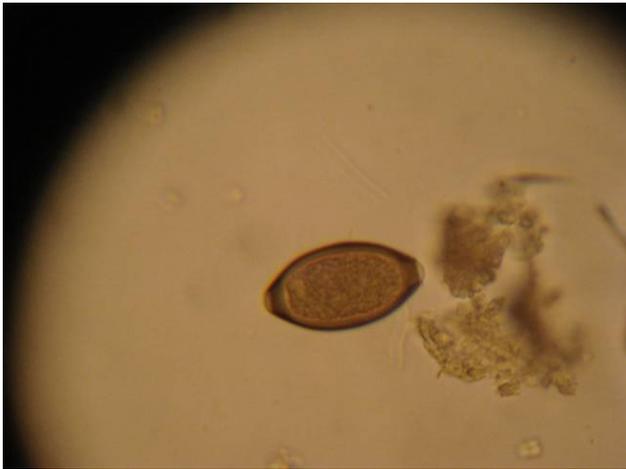


Figure 2.6: *Trichuris trichiura* eggs (x400) from human stool noted with bipolar prominences

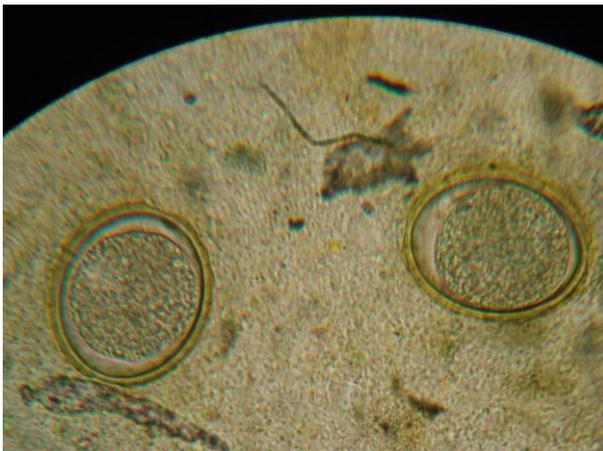


Figure 2.7: Fertile *Ascaris lumbricoides* eggs (x400) noted for the decorticated shells as observed on a Kato-Katz thick smear.

2.4.2 Sequencing of Parasites isolated from faecal samples of humans and baboons

Amplification using the nested PCR primers for the 18S rRNA gene and sequencing using the second round primers (SchF017 and SchR019) was not very reliable and so good sequences could only be obtained from a few host individuals. The 18S rRNA gene is a conserved region of the parasite's DNA and it is therefore suitable for identifying the organism's taxonomic group (Smit *et al.*, 2007). In total, amplification products from parasite eggs isolated from five children from Bugamba, three children from Mwamgongo, and two baboons were sent for sequencing but clearly readable samples were only obtained for six of the human samples and one of the baboon samples. All sequences were identical to one another and to a sequence deposited to Genbank from chromosome 5 of the strain of *S. mansoni*, for which the genome has been sequenced (from Puerto Rico, accession HE601628). Although only one of the children and one of the baboons were sequenced using the single round amplification 18S primers ETTS2 and ETTS17, they were again identical to one another and pulled up the same match in BLAST. The baboon individual that did not produce readable sequences with either primer had been diagnosed with *Paragonimus* sp. and not *S. mansoni* in the microscopic analysis but species identification could not be confirmed based on sequencing. The methods and preliminary results on the sequence analysis of humans and baboon schistosome DNA are shown in Appendix 2.

2.4.3 Variation of schistosome infection with host demographic characteristics and site

Analysis results for the impact of site, sex and age on schistosome infection that was based on wet season samples only (N = 406) to avoid the confounding influence of praziquantel treatment are presented in Tables 2.3 and 2.4 for prevalence and intensity, respectively. Hierarchical model selection suggested that the best fitting model included age, site and their interaction (i.e. these factors influenced the variation in parasite prevalence) but that

there was no effect of host sex (Table 2.3). Host sex did not affect prevalence because the AIC value of the model was lowered when it (sex) was included in the model (474.21 to 474.10) and higher without it (474.21 to 491.99; Table 2.3 Model 2). Similarly, AIC values for the model were lower due to sex-site interaction (from 476.77 to 474.88) and sex-age interaction (476.77 to 475.09) while AIC values were higher without these interactions (476.77 to 496.44; Table 2.3 Model 1). Hence, sex, sex-site interaction and sex-age interaction variables were dropped from the model while age, site and age-site interaction were retained. AIC values for the ZINB model containing egg counts as a response variable and all of the predictors as described above produced similar results, with the model AIC lowered by sex variable (1817.0 to 1815.3), sex-site interaction (1828.2 to 1818.5) and sex-age interaction (1828.2 to 1826.2; Table 2.4). As for prevalence, age-site interaction raised the value of the model (1828.2 to 1838.5; Table 2.4 Model 1) and therefore age, site and their interaction were retained in the final egg count analysis model.

The lack of the impact of host sex on schistosome infection can also be observed in graphical presentation in Figure 2.8. Infection prevalence was similar in male and female adults while it was marginally higher in girls than in boys and children tended to show higher prevalence than adults (Figure 2.8A). Similarly, the intensity of infection was similar between adult males and females and children showed higher levels than adults, but boys showed a higher number of parasites than girls (Figure 2.8). The effect of the age-site interaction was reflected in the relative variation in prevalence and intensity among sites (Figure. 2.9). For instance, although schistosome prevalence at Mwamongo and Bugamba sites was higher in children than adults, the opposite was true at the Kiziba, Gombe and Mtanga sites (Figure 2.9A). The prevalence of infection in children varied markedly across sites (after excluding data from Gombe, where only one child was examined), being lowest at Mtanga (13 ± 0.05) and highest at Mwamongo (88 ± 0.04).

Conversely, schistosome prevalence in adults stayed relatively constant among all sites, with the largest difference observed comparing Mtanga (28 ± 0.08) and Bugamba (44 ± 0.12). There was substantial variation in schistosome egg counts across sites, in both children and adults. For instance, intensity was higher in children than in adults across all sites except at Bugamba, where adults had higher intensity than children (Figure 2.9B). For children, the lowest mean intensity of infection (excluding Gombe) was at Mtanga (8 epg) while the highest was at Mwamgongo (30 epg). The lowest mean intensity in adults was again recorded at Mtanga (4 epg) but the highest was at Bugamba (22 epg). A scatterplot of intensity infection in relation to host age (excluding zeros) indicated a nonlinear relationship, with egg numbers increasing to a peak in children aged between 9 and 12 years (although this was the predominant age group sampled). The peak then declined until 20 years of age, after which it remained relatively constant (Figure 2.10).

Table 2.3: Goodness of fit models obtained through “drop one” selection processes in R for the binomial distribution model used for schistosome prevalence.

The table indicates the part of the model tested (Model used), the degree of freedom (DF), the AIC value and comments on the action taken (Remarks). Based on the results, there was no effect of host sex on parasite prevalence while age, site and their interaction influenced it.

Model 1 (Full model):			
Preval~ Age_group + Site + Sex + Age_group *Sex + Age_group * Site + Site * Sex, family= binomial			
Model used	DF	AIC	Remarks
Full model		476.77	Reference value (no term dropped)
Age_group*Sex	1	475.09	Age_group*Sex dropped
Age_group*Site	4	496.44	Age_group* Site retained
Site*Sex	4	474.88	Site*Sex dropped
Model 2 (excluding sex-site and sex age interactions):			
Prevalence~ Age_group + Site + Sex + Age_group * Site, family= binomial			
Full model		474.21	Reference value (no terms dropped)
Sex	1	474.10	Sex dropped
Age_group*Site	4	491.99	Age_group * Site retained
Model 3 (excluding sex, sex-site and sex age interactions) :			
Prevalence ~ Age_group + Site + Age_group * Site, family= binomial			
Full model		474.1	Reference value (no term dropped)
Age_group*Site	4	493.7	Age_group * Site retained
Best-fitting model for analysis of prevalence :			
Prevalence~Age_group+Site+Age_group*Site, family= binomial			

Table 2.4: Goodness of fit models obtained through “drop one” selection processes in R for the zero-inflated negative binomial distribution of egg count data.

The model tested (Model used) is shown followed by the degree of freedom (DF), the AIC value obtained and the justification (Remarks) for dropping or keeping that part of the model in the full model. The results show that host sex had no effect on parasite egg count counts while age, site and their interaction influenced that.

Model 1 (Full model):			
Egg_count ~ Age_group + Site + Sex + Age_group * Sex + Age_group * Site + Site * Sex, dist="negbin", link="logit"			
Model used	DF	AIC	Remarks
Full model		1828.2	Reference value (no term dropped)
Age_group*Sex	2	1826.2	Age_group*Sex dropped
Age_group*Site	8	1838.5	Age_group* Site retained
Site*Sex	8	1818.5	Site* Sex dropped
Model 2 (excluding sex-site and sex age interactions):			
Egg_count ~ Age_group + Site + Sex + Age_group * Site, dist="negbin", link="logit"			
Full model		1817.0	Reference value (no terms dropped)
Sex	2	1815.5	Sex dropped
Age_group*Site	8	1828.7	Age_group * Site retained
Model 3 (excluding sex, sex*site and sex *age interactions) :			
Egg_count ~ Age_group + Site + Age_group * Site, dist="negbin", link="logit"			
Full model		1815.5	Reference value (no term dropped)
Age_group*Site	8	1827.1	Age_group * Site retained
Best-fitting model :			
Egg_count~Age_group+Site+Age_group*Site, dist="negbin", link="logit"			

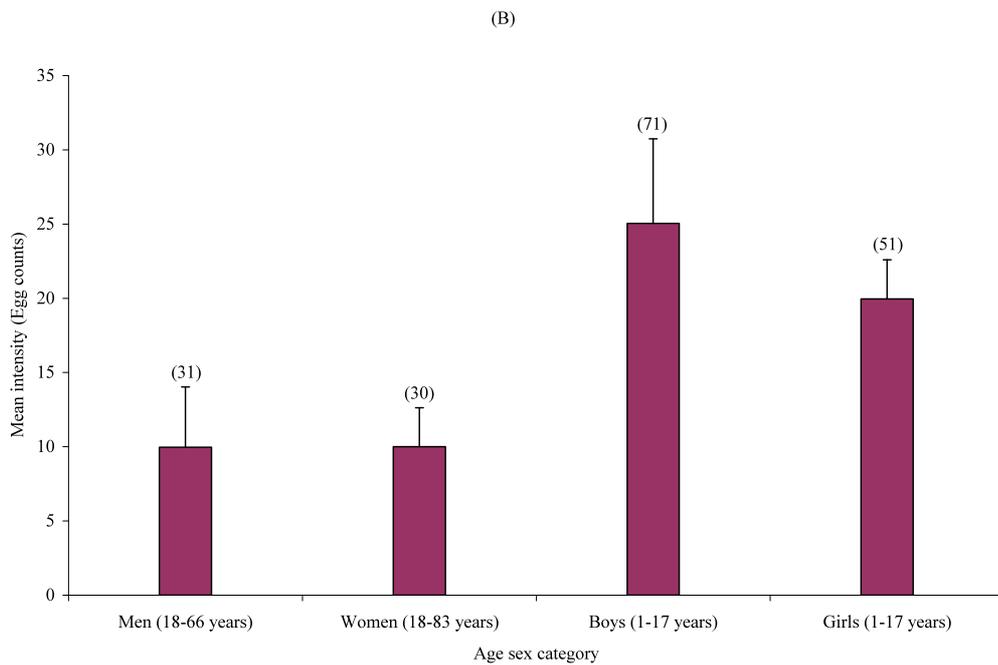
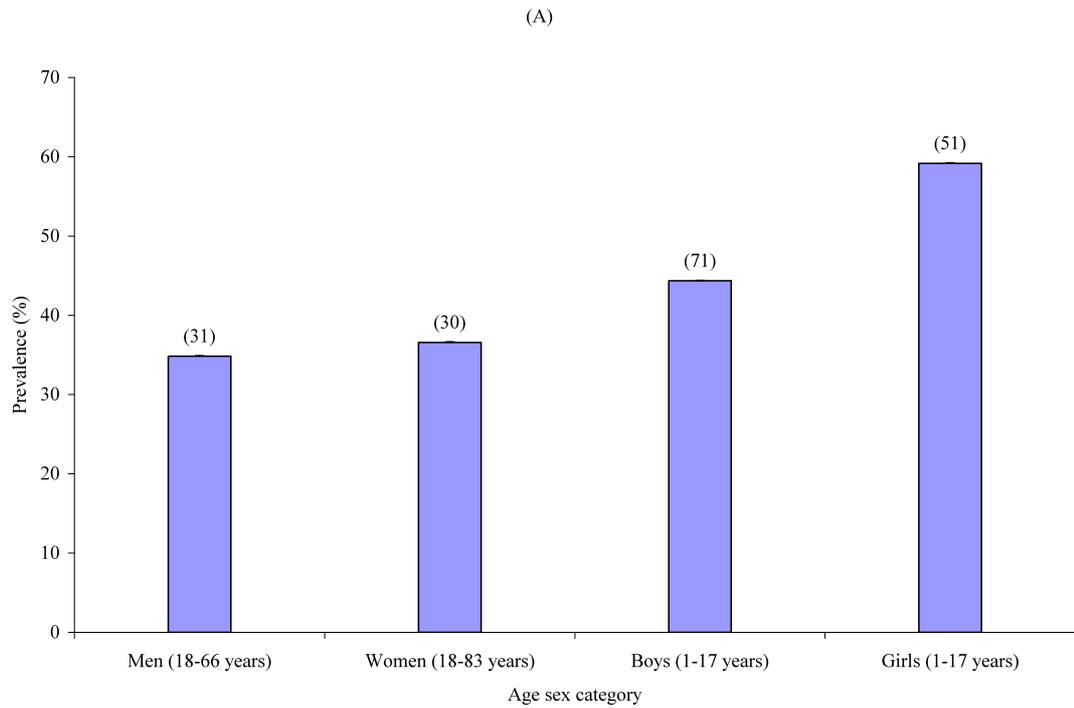


Figure 2.8: Variation in the prevalence (A) and intensity with untransformed egg counts data (B) of schistosome infection with individual sex humans.

The mean intensity shown in B was calculated by excluding zero egg counts (untransformed data). Bars on top of each histogram denote standard errors while numbers on the top of bar indicate the number of infected individuals. The sample size i.e. $n = 89$ (men); $n = 82$ (women); $n = 115$ (boys) and $n = 120$ (girls).

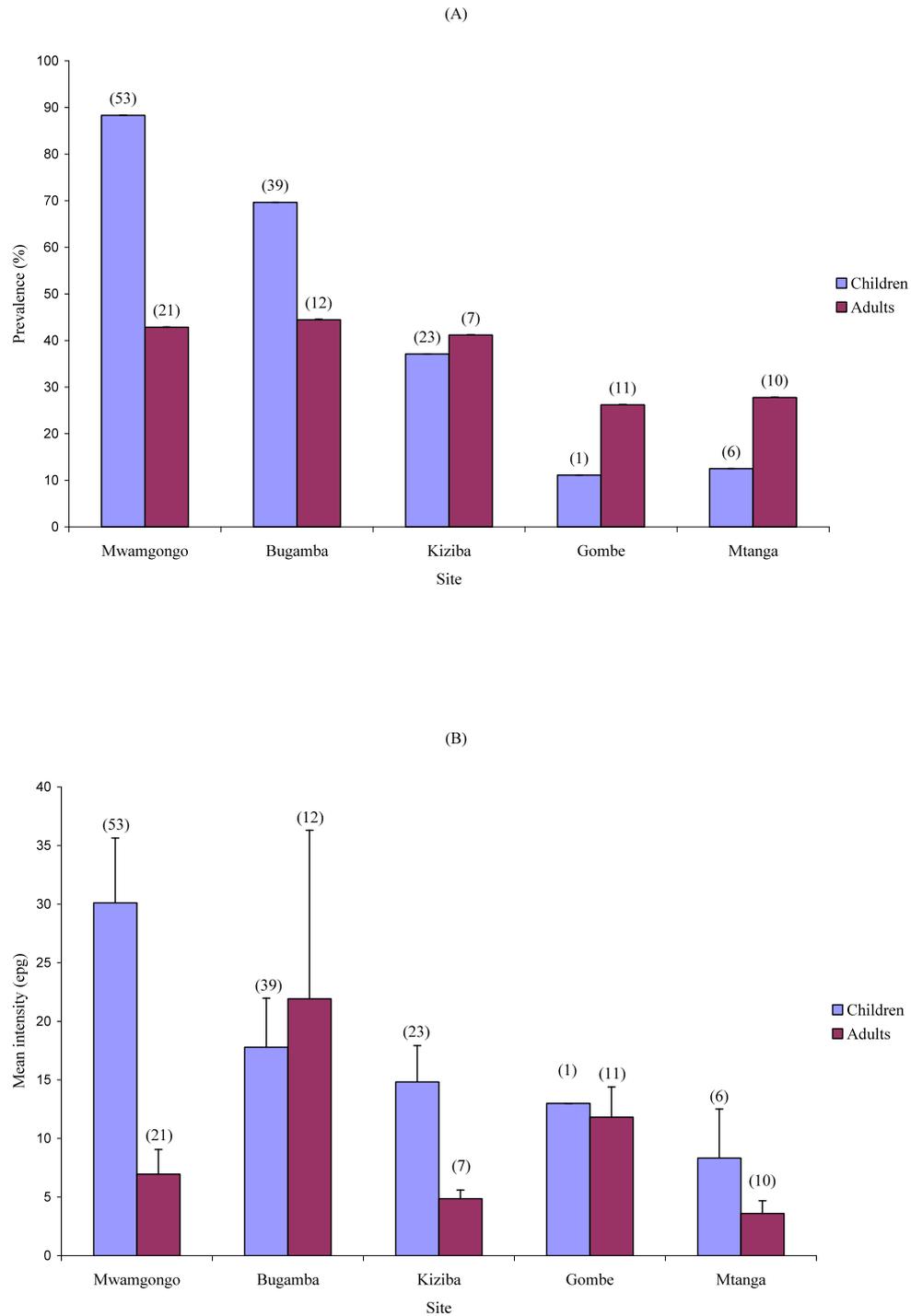


Figure 2.9: Influence of host age and site on the variation on schistosomiasis prevalence (A) and intensity (excludes zero egg counts) across study sites.

Standard errors are indicated by bars on top of histograms while the number of infected individuals is shown on top of bars (Mwamgongo: n = 60 children and 49 adults; Bugamba: n = 56 children and 27 adults; Kiziba: n = 62 children and 17 adults; Gombe: n = 9 children and 42 adults; Mtanga: n = 48 children and 36 adults).

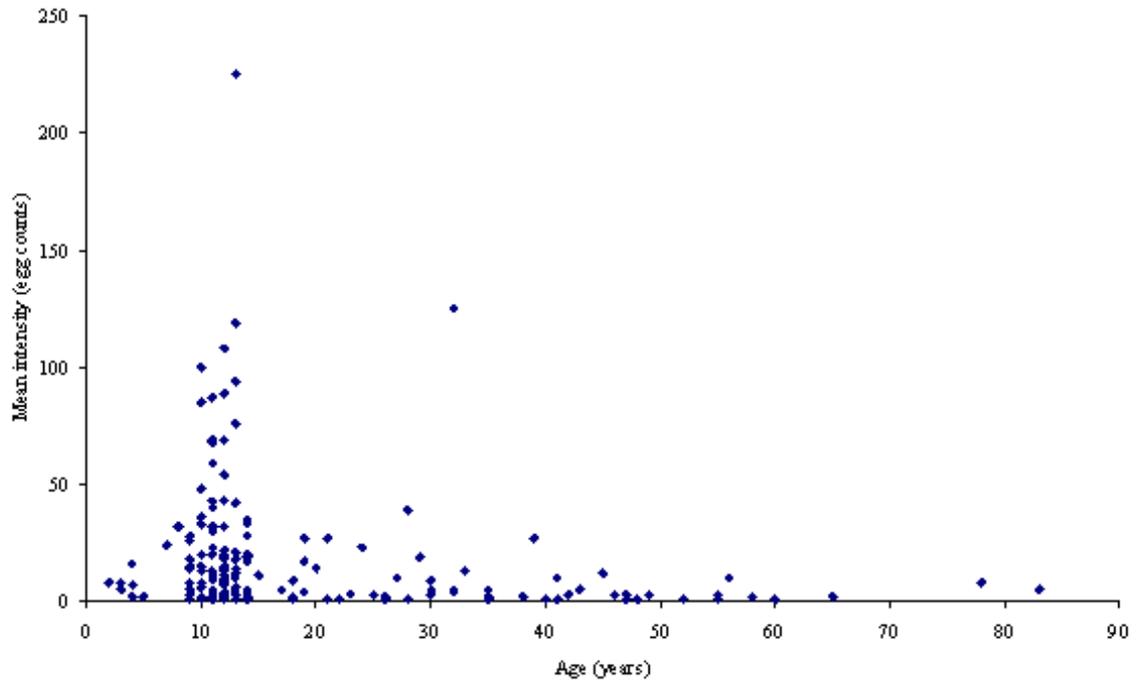


Figure 2.10: Variation in the intensity of *S. mansoni* infection (excluding zeros) with host age in humans.

The figure shows a general scatter distribution indicating that the intensity of infection peaks in children aged between 9 and 12. Intensity of infection then declines until age 20, after which it generally remains low.

2.4.4 Effect of season on schistosome infection in children and adults

Analysis of seasonal effect on schistosome infection in untreated children and adults from all sites combined ($n = 67$) showed no significant effects of age, season or their interactions on infection prevalence (Table 2.5) and intensity (Table 2.6). However, graphical analysis of these results suggested some interesting patterns. Whereas there were similar levels of prevalence of schistosome infection between wet and dry season in children, among adults prevalence was higher in the wet than the dry season (Figure 2.11A). In contrast, the opposite pattern was found for infection intensity, where higher

egg counts were found in the wet than the dry season in children but adults showed similar numbers in both seasons (Figure 2.11B). Wet and dry season samples from all study sites were combined and analysed together due to small sample size ($n = 67$).

Table 2.5: Results of a Generalized Linear Model binomial analysis evaluating the effect of age and season on schistosome prevalence

GLM binomial model coefficients:				
	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	-1.6864	0.4869	-3.464	0.000533 ***
Age group	-0.3613	0.7206	-0.501	0.616112
Season	0.4134	0.6480	0.638	0.523459
Age group: Season	-0.1575	0.9672	-0.163	0.870644

Table 2.6: Results of a zero-inflated negative binomial (ZINB) statistical analysis assessing the effect of age and season on schistosome intensity.

Count model coefficients (negbin with log link):				
	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	0.73958	0.71309	1.037	0.300
Age group	1.28521	0.91221	1.409	0.159
Season	0.04555	0.81196	0.056	0.955
Age group: Season	1.21039	1.20428	1.005	0.315
Log(theta)	-0.57828	0.77460	-0.747	0.455
Zero-inflation model coefficients (binomial with logit link):				
	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	1.0023	0.7860	1.275	0.202
Age group	0.7552	0.8592	0.879	0.379
Season	-0.4724	0.8611	-0.549	0.583
Age group: Season	0.3647	1.1522	0.317	0.752

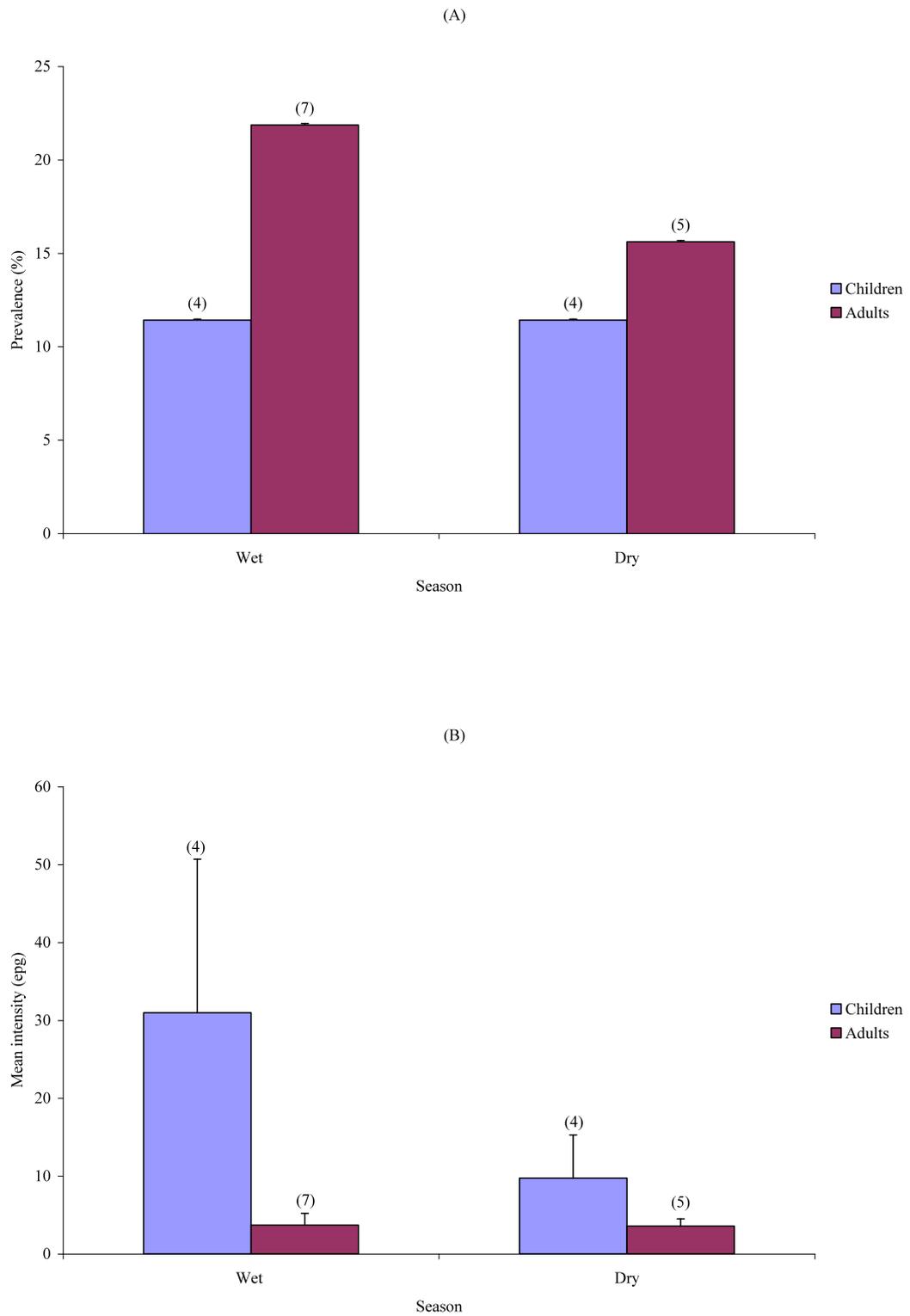


Figure 2.11: Seasonal variation in the prevalence (A) and intensity (B) of schistosome infection in humans.

Mean intensity excluding zero; numbers on the top of bars indicate the number of individuals infected (wet season: children, $n = 35$; adults $n = 32$; dry season: children, $n = 35$; adults, $n = 32$) while bars on top of histograms denote standard errors.

2.4.5 Effect of human occupation and health and on schistosomiasis infection

The observed trends of the impact of human activities on the outcome of schistosomiasis infection are indicated in Figure 2.12. For instance, people engaged in fishing had the highest prevalence of infection (about 50%), while domestic (home) workers and professionals (employed and salaried) had the lowest prevalence (Figure 2.12A). On the other hand, variation in schistosome prevalence among farmers, business people and people of other occupations were only marginally different. As depicted in Figure 2.12B, variation of *S. mansoni* intensity with occupation showed similar unclear trends as for prevalence. The intensity was highest among domestic workers and lowest in business people. The prevalence of infection among farmers was only slightly different from that of the people engaged in business while there was also very little variation among farmers, professionals and people of other occupations (Fig. 2.12A and B).

In terms of people's perception of illness in relation to infection status, people who complained of general illness had higher schistosome prevalence and intensity than participants who claimed to be in good health or had complained of abdominal pain. While schistosome prevalence showed only marginal variation among the three health states (Figure 2.13A), variation of intensity with health status was evident (Figure 2.13B).

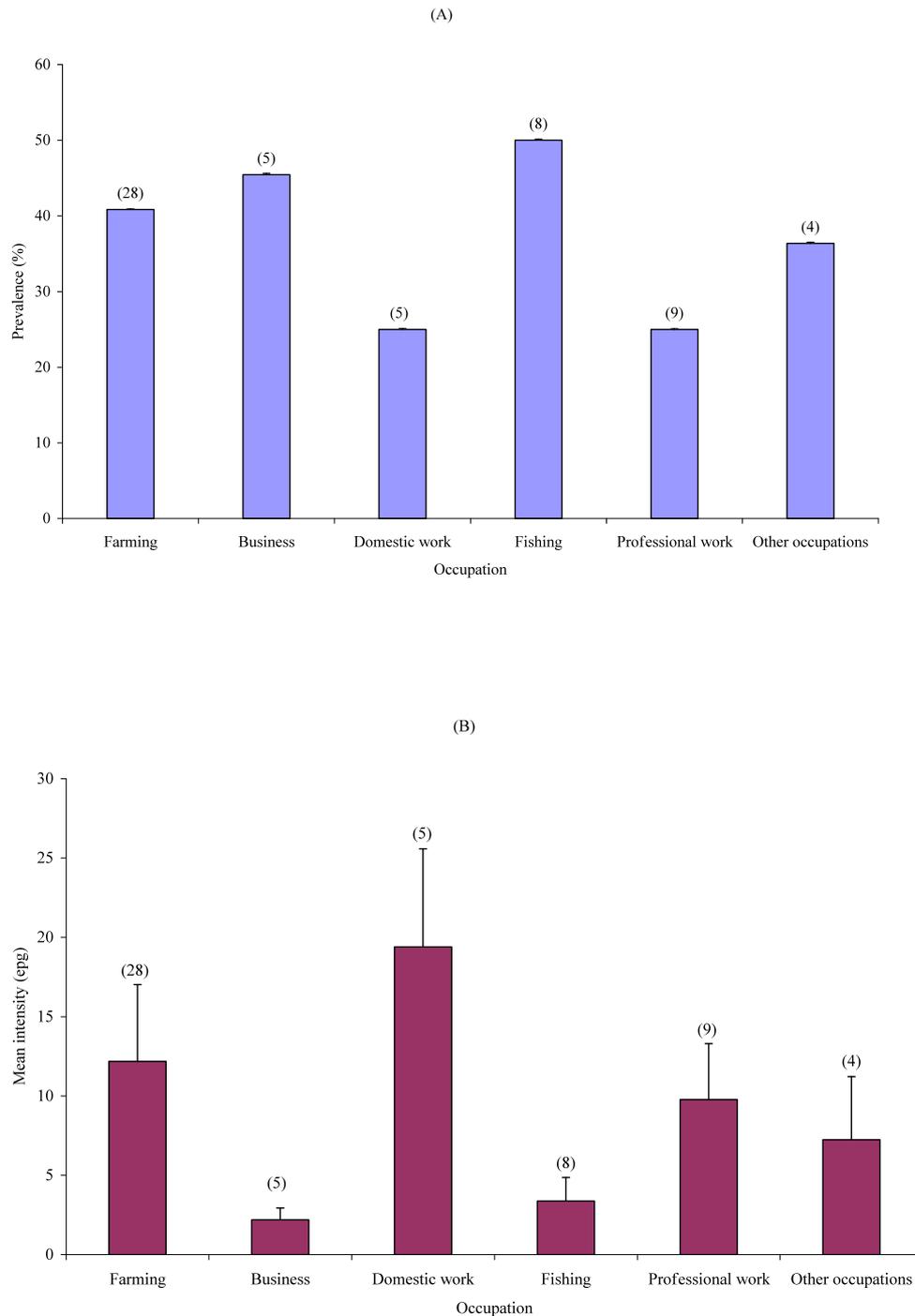


Figure 2.12: Relationships between occupation and schistosomiasis prevalence (A) and intensity (B) in humans in the Gombe ecosystem.

Mean intensity was calculated by excluding zero counts. Bars on top of each histograms denote standard errors while numbers on the top of each bar indicate the number of infected individuals (farming: $n = 70$; business: $n = 11$; domestic worker: $n = 20$; fishing: $n = 16$; professionals: $n = 36$; other occupations: $n = 11$).

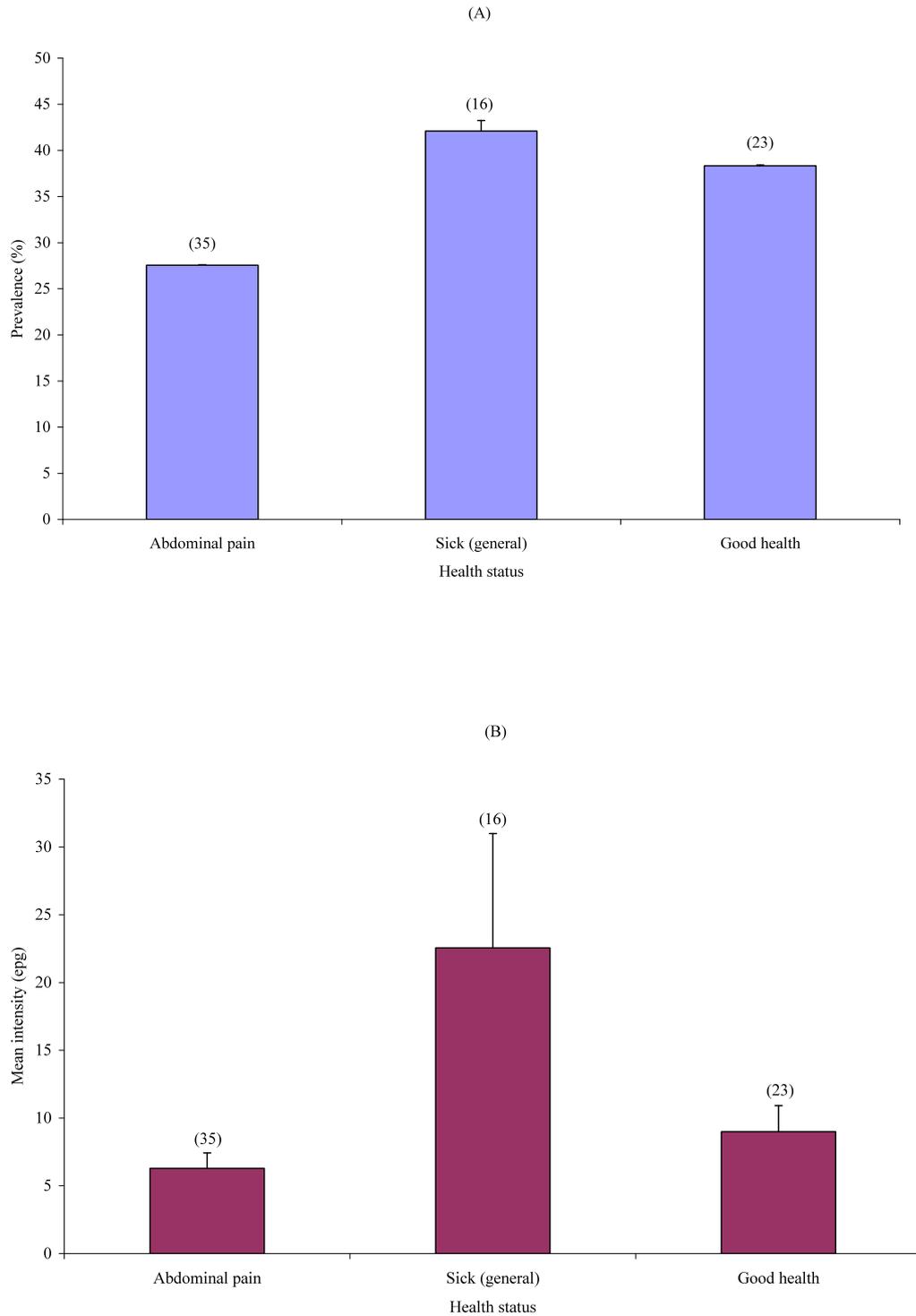


Figure 2.13: The prevalence (A) and mean intensity (excluding zero counts) (B) of *S. mansoni* infection and perceived impact of the infection on people's health status.

Numbers on top of each bar indicate the number of individuals infected (abdominal pain: $n = 89$; sick-general: $n = 38$; good health: $n = 60$) while error bars denote standard errors for each health category.

2.5 Discussion

2.5.1 Distribution of intestinal schistosomiasis: General observations

Sampling compliance was observed more in children than adults, as reflected by higher attendance in children than adults during the initial baseline sampling, at treatment and during post treatment follow-up study (Table 2.2). The same number of children and adults were initially enrolled for sampling for each site (except Gombe) but in the end only 59% of the adults who enrolled for the study returned their sampling with stools, compared to 94% of the children. Similarly, during praziquantel distribution, only 11 % of adults turned up for treatment compared to 48% of the children. Only 14% of adults participated in the post-treatment study compared to 48% compliance in children (Table 2.2). Perhaps there are exceptions in some settings, but these observations are crucial as they can have significant implications for the planning and success of epidemiological studies that target the entire population. As noted by Montresor *et al.* (1998), epidemiological data obtained from population sampling is more informative than that collected from subpopulations such as school children. The authors however cautioned that the sampling of entire populations is more difficult and can result in sub-optimal results compared to school children sampling. Most adults are likely to miss sampling as they may be working away from home or travelling. It is also possible that some may consciously decide not to provide samples. Children, on the other hand, particularly those enrolled in schools, are accessible at their schools and there is usually some cooperation from teachers and sometimes parents (Montresor *et al.*, 1998). It is also thought that data collected on disease infection in schools can not only help to gauge levels of infection in children but are also useful in planning for intervention needs for the community overall (Montresor *et al.*, 1998). This observation further indicates that children are an ideal target group for sampling, especially in resource-constrained projects.

The present study has systematically confirmed that intestinal schistosomiasis due to *S. mansoni* is endemic in western Tanzania and thus that the distribution of the disease in the country is more widespread than previously thought. This part of the country was earlier regarded as either schistosomiasis free or a low infection area (Rugemalila, 1991; Brooker *et al.*, 2009). The Gombe area, where an overall prevalence of 45% of *S. mansoni* infection was recorded, is close to the Rusizi plain in Burundi, where *S. mansoni* has been reported at 23% prevalence (Gryseels, 1991). The two areas are located in the same ecological zone in the Albertine Rift region and are thought to be influenced by the same environmental conditions (Plumptre *et al.*, 2007). It is also possible that movements of people between the two areas and other endemic sites in Tanzania may have facilitated the spread of the disease at equal rates. While humans are considered the key in spreading schistosomiasis, other host vertebrates such as rodents and primates have also been implicated (see section 1.8). A discussion on the implication of the molecular confirmation of *S. mansoni* eggs in both humans and baboons in Gombe is given in chapter four.

The current findings also indicate that intestinal schistosomiasis in the area exists at high prevalence, particularly among school children. It was observed that in some villages, such as Mwamgongo, more than 80% of the children were infected with the disease. The prevalence recorded in this area is a typical scenario for any schistosome endemic area (Rugemalila, 1991; de Vlas, 1996). Since prevalence indicates the proportion of infected members of the population, this measure is a key element in assessing the risk of schistosomiasis infection. It is thus very useful data for control efforts against schistosomiasis. The overly high prevalence of schistosomiasis observed in children in the present study is thus a guide to focus attention on this subpopulation. On the other hand, the intensity of infection indicates an impact the disease has had on or in an infected individual (WHO, 2002). Thus, people with high egg counts of schistosomiasis are likely to be more ill than those with lower egg counts of the parasite. This condition, which is

technically referred to as morbidity, is often used a guide for monitoring control programmes for schistosomiasis. In this case, lower egg counts after treatment signify success of the project while the same or higher egg counts after treatment indicate programme failure. Prevalence is thus a key indicator of transmission or infection risk (Montresor *et al.*, 1998) while intensity indicates morbidity risk and both are used for planning treatments in schistosome endemic areas (WHO, 1985).

A few species of soil-transmitted helminths were also identified alongside *S. mansoni* in human stool samples, including *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms. However, the parasites were detected at very low prevalence and hence only a few remarks will be given about them below. The worms are nonetheless important as they represent the three major soil-transmitted helminths currently affecting more than one billion people globally, with another two billion at risk of being infected (Montresor *et al.*, 1998). From a public health perspective, these soil-transmitted helminths and schistosome parasites have many features in common. They are usually found together in the same areas or individuals and all are mostly associated with inadequate sanitation and poor living conditions, poor personal and environmental hygiene, as well as the lack of health awareness among the general population (Montresor *et al.*, 1998; Fenwick, 2011). The infections are therefore a result of poverty and they also cause poverty because infected people become too weak to work (Fenwick, 2011). Both schistosomiasis and soil-transmitted helminths cause blood loss and hence anaemia and infection with these diseases causes malnutrition, growth retardation and susceptibility to other infections (Montresor *et al.*, 1998). These diseases do not kill patients instantly and their presence is mostly asymptomatic; hence, they do not receive as much attention as infections like malaria or TB or HIV. In addition, schistosomiasis and soil-transmitted helminthiasis occur in poor societies who lack the necessary resources to manage and control the diseases. However, there is some optimism, as governments in endemic countries have

taken various initiatives to combat these infections and currently there is a global movement to control and eliminate these neglected tropical diseases, headed by organizations such as the Bill and Melinda Gates Foundation.

Although the Kato-Katz technique is highly effective for detecting the presence and burden of *S. mansoni* in stools (WHO, 1991), it is not effective in diagnosing soil-transmitted helminths such as hookworms (Zamen and Cheong, 1967; WHO, 1991; de Vlas, 1996). The eggs of these parasites become disintegrated within the 30 to 60 minutes taken to prepare and process samples in this method.

2.5.2 The influence of host demographic attributes and site on schistosome infection

Host sex

In the current study, host sex was not found to influence either schistosome prevalence or intensity. The linking of host sex or lack of it to schistosome infections has been investigated and a few of them found no association between sex and schistosomiasis infection (Farooq *et al.*, 1966; Pugh and Gilles, 1978; Scott *et al.*, 2003). For instance, in their study on the impact of sex on schistosome infections in Senegal, Scott *et al.* (2003) did not find any evidence that sex determined exposure to *S. mansoni* infection and concluded that other factors played a more important role in determining infection levels.

Other studies have however reported strong link between sex and levels of schistosomiasis infection. In such cases where host sex has been associated with schistosome infections, the variation was attributed to occupational activities such as fishing and farming (Farooq *et al.*, 1966). According to Farooq *et al.* (1966), in areas such as the Nile delta in Egypt where fishing is solely men's job, higher prevalence of schistosomiasis were reported in males than females. Similar trends have been reported in areas where men are more

involved in water-related activities than women, such as in the Philippines (Pesignan *et al.*, 1958) and northern Nigeria (Pugh and Gilles, 1978). In areas where fishing or farming are mostly done by women, such as among the Mende people in Sierra-Leone, higher prevalence of schistosomiasis has been reported among females than males (White *et al.*, 1982). It is therefore possible that, men and women in the Gombe ecosystem did not have different levels of schistosome infection because, given their social and cultural duties, both groups are equally exposed to schistosome transmission in the local streams. As there is an insufficient supply of running tap water, both women and men use the stream, albeit for different purposes. Men come in contact with the stream water for bathing, ablution before prayers for muslims and while fording on foot. Women, on other hand, spend time in the stream water while washing up clothes and utensils, collecting water for domestic use, as well as bathing and fording the streams on foot.

Since sex differences in schistosomiasis infection have commonly been reported in endemic areas, the lack sex differences observed in the present study is unusual and interesting. This lack of significant difference between male and female participants may be attributed to the small sample size that was studied. Only one sample per person was collected per season and this is likely to have influenced the current observations. Studies involving small sample sizes may not reveal significant infection differences between sub-populations (Gregory & Blackburn, 1991). Thus the underlying ecological and host variables influencing the intensity and prevalence of schistosomiasis in the present study area could possibly be better understood by employing a bigger sample size of the host population for a relatively longer sampling period. It is also worthwhile looking at others factors that could influence the levels of schistosomiasis infection in human populations including immunity, water-contact patterns and ethnicity.

Host age

In the present study, overall, more children were infected with *S. mansoni* than adults and peaks in both prevalence and intensity were found in children aged between 9 and 12 years (Figure 2.10). Statistical results showed a significant age influence on schistosome prevalence and egg counts. The age effect on schistosome infection observed in the present study is consistent with reports by other workers such as Farooq *et al.* (1966) in Egypt and Kvalsvig and Schutte (1986) in South Africa. In these studies, the prevalence and intensity of *S. mansoni* were found to rise slowly in children and then slowly decline in older individuals. In their work, Butterworth and others (1985) found that susceptible children in Kenya were heavily exposed and continually re-infected with schistosomiasis.

Age is one of the host characteristics that can significantly influence the patterns of parasite transmission and distribution in host populations (Wilkins, 1987; Day, 2010; Harrington, 2010). Young people are susceptible to infection due to their risk behaviours, which bring them in contact with water more often than adults (Wilkins, 1987; Sama *et al.*, 2007). While adults would shun contact with water for fun, young people are more likely to engage in actions that expose them to infection. For schistosomiasis infection to occur, it is critical that humans come in direct contact with contaminated fresh water (Southgate and Rollinson, 1987; Coutinho *et al.*, 1997a). It is also thought that diminishing of schistosome infection with age is due to acquired immunity from parasite antigens that manifests itself in adulthood (Wilkins, 1987; Pinot de Moira *et al.*, 2010). Thus, since children would not have been exposed to schistosome infection before, they are more susceptible to the infections than adults.

Site

The differences in the levels of schistosome infection observed between children and adults varied across study sites. It was evident therefore, that both age and sites are jointly major determinants of schistosome infection in the study area. Higher prevalence and egg counts of *S. mansoni* were recorded among children than adults in the villages of Mwamgongo and Bugamba, while in other sites adults had higher infection levels than children. At Gombe, too few children were sampled to determine relative levels of infection compared to adults. As highlighted in studies elsewhere (Wilkins, 1987; Madsen, 1995; Lwambo *et al.*, 1999), the factors that could cause local variation in schistosomiasis infection include variation in local conditions such as environment and socio-economic level, as well as the abundance and distribution of snails. There is also a probable link between schistosome transmission in local conditions in some sites, including for instance settlement patterns, overcrowding, and socio-economic activities, which could also account for village variation in schistosomiasis infection observed in the present study. For instance, in Mwamgongo village, where the prevalence and egg counts of *S. mansoni* were highest particularly among children, housing conditions and settlement patterns put people at risk of acquiring the parasite. In the village, residential houses and latrines are arranged along the course of the river in a single file, while in other villages houses are spread out and situated far away from the streams. Most households in Mwamgongo village do not have latrines and thus use public areas as lavatories (*pers. observation*). Similar observations have been reported in communities living along the shores of Lake Victoria in northwest Tanzania, where local variation in schistosome infections was attributed to the patchy distribution of snails in the areas (Forsyth and Bradley, 1966). Unlike the distribution of malaria vectors, which can generally be ubiquitous in a local environment, the distribution of schistosome snail vectors is highly patchy (Wilkins, 1987). The presence or absence of the snails and their ability to transmit schistosomiasis depend on the existence of suitable habitats and the individual characteristics of these habitats, as well as

their proximity to human settlements (Lwambo *et al.* 1999). The epidemiological role of snail vectors in the transmission of schistosomiasis in the Gombe ecosystem is discussed in chapter five of this thesis. There is a possible link between the observed patterns of schistosomiasis infection in humans among study sites and snail abundance and distribution in the area.

The role of religion in this particular area cannot be ruled out. More than 90% of Mwamgongo residents are predominantly Muslim. It is believed that Islam was first introduced to the village almost a century ago by Arab traders and has since flourished, with Christianity being a recent introduction (Mung'ong'o, 1999). The neighbouring villages of Bugamba and Kiziba are overwhelmingly Christian. It was in Mwamgongo where highest infections of schistosomiasis were recorded. Cleanliness is an important matter among Muslims and this brings them close to water more than would be the case in non-Islamic communities. Epidemiological studies in other schistosome-endemic areas have found that people with frequent occupational contact with water such as Muslims, who also use water for ablution before praying, tend to have higher prevalence of schistosomiasis (Farooq *et al.*, 1966; Wilkins, 1987). However, to explore this factor, more data would need to be collected.

2.5.3 Relationship between season and schistosome infection

Paraziquantel drugs were distributed to infected participants after the initial sampling in the wet season. The second follow-up sampling was conducted after drug distribution in the dry season. It is therefore possible that the drugs that were distributed in the wet season influenced the infection results observed in the dry season. Because of the possibility of the confounding influence of praziquantel drugs on season, it may not therefore be possible to account for seasonal influence on schistosomiasis infection in the present study.

However, stool samples from a subset of people who did not receive praziquantel drugs and were sampled in both seasons indicate a possible season effect. Although the relationship was not statistically significant, there was some indication that the prevalence and egg counts of schistosomiasis were higher in the wet season than the dry season. The caveat here is the fact that only one sample per season was collected in the present and only two seasons were studied. On average three to four different seasons would need to be sampled in order to adequately estimate seasonal trends in schistosomiasis infections (Davis *et al.*, 2002). Similarly, larger sample sizes than what was achieved in the present study would be required to draw firm conclusions about this pattern. Climatic parameters such as temperature and rainfall are known to be key determinants of schistosome parasites and their intermediate hosts, particularly because they influence the snail fauna (Southgate and Rollinson, 1987). For instance, the fluctuations in snail population are known to be linked to changes in the levels of rainfall. In the few sites where snails were present, they were found in higher numbers at the beginning of the wet season (Chapter 5) and so it is possible that higher infection of schistosomiasis is caused by the sudden increase in snail abundance at the start of the wet season. A detailed overview on the role of snail intermediate hosts on the transmission of *S. mansoni* in the Gombe ecosystem is covered in chapter five.

2.5.4 Observed trends in schistosome infection with host occupation and health status

The results did not indicate a clear trend for any human occupation that is more risky for schistosomiasis infection than others in the present study; however, prevalence was highest among fishermen and business people and there was a reverse trend on these activities observed for egg counts. Fishing in the area is conducted in Lake Tanganyika, which is famously known for its lack of schistosomiasis and that schistosome-transmitting snails do

not survive in it (Clements *et al.*, 2006a, 2006b). However, fishermen are mobile so they might have fished in infested lakes in other areas in Tanzania such as Lake Victoria, which is known to harbour both *S. mansoni* and *S. haematobium* parasites. It could also be that these people are involved in other risky activities such as wading in the village streams, such as when fording the stream on foot or bathing. For example, in the River Ngonya in Mwamgongo village, most snails were diagnosed with schistosome larvae and so there would be a high risk of exposure. The high prevalence of schistosomiasis among the people engaged in business is unusual but not surprising. As suggested for fishermen above, water contact is the major exposure to them. Although businessmen and women spend most of their day time in their shops, they also have to come in contact with water when bathing or fording streams on foot. It could be argued that in places like Mwamgongo, where the stream is full of schistosome infected snails, every member of the community is at risk of acquiring the parasites. Similar conclusions have been made in other highly endemic areas such as in Uganda, where all individuals in the population had the same prevalence and egg counts of infection, suggesting that the high rates of transmission were sufficient to override any occupational or immunity differences (Wilkins, 1987)

There were indications that activities such as domestic work and farming had greater variance in the level of schistosome egg counts (Fig 2.13B). It is possible that domestic workers, due to their regular contacts with water during washing up, are more likely to encounter schistosome larvae in the streams (Fenwick, 2011). Many sites in the study area lack tap water and so washing of clothes, utensils and water fetching are normally done in the stream. Water taps are available in Mwamgongo village and Mitumba in Gombe but, given that these are not sufficient for the areas needs, people still tend to do their washing up in the stream water. Domestic activities such as washing of utensils and disposal of kitchen waste in stream waters are also known to encourage the survival of snails, the

intermediate host for schistosome parasites (Madsen, 1995), which would also increase infection risk. This is because contamination of the human contact sites with food particles and other debris may lead to alterations of the vegetation and increased trophic status (Madsen, 1995).

Although the data are sketchy, this study has shown the nature of people's perception on schistosomiasis and that most of them would not know that they are infected with the disease, unless they have very high parasite loads. Most people who claimed to be in good health had higher schistosome egg counts compared to those who reported abdominal pain (Figure 2.13B) but the highest counts were found in those who reported feeling unwell. The results also indicate the weakness of using questionnaires to assess schistosomiasis infection status from respondents. Due to limited resources in most endemic countries, questionnaires have been promoted as a possible proxy to laboratory diagnosis for gaining information on schistosomiasis infection status (Brooker *et al.*, 2001). The irony of this reality is that even if they were aware of their infection status; quite often people downplay an infection with schistosomiasis. Except in rare cases where skin itch (Swimmer's rash) accompanies the entry of schistosome larvae into the human body, infection with schistosomiasis is mostly asymptomatic and can not be detected easily (Fenwick, 2011). Infected patients may not be aware that they have schistosomiasis, leaving the disease to develop into advanced and serious conditions. Only advanced or chronic forms of schistosomiasis infection in humans can produce visible symptoms such as distended stomach (hepatosplenomegally). At this stage of infection, schistosomiasis effects are severe and usually irreversible as any treatment would only lessen the infection effect but not eliminate it (Fenwick, 2011).

However, in the present analysis, many of the study participants who reported some of the common symptoms of schistosomiasis infection such as abdominal pain or fatigue were

later diagnosed with *S. mansoni* eggs, suggesting that self-diagnoses can be possible. Some of these people were the first to volunteer for schistosomiasis testing, perhaps indicating the gravity of their suffering. This, however, suggests that people would be more willing to volunteer to participate in a schistosomiasis survey if there were an incentive or reward such as free treatment. Among the people found infected with schistosomiasis and treated at the beginning of the survey in the present study, only 48% children and 14% adults turned up during the follow-up study (Table 2.2). Given the asymptomatic nature of the infection with schistosomiasis, particularly in the early stages of the infection (Engels, 1997), laboratory testing for the presence of eggs in stool for intestinal schistosomiasis and blood in urine for urinary schistosomiasis should be the reliable diagnostic tests for the infection. In areas where the infrastructure does not permit these tests to be performed within a reasonable time, the distribution of praziquantel should be prescribed for patients with schistosomiasis related symptoms. This is because the drug is safe and it would do no harm to take it even if the patient did not have schistosomiasis (WHO, 2002). For monitoring purposes, the use of questionnaires would still be useful especially in resource-poor settings.

2.5.5 Conclusion

This study has indicated how intestinal schistosomiasis is locally distributed in communities along the shores of Lake Tanganyika in western Tanzania. Infection of schistosomiasis was affected by both host age and site, as reflected in the interaction between the two factors. Certain local specific environment and social factors such as settlements and human activities in relation to the village streams could account for infection variation between sites. These findings therefore offer some guidance on how to better distribute the limited resources for schistosomiasis control, particularly effort and drugs (praziquantel). Since intensity (egg counts) indicate the risk for morbidity due to

schistosomiasis (Fenwick, 2011), treatment and control priorities would be effectively directed in the villages of Mwamgongo and Bugamba, where the egg counts were highest, particularly among children. Since there was no link between schistosome infections and host sex across all study sites future studies should explore the role of other exposure factors such as human activities related and water contact in the streams that might be driving the transmission of schistosomiasis in the area.

Chapter 3: Tablet Pole Efficiency in Estimating Praziquantel Dose for Treating Intestinal Schistosomiasis among School Children in Tanzania

3.1 Abstract

The use of body height as a proxy measure for body weight, also known as dose pole, is popular during mass administration of praziquantel drugs against schistosomiasis. Dose poles are simpler, cheaper and not as susceptible to environmental conditions compared to weighing scales. However, more research is still needed to test the accuracy and practicality of dose poles in various field conditions. This study examined the dosage and number of praziquantel tablets administered to school children in western Tanzania based on weight and compared this to the dosage they would receive based on their height (i.e. if a tablet pole approach had been used). Stools were collected from 113 children aged 7 to 15 years old from four schools in Kigoma District with one stool sample obtained from each child in the wet and the dry season. The samples were examined for *S. mansoni* parasites using the Kato-Katz technique. Children diagnosed with schistosome eggs in the wet season were treated with praziquantel drugs based on their bodyweight. A follow-up study after treatment was conducted in the dry season to determine the status of schistosomiasis infection and the effectiveness of the drug. A paired t-test analysis showed that the prevalence of schistosomiasis decreased significantly from 79.0% before the treatment to 19.4% after treatment ($p > 0.0001$). Similarly, the intensity of infection before treatment (mean: 81.5 ± 0.04 eggs per gram of faeces) was significantly higher than that after treatment (mean: 2.3 ± 0.05 eggs per gram of faeces; $p > 0.0001$). The number of praziquantel tablets given to children based on their weight (mean: 1.7) was significantly lower than that estimated by each child's height (mean: 2.2; $p > 0.0001$). The dosage range of praziquantel in mg per kg of bodyweight predicted by weight was 23-43 (average: 35.2) while the dosage range given to children based on their height was 29-78 (average: 45.2) and this variation was statistically significant ($p > 0.0001$). Overall, six children (5.3%) received a praziquantel dosage under the recommended range (30-60 mg/kg) based on weight i.e. underdose, while two children (2.6%) would have received the drugs above

the optimum range based on their height. The study shows that while both weight and height estimate the amount of praziquantel dosage that is within an acceptable range, weight tends to underestimate the amount of drug dose while height slightly overestimates it. It is therefore essential to conduct further field studies to test the usefulness of the dose pole in praziquantel distribution and evaluate the extent to which the method could be wasting the drug by giving more than the necessary dosage. Similarly, the use of recipient's weight in praziquantel administration should be scrutinized to determine the magnitude of its tendency to underestimate the drug dosage and the implications this has on the expected treatment outcomes.

3.2 Introduction

As highlighted in chapter one, schistosomiasis constitutes a significant public health problem in many developing countries, particularly in sub-Saharan Africa. A number of programmes have therefore been implemented to control the disease including mass distribution of antihelminthics such praziquantel particularly among school children (Reddy *et al.*, 2007; Fenwick, 2011). Praziquantel is currently regarded as the most efficient drug for combating schistosomiasis and to reduce its resulting morbidity (WHO, 2002). The World Health Organization (WHO, 2002) for instance recommends regular use of praziquantel in the treatment of schistosomiasis through schools and existing health services (Montresor *et al.*, 2002). The drug is cheap and safe, with limited and transient side effects that would not need medical attention (Midzi *et al.* 2008). Sustained mass distribution of praziquantel has substantially reduced schistosomiasis in many countries, most notably in Brazil and Egypt (Salem *et al.*, 2011). In recent years, studies have shown that it is possible and safe to give praziquantel drugs to young children and infants as this age group was initially not covered in the original dose pole (Sousa-Figueiredo *et al.*, 2010; Stothard *et al.*, 2011).

However, the distribution of praziquantel drugs has some inherent problems such as dosage estimation, which can potentially jeopardize its usefulness (King *et al.*, 1989). The effective delivery of the drugs requires that the medication be given at the correct dosage, depending on an assessment of the weight of the recipient. Individual assessment of weight requires appropriate use of accurate weighing scales. Standard weighing devices are susceptible to environmental conditions such as temperature and humidity and this can make it difficult to maintain their sensitivity and accuracy in many endemic settings in the tropics, where users have also found them difficult to operate (Fenwick, 2011). For example, the scale, which is placed on the ground to measure bodyweight, can also give

inaccurate readings if the surface is not flat. These problems make weighing scales less dependable for use in delivering anti-schistosomiasis drugs in developing countries (Montresor *et al.*, 2001).

Following the successful use of height as a proxy measure of weight for treating onchocerciasis with ivermectin in Africa, Hall *et al* (1999) recommended its use in praziquantel distribution. Subsequent studies and improvements on the method have found that height can reasonably predict bodyweight and could be used to determine the correct praziquantel dosage required to treat schistosomiasis i.e. 40mg/kg (Montresor *et al.* 2001, 2002, 2005; Sousa-Figueiredo *et al.*, 2010). Thus, the measurement of height for praziquantel administration, which is variably called “height pole” or “tablet pole” or “dose pole”, is increasingly becoming popular due to its easier operation (Fenwick, 2011). In recent years, the World Health Organization has also supported the use of “dose pole” to determine the dosage of praziquantel during treatments and in control programmes (WHO, 2002). A “dose pole” is a tape measure or ruler that is modified to show the numbers of tablets that should be given to each recipient as shown in figure 3.1. The pole is positioned upright against the back of the individual and the number of tablets determined from the pole’s interval that corresponds with the respective height (Fig. 3.1).

The ease of calibration and use means that non-clinician personnel such as teachers and community drug distributors can be used in delivering the drug, especially in school-based mass treatment (WHO, 2002). In fact, height is now considered more efficient than weight for praziquantel distribution, particularly in low resource settings (Fenwick, 2011).

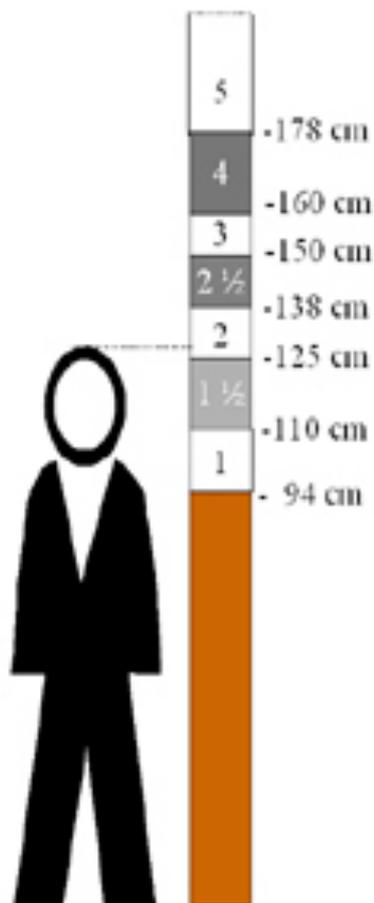


Figure 3.1: Tablet or height pole for estimating praziquantel tablets.

The person in this illustration should be given two tablets. The figure was reproduced from WHO (2011) at http://www.who.int/wormcontrol/statistics/useful_info/en/index1.html, with permission from the World Health Organization.

Nevertheless, there have been some misgivings on the use of height in praziquantel delivery especially from the general public who are key stakeholders in the implementation of schistosome control programmes through drug administration (WHO, 2002). There is a reasonable section of the public that believes that height may not accurately correspond to weight and can therefore give inaccurate estimations of the patient's dosage. Recently, during mass distribution of praziquantel to school children in Tanzania, there were reports of widespread cases of severe adverse effects of the drugs to the children, including prolonged unconsciousness (Tanzania Daima, 2005; Allen and Parker, 2012). Many parents were concerned by this and complained about the use of height in estimating the

dosage of the drug, since they thought that height was overestimating the number of tablets and hence overdosing the children (Tanzania Daima, 2005; Allen and Parker, 2012). This perception, which is perhaps not limited to Tanzania, is attributed to inadequate information about the applicability of height in determining the number of praziquantel drugs for children.

The purpose of this chapter is to establish the effectiveness of height as a proxy for estimating the dosage of praziquantel drugs among school children, by comparing the number of tablets given to each child based on their weight and assess the drug dosage that would have been recommended based on their measured height. Field-based studies to provide further evidence on the usefulness of height for the estimation of praziquantel dose in treating schistosomiasis have been called for by various authors (Montresor *et al.* 2001, 2002; WHO, 2002; Sousa-Figueiredo *et al.*, 2010). The findings of the study will provide useful information on the better management of weight and height to improve praziquantel delivery among school children in resource limited settings. The study will also assess the variation in the prevalence and intensity of schistosomiasis among patients after a single treatment incidence. A significant reduction in the infections will help to indicate the efficacy of praziquantel drugs to cure schistosomiasis mansoni and reduce its potential effects.

3.3 Materials and Methods

3.3.1 Experimental design for stool collection

Information on the study area is described in chapter one of this thesis while detailed descriptions on ethical approvals and clearance for sampling children are given in chapter two. Similarly, the criteria and procedures for selection of schools and children are given in chapter two. In brief, the study was conducted at the primary schools in the villages of Mwangongo, Bugamba, Kiziba and Mtanga located along the eastern shores of Lake Tanganyika north of Kigoma town, Tanzania. After obtaining teachers and parental consent, school children aged between 7 and 15 years were selected for sampling (Table 3.1). The selection followed the WHO's recommendations on schistosomiasis survey (Montresor *et al.*, 1998) by choosing from each school classes III and IV of 40-50 children, resulting in a sample size of 200 children. Where possible, the same number of boys and girls was chosen from each school (Table 3.1).

As detailed in chapter two, stool sampling involved visiting school children at their respective schools a day before stool collection day. The children were briefed about the stool collection exercise and its significance in general for understanding schistosomiasis in their area and also as an assessment of their health. Each child was then given materials for collecting stool samples and was requested to provide a single sample. Sampling materials included a wooden spatula for picking up stool, a plastic vial (120 ml) for depositing the stool and a polythene plastic bag for carrying the samples. The vials were labelled with the provider's name and school and collection date. Sampling materials were distributed in the morning of day one and collected in the early hours of the following day. Demographic features such as age, height and sex are known to influence trends in schistosome infection (Wilkins, 1987) and were therefore collected to test whether they have effect on levels of infection in the children. These characteristics were recorded on a

WHO-adapted questionnaire, as described in chapter two. Initial stool sampling was conducted in the wet season (January-May, 2010) and a follow-up study was conducted after treatment in the dry season (July-September, 2010) to determine the status of schistosomiasis infection after praziquantel distribution.

3.3.2 Stool examination for *S. mansoni*

Detailed description on stool examination for parasites was described in chapter two, so only short explanations of the procedures are given here. In the laboratory, stool samples were examined for *S. mansoni* and other helminths within three to ten hours of collection, using the Kato-Katz kit (Indústria Brasileira; Rio de Janeiro), as recommended by others (Ebrahim *et al.* 1997; WHO, 1991). Briefly, about 5g of each faecal sample was passed through a mesh screen to remove large particles and the filtrate transferred onto a template hole placed on a microscope slide. The template was removed and a cellophane previously soaked in glycerol-malachite green solution was placed on the sample. The sample was then pressed between two microscope slides to spread out the stool for easy observation of parasite eggs. This was left to clear for 30-60 minutes and slides were then examined for parasites under a compound microscope. One slide was examined per child sampled and parasite species present, including *S. mansoni*, were identified as described in chapter two.

3.3.3 Praziquantel distribution

The children diagnosed with schistosome eggs were treated with praziquantel drugs (S Kant Healthcare Ltd, India) at the recommended dosage of 40 mg/kg and counselled about schistosomiasis transmission and prevention. Drugs were distributed to children at their respective schools by health personnel from the village health centres and clinics (Figure 3.2). An exception to this treatment arrangement was Mtanga village where only three children were infected and these were referred to the doctor as outpatients. The dose of the

drug for each child was estimated based on their bodyweight, which was measured in kg using a digital weighing scale (KINLEE ® Cap. 120, Grad.1kg). At the same time the height of each child (in cm) was taken by placing a 30m/100ft carpenter tape measure against an upright standing child. A common formula was used by the doctors to determine the number of praziquantel tablets appropriate for each child's weight: number of 600 mg tablets: = $[(40\text{mg/kg} \times \text{weight in kg})/600\text{mg}]$. The number of praziquantel tablets that would be predicted by children's height were obtained following guidelines in Montresor *et al.* (2001) and Sousa-Figueiredo *et al.* (2010) as illustrated in Fig. 3.1 above. This involved categorizing the measured height for each child in one of the intervals as given in tablet pole (Fig. 3.1). The dosage of praziquantel that would have been given in terms of mg/kg was determined by multiplying the number of tablets by 600mg and divide that by the child's weight in kg (Montresor *et al.*, 2005). As highlighted above, drug distribution was conducted in the wet season (April, 2010) and a follow-up study was conducted after treatment in the dry season (July-September, 2010) to determine the status of schistosomiasis infection after treatment.



Figure 3.2: Registration of school children for praziquantel distribution.

A medical doctor (in pink shirt) and a nurse (in blue attire) can be seen on the left administrating the drugs to school children (in white and blue uniforms).

3.3.4 Data analysis:

Children who were not treated with praziquantel in the initial stage but turned up for the follow-up survey have been excluded from the analysis. Also excluded were those who were infected with schistosomiasis in the initial sampling but were not available at the time of drug distribution. All children from Mtanga village where only three of them were infected with schistosomiasis and their treatment at an outpatient clinic could not be confirmed have also been excluded from the analysis. The terms prevalence and intensity of infection were used as previously described in chapter two. The percentage reduction in the prevalence of schistosomiasis infection after praziquantel delivery was determined using the formula: $\{[(\text{Prevalence1}-\text{Prevalence2})/\text{Prevalence1}] \times 100\}$, where Prevalence1 indicated infection level before treatment while Prevalence2 was recorded after treatment.

Mean intensity of infection was calculated using arithmetic mean using direct egg counts
 $\text{mean} = \Sigma \text{epg}/n$, where:

epg is the egg count from each infected individual and n is the number of infected subjects (Montresor *et al.*, 1998). Percentage reduction in the intensity of schistosomiasis after treatment was calculated using the following formula: $\{[(\text{epg1}-\text{epg2})/\text{epg1}]*100\}$ where epg1 and epg2 are mean intensities (excluding zero counts) before and after treatment, respectively (Montresor *et al.*, 2005). Variation in the prevalence of schistosomiasis before and after treatment and the number of tablets given to each child based on their weight was compared to those predicted by their respective height using a simple paired t-test. The relationship between children's weight, height and age were analysed using a correlation test (Hall *et al.*, 1999). A statistical significant level was taken at <0.05 . Scatter plots of children age and weight against their height indicated a strong positive correlation between age and height (Figure 3.3; Pearson's product-moment correlation $r = 0.57$, $p = 4.305e-11$) as well as weight and height (Figure 3.4; $r = 0.74$, $p < 2.2e-16$). This correlation suggested that the effects of weight and age on schistosome infection in the children were confounded by height. Weight and age were therefore not analysed for their influence on schistosomiasis infection in children. Statistical analysis on the possible impact of study site and children's sex and age on the prevalence and intensity of schistosomiasis indicated no significant influence ($p > 0.05$).

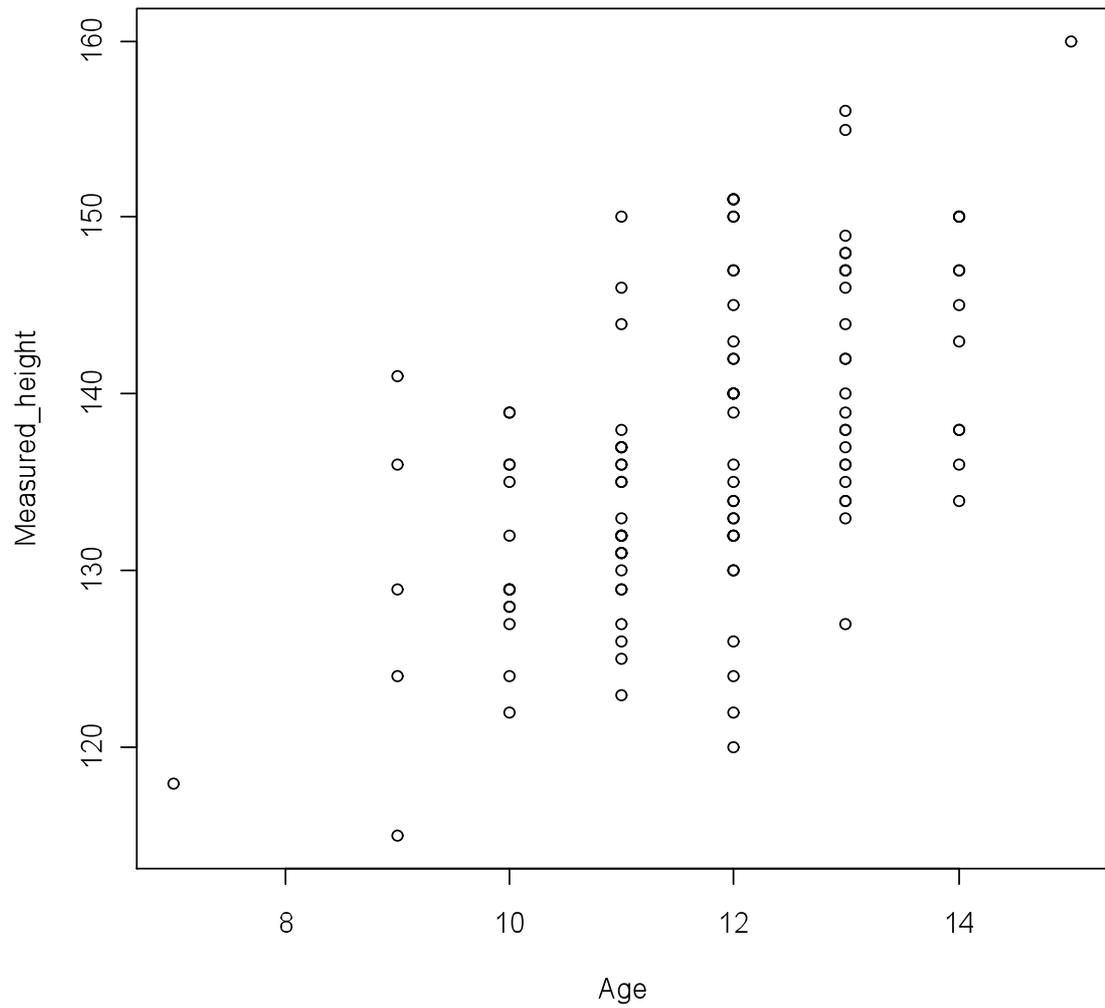


Figure 3.3: Scatter plots indicating some degree of positive correlation between host age and the confounding factor of height in children.

The children were aged between 7 and 15 years and the correlation was positive (Pearson's product-moment correlation $r = 0.57$, $p = 4.305e-11$). Age was thus excluded from the analysis because its effect was confounded by, height, which was the main factor.

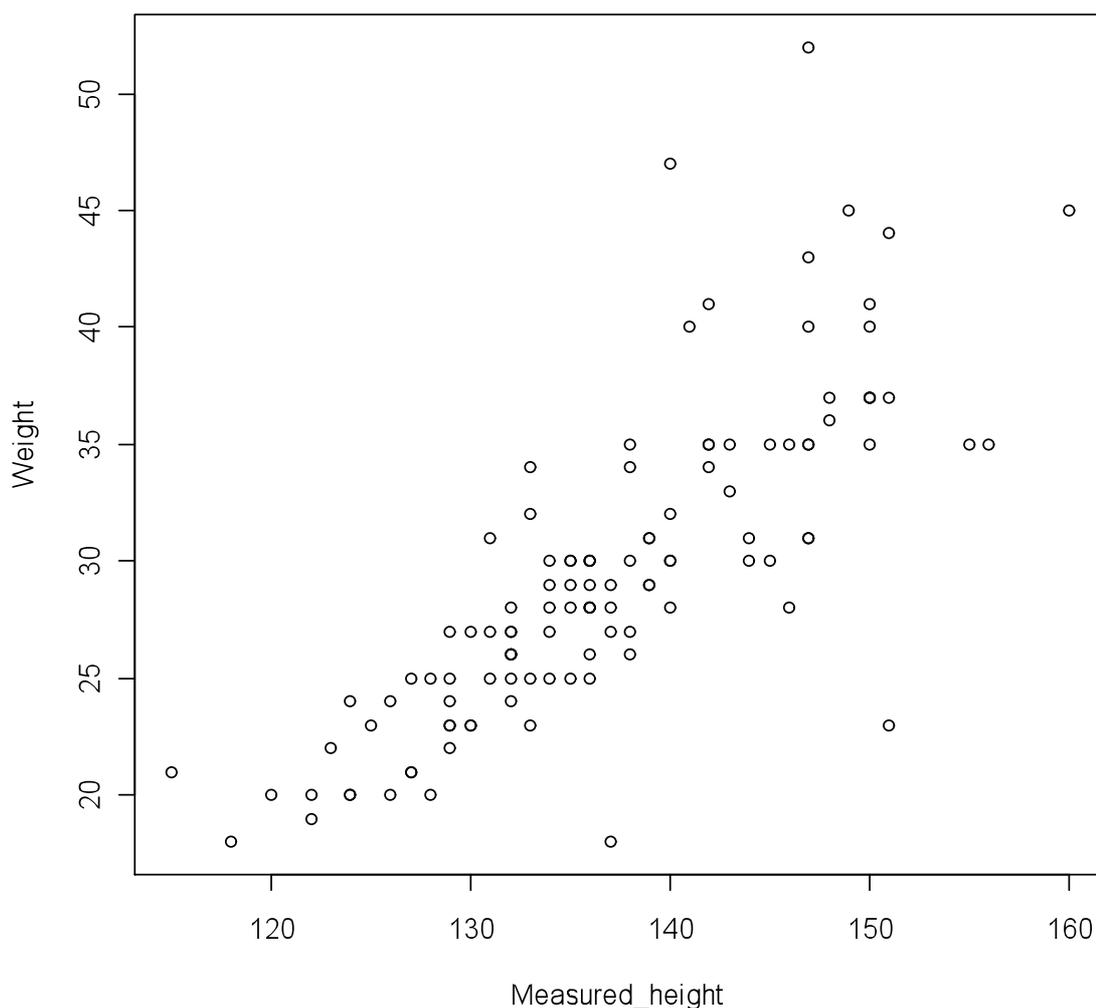


Figure 3.4: Scatter plots of children’s weight against their height indicating a strong correlation.

This correlation between the two factors ($r = 0.74$, $p < 2.2e-16$) was essential to emphasize the predictive relationship between weight and height, which is the basis for using height as a proxy measure of weight during praziquantel distribution. The strong correlation between these factors meant also that height had a confounding influence on weight. Because of that weight was excluded from the analysis as its influence was confounded by height. There were no children aged less than 7 years at school so height measurements start at approximately 100cm.

3.4 Results

One hundred and thirteen children turned up for re-sampling during the follow-up study after praziquantel distribution, representing 57.9% of participants in the initial sampling (Table 3.1). A minimum of 0.75 tablets and a maximum of three tablets were provided to children based on weight while 1 and 3 were minimum and maximum tablets predicted by height, respectively (Table 3.2). The minimum and maximum praziquantel dosage of 23mg/kg and 43mg/kg (mean: 35.2mg/kg) were given to the children based on their weight while between 29mg/kg and 78mg/kg dosage (mean: 45.3mg/kg) would be provided based on their height (Table 3.3). Nonetheless, six children (5.3%) received less than the optimal dosage of praziquantel (i.e. <30mg/kg) based on weight while two children (2.6%) would have received the drugs above the optimum amount (>60mg/kg) based on height (Table 3.3). The dosage of praziquantel drugs provided to the children based on their bodyweight were significantly lower than those predicted by their height ($z = -13.7$, $df = 227$, $p < 2e-16$). The prevalence and intensity of schistosomiasis were significantly lower after praziquantel distribution than before treatment (prevalence: $z = 7.1$, $df = 180$, $p = 9.89e-13$; intensity: $z = 4.5$, $df = 5$, $p = 7.69e-06$).

Table 3.1: Age range (in years) of school children surveyed for schistosomiasis. Numbers in brackets indicate the sample size for each school (n).

Sex	School		
	Mwamgongo	Bugamba	Kiziba
Boys	10-15(29)	7-15(22)	9-13(9)
Girls	9-14(19)	9-13(27)	9-13(7)

Table 3.2: Minimum and maximum number of praziquantel tablets estimated by weight and height among Tanzanian school children

Praziquantel numbers (tablet numbers/child)	Provided based on weight	Predicted by height
Minimum	0.75	1
Maximum	3	3
Average \pm SE	1.73 \pm 0.04	2.17 \pm 0.04

Table 3.3: Praziquantel dosage estimations based on children's weight (Average for weight column) and height (Average for height).

The table indicates that the average optimum dosage based on weight (35.7 mg/kg) was lower than that predicted by height (44.9 mg/kg). There was also an underdosage based on weight in six children while a slight overdosage was recorded based on height for two children. Numbers in brackets indicate the children who received an underdose, optimum or overdose of praziquantel drug based on weight and those predicted by height (N = 113). Dosage description was treated according to Montresor *et al.* (2005).

Dosage (mg/kg)	Dosage description	Average for weight in mg/kg	Average for height in mg/kg
<30	Underdose	26.8 (6)	29(1)
≥30≤60	Optimum	35.7(107)	44.9(110)
>60	Overdose	0	72.5(2)

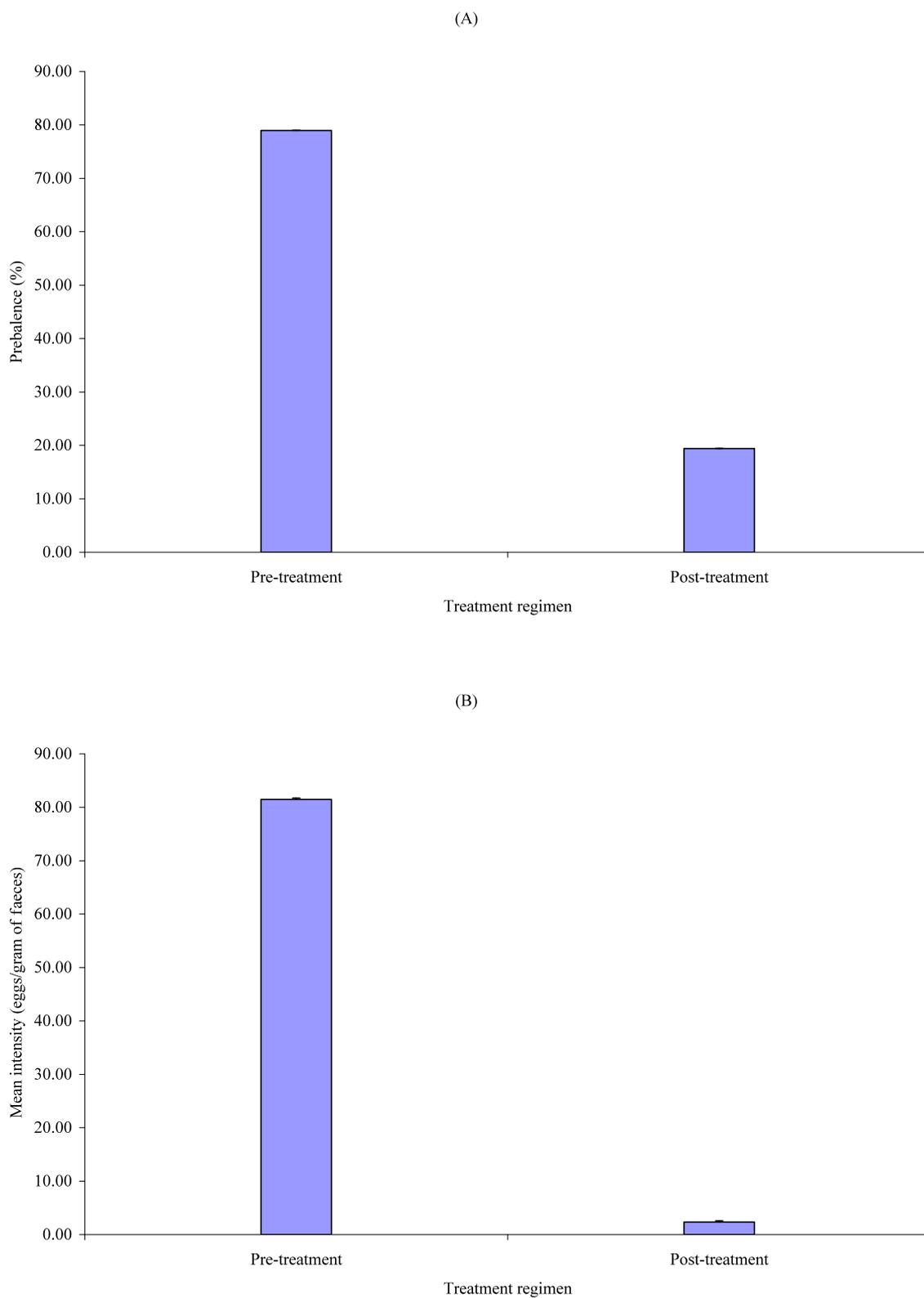


Figure 3.5: Comparison of the prevalence (A) and intensity (B) of schistosomiasis before and after praziquantel distribution among school children.

Pre-treatment n = 113 and post-treatment n = 67.

3.5 Discussion

3.5.1 Comparison of tablet pole and weight-based praziquantel distribution

In general most children (94.7%) received praziquantel dosage within the acceptable or optimal range (i.e. 30-60mg/kg) based on weight while 97.4% of them would have received the drugs within this range based on the children's height (Table 3.3). The overall majority of the children (92.9%) received the optimum dosage of praziquantel based on both weight and height. However, there was an indication of drug underdosage based on children's weight although this value was marginal (Table 3.3). On the other hand height appeared to predict a dosage within the accepted range, except for a small proportion of cases where it over-inflated the dosage that would be considered to be above the recommended based on weight (Table 3.3). Both weight and height therefore estimated the dosage of praziquantel within the range acceptable by the World Health Organization (30-60mg/kg). This is similar to a report by Montresor *et al.* (2001) who tested 1289 children in Zanzibar and concluded that although weight measurements performed better than dose pole in estimating the optimal praziquantel dosage (40-60mg/kg), the overall achievement of the two methods was similar in estimating acceptable dosage of the drug (30-60mg/kg). Nonetheless, weight measurement remains the most preferred method or gold standard for praziquantel distribution (WHO, 2011).

The percentage of children indicated by height (97.4%) in the present study that they would receive praziquantel dosage within the acceptable range of 30-60mk/kg is consistent with findings from other studies such as 99.2% and 98% by Montresor *et al.*, (2001; 2005). Using data from over 25000 children from 10 African countries, Montresor *et al.* (2005) for instance found that based on dose pole, 98% of the children would have received acceptable dosage of praziquantel. This perhaps indicates the usefulness of the method for this purpose despite the overdosage that was observed. A slight overdosage of

praziquantel drugs distributed according to height (0.8%) was also reported by Montresor *et al.* (2005) compared to the 2.6% observed in the present study. According to the report there was also some variation in the performance of dose pole according to the country of origin for the children examined. For instance, the performance of dose pole was not good in China where only 94% of the children would have received acceptable dosage. This discrepancy was attributed to the fact that 20% of the children were overweight and this condition affected the body mass index. Montresor *et al.* (2001; 2005) reported an underdosage of praziquantel drugs by 0.2% and 1% based on weight and height, respectively but considered this effect to be too minor to have any meaningful impact on the individual. Overall, both weight and height measurements estimate the dosage of praziquantel within the acceptable range. Further studies are however necessary to investigate how the applicability of these methods could be improved as it has also been argued by other authors (Montresor *et al.* 2001, 2002; WHO, 2002; Sousa-Figueiredo *et al.*, 2010).

The presence of excess dosage (overdose) based on height observed in the present and in previous studies such as Montresor *et al.* (2005) can have reasonable logistical and medical implications. Logistically, although no study has investigated the cause of this excess dosage, it could be attributed to an inherent subjectivity of the tablet pole method, which provides dosages in increments of half tablets rather than lower increments of praziquantel that are used with weight. For instance, if the ruler that is used to find the height of a child exactly touches the line between two doses of tablets (e.g. on the line between 1½ and 2), it is suggested that the child be given the higher dose (i.e. 2 tablets): (http://portal.unesco.org/education/en/files/37083/11018312303FINAL_cc4-helmt03praziquant.doc/FINAL%2Bcc4-helmt03praziquant.doc). On the other hand, the lower dose of 1½ tablet would be given to the children based on their weight. A study by Swar and Amin (2011) on dose pole application among children in Sudan, found that

among older children (over 12 years of age) dosage estimated by height tended to be higher than those from weight. The authors argued that growth velocity slows down at age ≥ 18 years, and the body surface area to weight is impaired compared to body surface area to height (Swar and Amin, 2011). In this process the fat and muscle mass of the body change quicker than the body height. They therefore concluded that height alone is unreliable measure for estimating the dosage of praziquantel drugs (Swar and Amin, 2011).

The overdose can also have significant medical or pathological impact to individual patients and the population at large. Although side effects of praziquantel are transient and self-limiting, its dosage above the acceptable levels may worsen the drug's side effects. If the inflation of praziquantel dosage is real, health of the children could be compromised since high doses of praziquantel can cause adverse side effects (Midzi *et al.*, 2008). This may ultimately reduce compliance due to the negative perception of the impacts of the drug. Although the inflated dosage of the drugs based on height was marginal it could be substantial if the inflation is extrapolated to reflect the reality in the field. The extrapolation is important because in most cases many children are normally involved in the treatment and quite often each child would need more than one treatment (Sousa-Figueiredo *et al.*, 2010). A recent study on the efficacy of 40mg/kg and 60mg/kg dosages found no significant differences to suggest that the higher dosage was required for treating intestinal schistosomiasis caused by either *S. mansoni* or *S. japonicum* (Olliaro *et al.*, 2011). It is thus recommended that lower doses of praziquantel (<40mg/kg) are as effective in morbidity control as the higher doses of the drug and so alternative means should be employed to reduce the wastage of drugs (overdose) resulting from dose pole estimations. This is important given the scarcity of praziquantel drugs in most schistosome endemic areas. Given the public reactions to the perceived overdosing of praziquantel based on dose pole as previously reported (Tanzania Daima, 2005; Allen and Parker,

2012), more investigation on the efficiency of this method would help to allay these concerns and misconceptions .

The underdosage of praziquantel reported in the present study based on weight estimations and that reported by Montresor *et al.* (2005) based on height estimations, are minor although they could have some ramifications including inducing drug resistance. Chaiworaporn *et al.* (2005) reported that, although that there was no evidence to support the inefficiency of sub curative doses of praziquantel, drug resistance was more sustained in worms treated with subcurative doses of praziquantel than those treated with a full dose. Prolonged underdosage in treating schistosomiasis can lead to an emergence of disease strains that become resistant to the drugs (Brindley, 1994). On the other hand, Taylor *et al.* (1988) found no significant differences in egg reduction of *S. mansoni* between the low and high praziquantel dosages based on bodyweight (i.e. 20-60 mg/kg). It was therefore concluded that morbidity control against schistosomiasis can be achieved with lower dosages of praziquantel (Taylor *et al.*, 1988). This means therefore that the marginal underdosage observed in the present study may have no long term effect on the recipients.

3.5.2 Variation in schistosome infection before and after praziquantel treatment

There was a significant variation in the prevalence and intensity of schistosomiasis infection in children before and after praziquantel distribution (Figure 3.5). Since treatment was conducted during the wet season, it was not possible to distinguish between the effect of drugs and any possible seasonal impact on the decrease in schistosomiasis infection after treatment. However, the findings corroborate with findings recorded elsewhere during mass treatment with praziquantel. For instance, Midzi *et al.* (2008) found similar reduction levels in prevalence and intensity after treating school children with praziquantel based on weight and attributed the drop in infection to the drug's action.

A separate analysis of seasonal effect on schistosomiasis in untreated adult participants in chapter two showed that season had not significant effect on schistosome infection. Therefore, most likely, the reduced low infection recorded in the current study was a result of the treatment provided to the children.

Reduced prevalence and intensity after praziquantel treatment have wider implications for schistosomiasis control programmes. Since high intensities of schistosomiasis are directly related to high morbidity (Magalhães *et al.*, 2011), morbidity is also expected to have decreased in the individuals treated in our study. In other areas such as Uganda where the drug has been properly delivered, noticeable improvements have been observed for schistosomiasis related morbidities such as improvement in periportal fibrosis and hepatosplenomegally (WHO, 2002). Evidence of reduced prevalence and sickness due to schistosomiasis after praziquantel distribution has been reported in various endemic countries particularly in Asia and South America (WHO, 2002; Fenwick, 2011). This success has been attributed to sustained mass distribution of the drugs to children and adult patients (WHO, 2002). Thus successful efforts to have the drugs accessible to most people in poor areas of endemic countries in Africa can have substantial impact in reducing the problem of schistosomiasis as a major public health problem in those countries (Fenwick, 2011).

3.5.3 Conclusion

Both weight and height estimated the dosage of praziquantel within recommended ranges with slight overdose based on height and a marginal underdosage based on weight. Further studies should be conducted on how to improve the performance of these methods so as to increase their usefulness for praziquantel delivery. Sustainable and effectively managed distribution of praziquantel drugs such as through mass administration and school based

treatment has the potential to reduce the prevalence of and sickness of schistosomiasis in endemic countries.

Chapter 4: Trends in Parasitic Infections in Baboons and Vervet Monkeys at Gombe National Park, Tanzania

4.1 Abstract

Increased interactions between humans and wild animals in and around protected areas have raised the risks for sharing diseases and parasites among them. Regular surveillance and monitoring of these infections in such areas is important for improving animal health and controlling any spill-over of animal diseases into nearby human populations. Although parasite infections in habituated baboons at Gombe National Park have previously been investigated, it is currently not known whether the infections have changed over time and how the current levels of infection are related to previous observations in the area. The parasites of non-habituated baboons and those of vervet monkeys in the park have also not been assessed. Faecal samples from habituated and unhabituated baboons and vervet monkeys at Gombe were examined for parasites using the formo-ethyl technique. Season, baboon sex, habituation and troop membership were examined for their influence on parasite infections. The parasites identified in baboons and vervets included *Trichuris* spp., *Physaloptera* spp., hookworms and unidentified nematodes, while *Paragonimus* spp., *Streptopharagus* spp. and *Schistosoma mansoni* were exclusively detected in baboons. Molecular analysis of baboon schistosome eggs confirmed them to be *S. mansoni*. Comparison of parasite occurrence showed that *Trichuris* sp. had the highest prevalence (35%) while the least common parasite was *Streptopharagus* sp. (5%). A GLM analysis indicated that the interaction between season and baboon troop was a significant predictor of parasite prevalence and intensity. Habituated baboons in Gombe had relatively higher parasite richness and parasite load than non-habituated baboons ($z = -4.286$, $p = 1.82e-05$), except for nematodes, whose prevalence was significantly higher among non-habituated baboons than habituated populations ($z = 2.922$, $p = 0.00348$). Most baboons currently infected with schistosomiasis in Gombe were born and infected with the disease after the last schistosome sampling in the area in June 1992, suggesting either that, humans have

continued to re-introduce the disease into the park or that baboons themselves can maintain the infection. Parasite intensity in the vervets was significantly higher among those residing near Mwamgongo village compared to those at Mtanga village (*Trichuris* spp.: $z = 2.443$, $p = 0.0146$; Hookworms: $z = 2.084$, $p = 0.0371$). However, the prevalence of these parasites did not show significant variation between the two vervet communities. As most parasites diagnosed in baboons and vervets are capable of infecting humans, these animals can potentially serve as reservoirs of human helminths given the regular human-wildlife interactions in the area. The implication of these observations to wildlife conservation and public health issues in the area has been explored.

4.2 Introduction

The shrinking of wildlife habitats in many parts of the world has brought people and wild animals close together, increasing the risks for sharing diseases and parasites among them (Wallis and Lee, 1999; Woodford *et al.*, 2002). In Gombe National Park in Tanzania, non-human primates such as chimpanzees, baboons and vervet monkeys interact regularly with humans, including sharing resources such as stream water and forests (Wallis and Lee, 1999). The park is comparatively small in size and is surrounded and threatened by human settlements and farms on all its borders except to the west, where it borders Lake Tanganyika (Pusey *et al.*, 2007; Inskipp, 2005). Humans residing in protected areas and those visiting them have been implicated in introducing new infections into those areas (Kortlandt, 1996; Mudakikwa *et al.*, 1998; Pusey, 1998; Mlengeya, 2000; Murray *et al.*, 2000). For instance, a polio outbreak that occurred among chimpanzees in Gombe National Park in the 1960s was later confirmed to have originated from the nearby human population (Wallis and Lee, 1999; Woodford *et al.*, 2002). Thus, regular surveillance and monitoring of infections in such areas is important for improving animal health and controlling possible spill over of animal diseases into nearby human populations.

The parasites of baboons at Gombe have previously been studied and various intestinal parasites that also infect humans, such as *Trichuris* sp., *Strongyloides* sp. and hookworms, have been found in the animals (McGrew *et al.*, 1989; Müller-Graf *et al.*, 1997; Murray *et al.*, 2000). However, it is currently not known whether these infections have changed over time and how the current infections are related to previous observations in the area. Recent demographic and ecological changes in the park may have also influenced parasitic infections in animals (Gillespie *et al.*, 2010). For instance, since 2000, the number of human residents in the park has been halved, which, together with improved sanitation and hygiene, may have lowered chances for disease and parasite transmission between people

and animals in the area (Collins, 2003; Lukasik-Braum and Spelman, 2008; Pusey *et al.*, 2008). However, the number of tourists visiting Gombe has been increasing since 2000 (Figure 4.1) and this may also have influenced disease dynamics among the baboons at Gombe, as recently reported for chimpanzee parasites in the park (Gillespie *et al.* 2010). Baboon troop membership was reported by Müller-Graf *et al.* (1997) as a major determinant of parasite infections in the Gombe baboons in the 1990s but no studies have been conducted since that time to assess the generality of this observation or whether infection dynamics have changed over time with changing human demographics.

Baboons at Gombe have constantly been monitored to study their behaviour since 1967 (Ransom, 1981). Together with chimpanzees, the animals have been deliberately habituated to humans to make them available for scientists to study them and for tourists to view them easily (Goodall, 1986). As a result of habituation, these animals have lost fear of people and they interact with humans regularly. For instance, some of the baboon groups spend most of their time around human residences in the park, scavenging for food and other domestic items (Müller-Graf *et al.*, 1997). Due to these interactions and given also that there are genetic similarities between humans and baboons (Shoshani *et al.*, 1996), there is a potential threat for sharing diseases between them. A study by Gillespie and Chapman (2006) found that habituated primates that interacted regularly with humans had higher parasites than unhabituated populations in western Uganda. It is currently not known how parasites harboured by unhabituated baboon populations at Gombe are related to those of habituated baboons or to human diseases. There are currently six study baboon troops with troop membership of 18-60, located in the Kasekela area of Gombe National Park (Figure 4.2). Since their first description by Müller-Graf (1994), baboon troops have been splitting to form new ones. The current troops include AC, which was originally the A-troop, BA and BB that split from the B-group and DA, DB and DC, which were originally the D-group. The L and C troops that were investigated by Müller-Graf (1994)

have since moved into inaccessible areas and are no longer studied. Detailed descriptions on the Gombe baboon troop are found in Müller-Graf (1994). Unstudied or unhabituated troops are scattered in other parts of the park, particularly around the park's boundaries, with their numbers approximated to 3000 individuals (A. Collins, *personal communication*).

A parasite of major medical significance that has also been reported in habituated baboons at Gombe is *S. mansoni* (Nutter, 1993; Müller-Graf *et al.*, 1997). The parasite is also known to occur among local human communities around Gombe (Mung'ong'o, 1999; A. Collins, *personal communication*), where it causes widespread intestinal schistosomiasis. The parasite was first reported in humans at Gombe in 1983 (A. Collins, *personal communication*) and a systematic study by Müller-Graf *et al.* (1997) in 1992, reported high prevalence in the baboons that interacted regularly with humans in the park. It is therefore believed that *S. mansoni* was probably brought into Gombe by people and it is possible that re-introduction has persisted today as people continue to move in and out of the park. However, it is also possible that baboons themselves have been maintaining the parasite without any link to humans. It should be remembered that as described in chapter one, adults of *S. mansoni* parasites can survive in humans up to 30 years (Brown, 1969; Gryseels *et al.*, 2006), and possibly they have the same life span in other definitive hosts such as baboons. Thus, knowledge about current levels of infection, compared to the previous study would therefore be informative about transmission dynamics of the disease and its longevity.

The history of schistosomiasis in Gombe and the reported human-baboon interactions also suggest that baboons and humans in the area could be infected by the same strain of *S. mansoni* parasites. Baboons are widely known to be good hosts for schistosome parasites in nature and it has been speculated that they could serve as reservoirs for human

schistosomiasis in shared habitats (Miller, 1960; Nelson, 1960; Fenwick, 1969; Else *et al.*, 1982; Ouma and Fenwick, 1991; Erko *et al.*, 2001; Legesse and Erko, 2004). Baboons could be more susceptible to this disease than other primates because of their close association with water (Figure 4.3) and human settlements, but it is not known whether they are primary hosts for the parasites (Ouma and Fenwick, 1991; Nunn and Altizer, 2006). Experimental studies have also shown that schistosome infections in baboons produce features that are similar to those of the parasite infection in humans (Nyindo and Farah 1999; Farah *et al.* 2001). Nonetheless, it is still not exactly known whether baboons harbouring *S. mansoni* in the wild could serve as potential reservoirs for human schistosomiasis (Ouma and Fenwick, 1991). It is therefore important to establish whether baboon schistosomes in Gombe are genetically or epidemiologically distinct from those of humans in the area (Miller, 1960; Fenwick, 1969; Else *et al.*, 1982; Legesse and Erko, 2004).

Like baboons, vervet monkeys are also known to acquire higher levels of water-borne infections, such as schistosomiasis, than most other primates because of their habitual contact with water bodies (Ouma and Fenwick, 1991; Legesse and Erko, 2004). Legesse and Erko (2004) noted in particular that helminth parasites (including schistosomes) from vervets in Ethiopia posed a high risk to public health in areas where the animals interacted closely with humans. It is therefore essential that the parasite fauna of vervet monkeys is well understood and monitored, particularly in areas where these animals interact with humans. In Gombe National Park there are two small communities of vervet monkeys (*Cercopithecus aethiops*) that are currently confined to the northern and southern boundaries of Gombe National Park (Figure 4.4). The vervets constitute a key component of the Gombe's ecosystem through their interactions with other animals and humans (Goodall, 1986). Quite often they leave the park to go into the nearby villages, visiting particularly dumping sites and other residential areas in search of food (Figure 4.5). This

puts the animals at risk of spreading or acquiring parasites and diseases from the human population. Despite these potential risks, the parasite fauna of vervets at Gombe has not been examined.

The purpose of this chapter was to determine the parasite species currently present in baboons and vervet monkeys at Gombe, in the context of what has been found by previous researchers and in relation to predicted levels of interaction with humans in the area. Specifically, measures of infection in stool samples based on parasite prevalence, intensity, species richness, total parasite load and species diversity were assessed to determine how they are influenced by season, baboon sex, troop membership, and habituation. Retrospective age analysis was also used to assess whether baboons currently infected with schistosomiasis were covered in the sampling report by Müller-Graf *et al.* (1997), in order to assess whether new infections have occurred, either due to human introductions or maintenance of the parasites in the baboon populations. Finally, a molecular approach was used to assess whether baboons share the same strains of *S. mansoni* as humans, which would provide conclusive evidence for cross-species transmission.

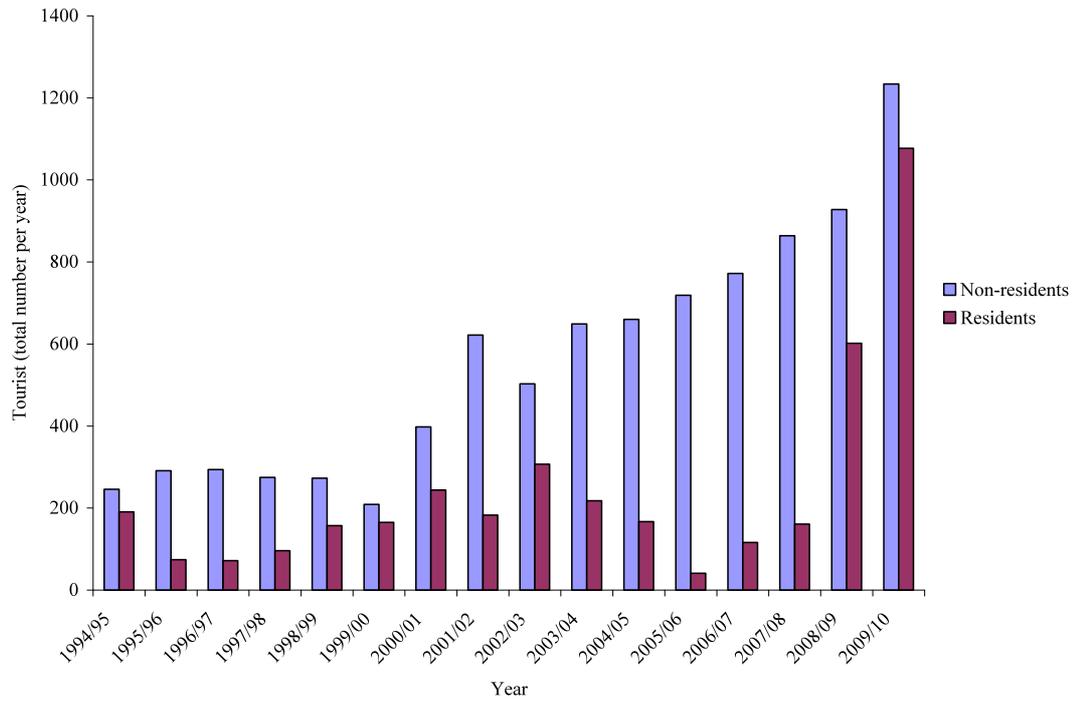


Figure 4.1: Trends in the number of resident and non-resident tourists visiting Gombe National Park between 1994 and 2010.

The figure shows a steady increase in the number of non-resident tourists visiting Gombe National Park after 2001. Data adapted with permission from Tourism Warden, Gombe National Park.

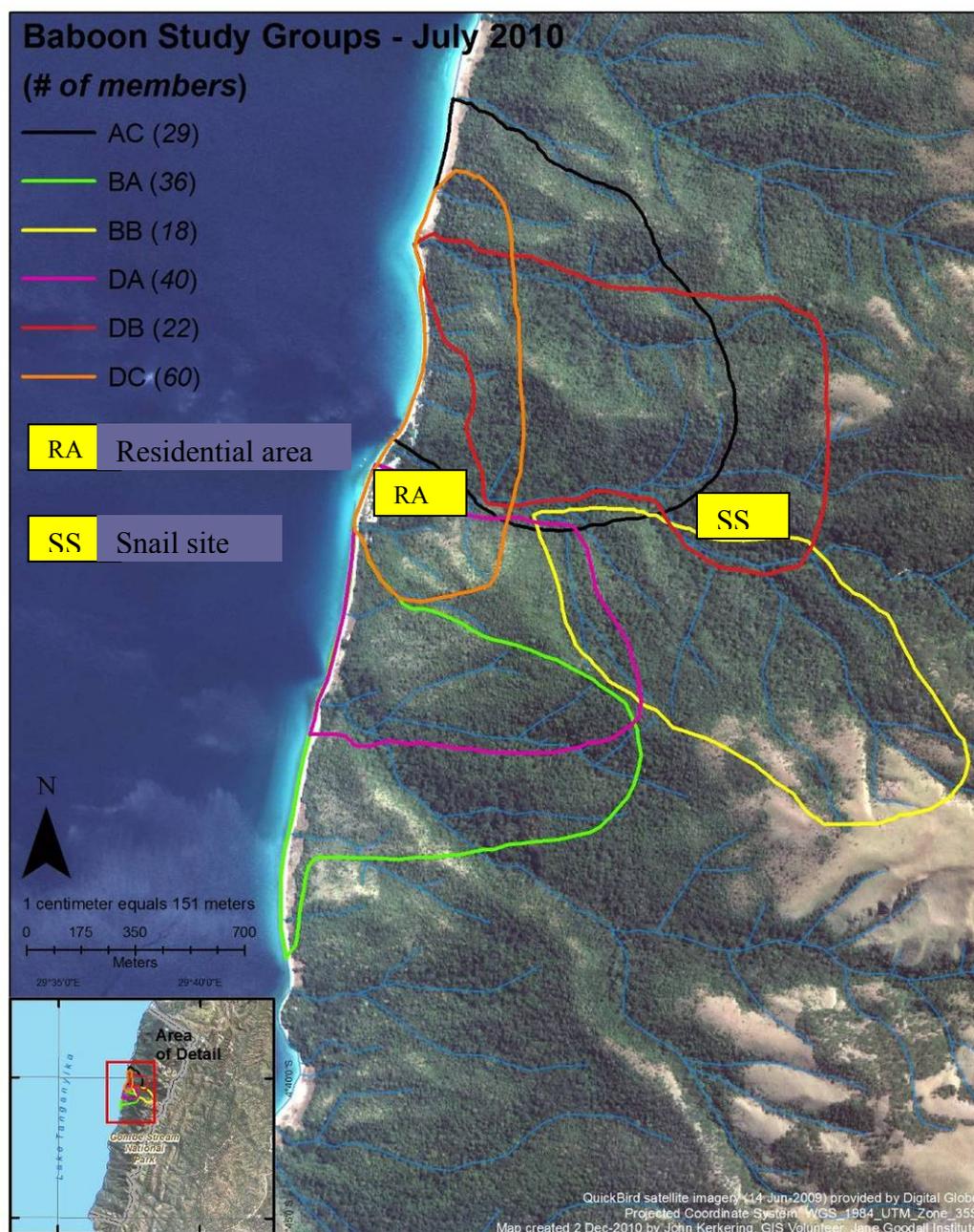


Figure 4.2: Detailed spatial distribution and home ranges of habituated baboon troops at Gombe National Park.

There is some degree of overlap between home ranges of different groups and the home ranges of some troops, such as DA, DC and AC overlap with human residential areas (RA). The boundaries of other troops also include snail sites (SS), where schistosome transmitting *Biomphalaria* snails have been found. Numbers in brackets on the left side of the map indicate the size of each troop. (Reproduced with permission from the author, John Kerkerling at the Jane Goodall Institute).



Figure 4.3: Water contact as one of the risk behaviour among baboons at Gombe National Park.

Such tendencies expose the animals to water-borne diseases such as schistosomiasis.

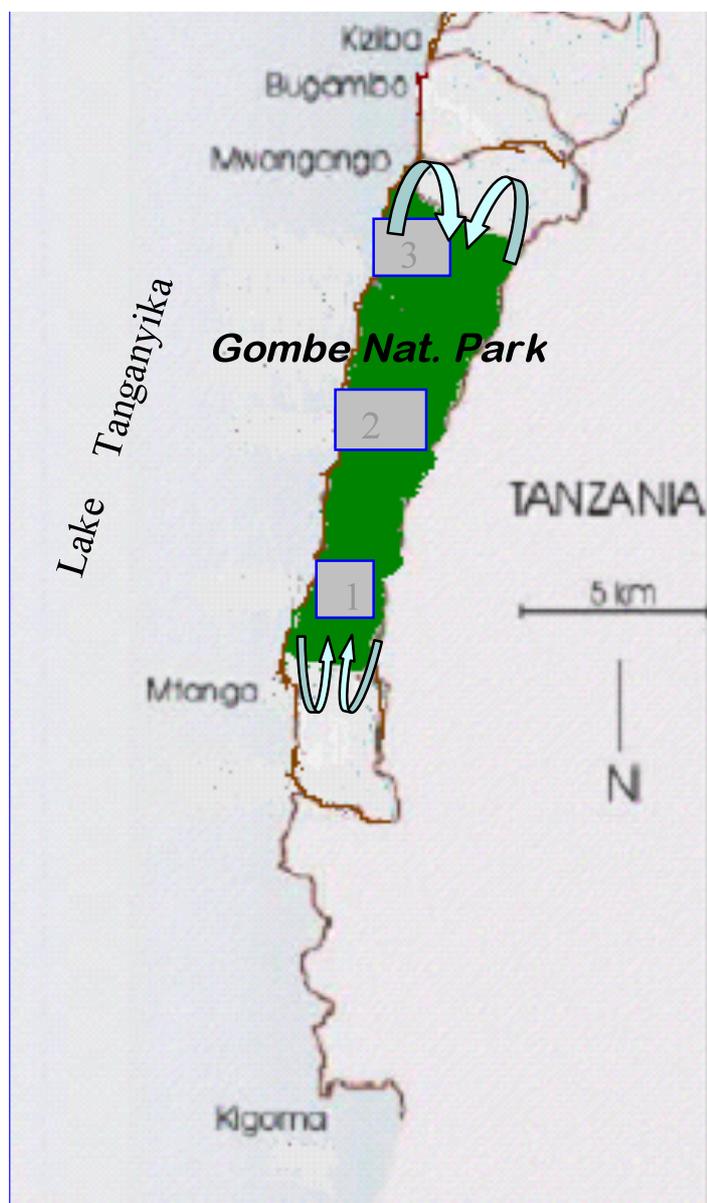


Figure 4.4: A sketch map of Gombe National Park, showing the distribution of baboon and vervet monkey communities.

The localities of these communities in the study area indicated by numbers 1-3 in grey boxes; unhabituated baboons and vervet monkeys at Kalande to the park's southern boundary (1), habituated baboons at Kasekela in centre of the park (2) and unhabituated baboon and vervet monkeys at Mitumba near the northern boundary of the park (3). Blue-green curved arrows represent movements of unhabituated baboons and monkeys between Gombe and neighbouring villages of Mwamgongo and Mtanga, respectively. Map adapted with permission from Lombardozi, 2003.



Figure 4.5: A vervet monkey from Gombe National Park.

The vervet can be seen passing by a kitchen in the backyard of a residential house in Mwangongo village, near the park's north boundary.

4.3 Materials and Methods

4.3.1 Experimental design for stool collection from baboons and vervet monkeys

The permission to conduct this study in Gombe National Park was granted by the Tanzania Wildlife Research Institute and Tanzania National Parks (Permit Ref. No. TNP/HQ/E.20/08B). Samples were obtained from animals in accordance with the accepted animal welfare and guidelines for working with wildlife subjects for research purposes (<http://www.tawiri.or.tz/images/Conference/guideline.pdf>). Baboons in study groups at Gombe are habituated to humans and this makes it possible to obtain stool samples from known individuals. Unlike those in unhabituated populations, baboons in habituated troops have constantly been monitored to study their behaviour since 1967 (Ransom, 1981). They are thus individually recognized and accessible for sampling. This makes it possible to accurately relate their demographic information such as age and sex to parasitological data obtained.

In habituated baboons, a target was set for sampling 30 individuals per troop (for troops BA, DA and DC with troop sizes exceeding 30 individuals) consisting of 10 juveniles, 10 adolescents, 10 adult with at least one half of them being males). In baboon troops AC, BB and DB whose sizes are below 30 (Figure 4.2 above, also Table 4.1), all individuals in each of those troops were targeted. Randomisation for sampling in troops BA, DA and DC was achieved by putting names of known individuals into a container and randomly selecting those to samples. Age groups were classified according to Pereira (1989) and Müller-Graf (1994), with individuals aged 3-5 years considered as juveniles, 5-8 years as adolescents and >8 years as adults. This sampling design was used because the number of juveniles and adolescents are usually higher than adults in baboon populations (Pereira, 1989). Faecal samples from habituated baboons were collected by the Gombe field assistants, who can identify all individuals in a troop. The animals were watched as they

fed in the forest and approximately 5g of the sample was picked up immediately after defaecation and preserved in 20ml vials containing buffered 10% formalin (Figure 4.6A). At least two stool samples were targeted from each individual baboon in the habituated troops, with one sample in the wet season (January-April) and another in the dry season (July-September) periods in 2010. The number of samples collected from each troop of habituated baboons in the wet and dry seasons is shown in Table 4.1 with troop DC sampled in the wet season only. Seasons were defined as described in Collins and McGrew (1988), Goodall (1986) and Müller-Graf (1994), where the wet season runs from October to May, and the dry season from June to September.

Unhabituated baboons and monkeys were sampled from two sites, the park boundaries near Mwangongo (to the north) and Mtanga (to the south). In consistence to the sample size in habituated animals, a total of 30 independent samples was aimed to be collected from each community of unhabituated baboons and vervet monkeys and Tables 4.2 and 4.11 respectively shows the number of samples that were obtained in the end. Stool samples from unhabituated populations were collected following guidelines in Lilly *et al.* (2002) with some modifications as described below. Each community except baboons at the northern park border (Table 4.2) were followed at dusk a day before sampling and a site where they slept noted. The animals were watched as they woke up in the early hours of the following day. As each animal left its nest, it defaecated and the sample, deemed therefore to be independent, was picked up and preserved as described above for samples from habituated animals. For the unhabituated baboons at the northern border their sleeping sites were not accessible and so they were lured out of the forest during the day by spreading maize corns or bananas on an open area (Collins, *personal advice*; Figure 4.6B). As they ate, the animals defaecated and the samples were picked up immediately and preserved as described above. The number of stool samples collected in the wet and dry

seasons from unhabituated baboons and vervet monkeys is respectively shown in Tables 4.2 and 4.11.



A



B

Figure 4.6: Collection of stool samples from wild animals at Gombe National Park. Picking up of faeces immediately after defaecation is possible for habituated baboons (A) while unhabituated individuals may need to be attracted out of the forest through temporary provisioning of maize cobs (B).

4.3.2 Retrospective analysis of baboon ages

The age of the baboons included in the study was used to determine whether individuals currently infected with schistosomiasis had also been studied in 1992 (Müller-Graf, 1994). Sampling by Müller-Graf (1994) was completed in May 1992, and June 1992 was therefore set as the reference time for whether individual baboons were either born before or after. If individuals presently infected with schistosomes were born after June 1992 they were deemed not have been studied by Müller-Graf and therefore to have acquired the disease after the study. To achieve this current ages of the individual baboons investigated were retrospectively traced to see if they were born before or around June 1992 when the first sampling on schistosomiasis was carried out in Gombe (Müller-Graf, 1994).

4.3.3 Microscopical examination of animal stools

Formalin-preserved samples were examined for helminth eggs and larvae, and protozoan trophozoites and cysts, using the formal-ethyl concentration technique (Allen and Ridley, 1970; WHO, 1991; Cheesbrough, 1998), in the Department of Zoology and Wildlife Conservation at the University of Dar es Salaam, Tanzania. This technique was chosen because of its reported superiority over other techniques in detecting a wide variety of parasites (Allen and Ridley, 1970; Müller-Graf, 1994). Each 20 ml universal plastic container containing unweighed faecal sample 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 (Cheesbrough, 1998; WHO, 1991). Retained materials on the strainer were examined for any parasites under a dissecting (stereo) microscope and then discarded. The filtrate was transferred into a 15 ml centrifuge tube and the solution centrifuged at 3000 rpm for 1 minute to get rid of the formalin preservative. This was achieved by decanting the supernatant, while retaining the debris at the bottom of the centrifuge. To obtain a stool sample with a known weight, one gram of the debris was picked up and weighed using a digital scale (Sartorius, Göttingen Germany). The sample was then mixed with 6ml of 10% formalin, followed by addition of 3ml ethyl acetate. The formalin-ethyl-faeces mixture was then re-centrifuged at 3000 rpm for one minute to isolate the parasites. The supernatant was discarded while the debris at the bottom of the centrifuge tube was retained. The debris was spread onto a microscope slide and the whole material examined for parasites under a 10x objective of the compound microscope. Each microscopical field on the slide was systematically and completely examined in a zigzag fashion, as described in chapter two. Since each examined stool sample weighed one gram, observed parasite eggs or larvae were counted and reported as eggs per gram or larvae per gram of stool, respectively (WHO, 1991; Müller-Graf, 1994).

4.3.4 Identification of parasites

Parasites were identified based on morphology, size and appearance of eggs/larvae by using identification guidelines, keys and photographs in the literature (WHO, 1991; Müller-Graf, 1994; Cheesbrough, 1998). Due to lack of published materials on wild animal parasites, identification of the various parasite stages in the present study was made in reference to characteristics of human parasites. Representative parasite materials were photographed by holding a digital camera onto one of the microscope eyepieces. Measurements of each parasite stage observed (eggs or larvae) were made using an eyepiece micrometer (Cheesbrough, 1998). Photographs of all identified parasite eggs and larvae were sent to the Scottish Parasite Diagnostic Laboratory (SPDL) in Glasgow, UK to confirm their identities. For baboons that were diagnosed with two or more trematode eggs (*S. mansoni* or *Paragonimus* sp.) per slide based on microscopy, additional samples were collected in order to obtain samples that could be used to isolate fresh eggs and larvae for molecular analysis. Isolation of trematode eggs from baboon stools and subsequent extraction, PCR amplification and sequencing were performed as described for human schistosome larvae and eggs in chapter 2.

4.3.5 Data analysis

Season, baboon troop membership and habituation were evaluated for their influence on parasite prevalence, intensity, mean intensity, species richness, total load and species diversity. Prevalence was calculated as the percentage of infected individuals out of all examined individuals (Bush *et al.*, 1997). Parasite intensity (eggs per gram of faeces) was assessed as the number of parasite eggs or larvae in 1gm of stool for each individual host (WHO, 1991). Mean intensity was calculated at the population level, as suggested in Montresor *et al.* (1998). Arithmetic mean was calculated for individual egg counts (excluding zeros from uninfected individuals) as shown below: arithmetic mean = Σ

(epg/n), where: epg is the direct egg counts for each infected individual, and n is the number of subjects investigated. Parasite species richness was taken as the total number of parasite taxa recorded in each individual host, as suggested in Bush *et al.* (1997) and Rózsa *et al.* (2000). Total parasite load was calculated as the sum of all parasite eggs and larvae in an individual host. Parasite species diversity was expressed as the Shannon-Wiener (SW) index and calculated as species richness and a factor that measures the relative proportion of members of each species present in each host individual as calculated by the formula: $SW = -\sum P_i \times \ln(P_i)$, where: SW is the Shannon-Wiener index and P_i represents the proportion of individual eggs or larvae belonging to the i th parasite taxon (also see Bush *et al.*, 1997 for more details).

Statistical analysis on variation in prevalence and intensity was performed for all parasite taxa except *Streptopharagus* sp. and *Schistosoma mansoni* due to small sample size; i. e. <10% prevalence. The taxa were, however, included for analysis of trends in parasite richness, parasite load and species diversity. Analysis of seasonal effect (wet or dry season) and baboon troop membership was based on samples obtained from habituated or known individuals only to avoid sampling bias in unidentified hosts. The effect of habituation was analysed based only on wet season samples (for which the largest sample sizes were obtained) because the same individuals could not be compared in the wet and dry seasons for the unhabituated animals, whereas paired samples were available for habituated samples. This could have created a bias in assessing the interaction between season and habituation.

The same response variables were evaluated to assess the influence of location in vervet monkeys except that species diversity was excluded due to insufficient data for calculating it. Similarly, as individual monkeys could not be individually identified, their repeated samples lacked independence. Hence, no seasonal comparison of the samples was

performed; instead dry season samples were analysed to elucidate the parasite infection patterns in vervets because too few samples were available in the wet season.

Data analysis was performed using Generalized Linear Models (GLM) in the R programming environment, as described in chapter 2. Prevalence for each parasite taxa was coded as 1 if a particular parasite species was present and 0 if it was absent and this binary data was analysed using GLMs under a binomial distribution, as suggested by Zuur *et al.* (2009). Frequency distribution of parasite egg counts in baboon and vervet showed a Negative Binomial (NB) distribution with excess zero counts for all parasites except *Streptopharagus* sp. and *S. mansoni*, which were excluded due to small data sets. Only the frequency distributions (negative binomial) of the egg counts of *Trichuris* sp. and *Physaloptera* sp. are given as examples in Figure 4.15 as all other parasite taxa showed the same type of distribution. As discussed in previous chapters, the Zero-Inflated Negative Binomial models (ZINB) were used to analyse variation in parasite egg counts, as the models can accommodate the overdispersion and excess zeros parts of the data. This was also true for parasite load and species diversity (Shannon-Wiener index; Figure 4.16) but not for species richness, which did not show an excess of zeros and was not strongly skewed (Figure 4.17). As for chapter 2, the best-fitting distribution model to use in GLM analyses of these factors was selected by comparing three candidate distributions: 1) Negative Binomial regression (NB regression) models 2) the zero-inflated poisson model (ZIP) and 3) the Zero-Inflated Negative Binomial models (ZINB). As described above, the models were run in R with the ZINB model performed under the *pscl* package (Jackman, 2011; <http://CRAN.R-project.org/package=pscl>). A likelihood ratio test indicated that the Zero-Inflated Poisson model (ZIP) fitted best for total parasite load and species diversity. Throughout the analysis, statistical significance level was taken at <0.05 .

4.4 Results

4.4.1 Baboon parasites identified through microscopy

A total of 126 individuals were sampled from habituated baboons (Table 4.1) and 105 of them (83%) were infected with one or more parasite taxa, while no parasites were detected in the other 21 (17%) baboons. As described in the previous sections, one gram of faecal material from each host was concentrated and whole concentrate was examined for parasites under a 10x objective of the compound microscope. No stool samples were obtained from the unhabituated baboons at Mwamgongo in the dry season while thirteen samples were collected from them in the wet season at Mtanga (Table 4.2).

Table 4.1: The troop sizes of habituated baboons at Gombe and the numbers of individual baboons sampled in the wet and dry seasons from each troop.

Some baboons that were sampled during the initial sampling in the wet season could not be traced for a second sample in the wet season. Troop DC was sampled in the wet season only and given here for comparison only but was excluded from statistical analysis on seasonal effect. There is therefore a discrepancy in the number of samples collected between the wet and dry season from each troop.

	Baboon troop					
Age group	AC	BA	BB	DA	DB	DC
Troop size	29	36	18	40	22	60
Sample size	18	30	11	23	22	22
Wet season	18	28	10	22	21	22
Dry season	10	18	6	16	12	0

Table 4.2: The number of independent stool samples collected from unhabituated baboons at Gombe National park in the wet and dry season.

Due to inaccessibility of the animals, no stool samples were obtained from the unhabituated baboons at Mwamgongo in the dry season while most samples were collected from them in the wet season at Mtanga.

	Season	
	Wet	Dry
Community		
Mwamgongo	12	0
Mtanga	13	12

Seven types of parasite taxa, comprising five nematodes and two trematodes were recorded in baboons. Three genera of nematodes were identified (*Trichuris*, *Physaloptera* and *Streptopharagus*), but other parasites found could only be assigned to hookworms or unknown nematode groups. One of the trematodes was identified to species level as *Schistosoma mansoni*. The other trematode was identified to genus level (*Paragonimus* sp.). All parasite taxa recorded in the present study had previously been reported in baboons, except for *Paragonimus* spp. The parasite, which was previously described as an unidentified trematode (McGrew *et al.* 1989; Müller-Graf, 1994; Murray *et al.* 2000), was confirmed to be *Paragonimus* based on the best currently available information from the literature and from the parasite identification database at the CDC in Atlanta. An original and detailed morphological description for each of these parasites is given in Müller-Graf (1994), which was used to aid in taxon identification. The characteristics of the parasites identified in baboons are summarised under each taxon below.

4.4.1.1 *Trichuris* sp.

Phylum: Nematoda

Family: Trichuridae

Genus: *Trichuris* Roederer, 1761.

Description: Parasite eggs from baboons with a length range of 47.5 - 57.5 μ m and a width ranging from 25.0 - 27.5 μ m were identified as *Trichuris* (Table 4.3). The eggs were barrel shaped with protruding ends and plugs on both poles (Figure 4.7) and smooth shells enclosing unsegmented granular contents, which are typical characteristics of *Trichuris* eggs (Ash and Ariel, 2007). Similar eggs have been reported in various baboon populations, including that of Gombe (File *et al.*, 1976; Huffman *et al.*, 1997; Müller-Graf, 1994).

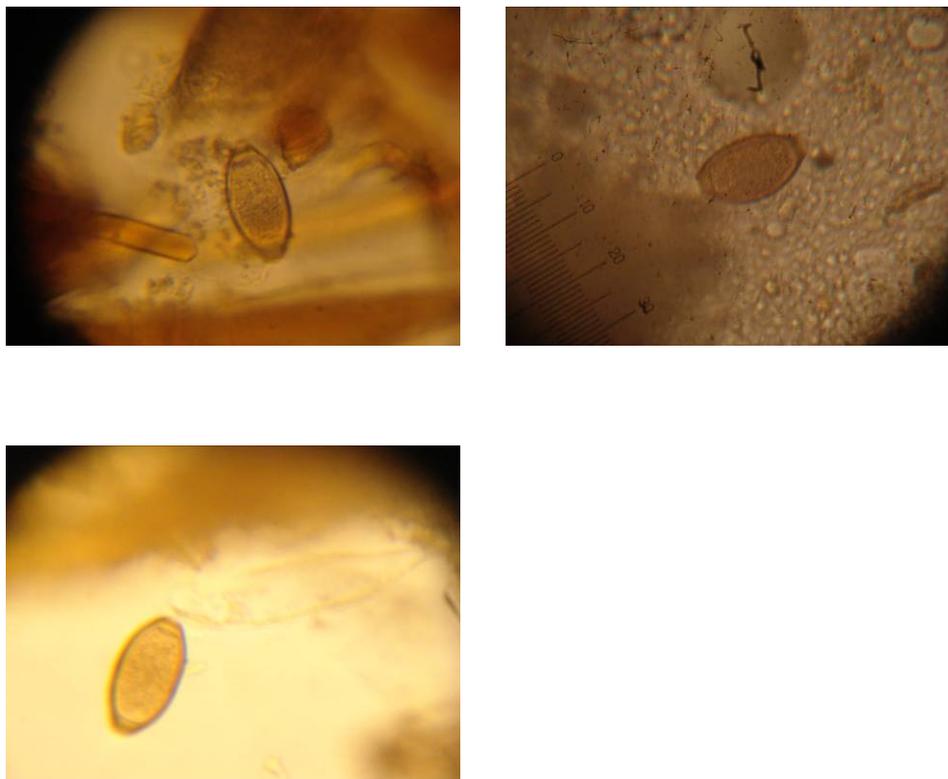


Figure 4.7: *Trichuris* sp. eggs (X400) with a characteristic barrel-shape and protruding poles on both ends of the egg.

4.4.1.2 Physaloptera spp.

Phylum: Nematoda

Family: Physalopteridae

Genus: *Physaloptera* Linstow, 1902

Description: Eggs resembling those of *Physaloptera* spp. (Ash and Orihel, 2007), with a length range of 47.5 - 65.0 μ m and width range of 36.3 - 50.0 μ m, were isolated from baboons (Table 4.3). Some of the eggs had a thick, mamillated outer layer that looked like debris (Figure 4.8). The eggs were also round in shape, with a thick but smooth wall

surrounding a coiled larva inside (Figure 4.8). Although *Physaloptera caucassica* can infect humans, most members of the genus *Physaloptera* are natural parasites of monkeys (Brown, 1969; Ash and Orihel, 2007). *Physaloptera* sp. was first reported in baboons at Gombe in the 1990s by Müller-Graf (1994).

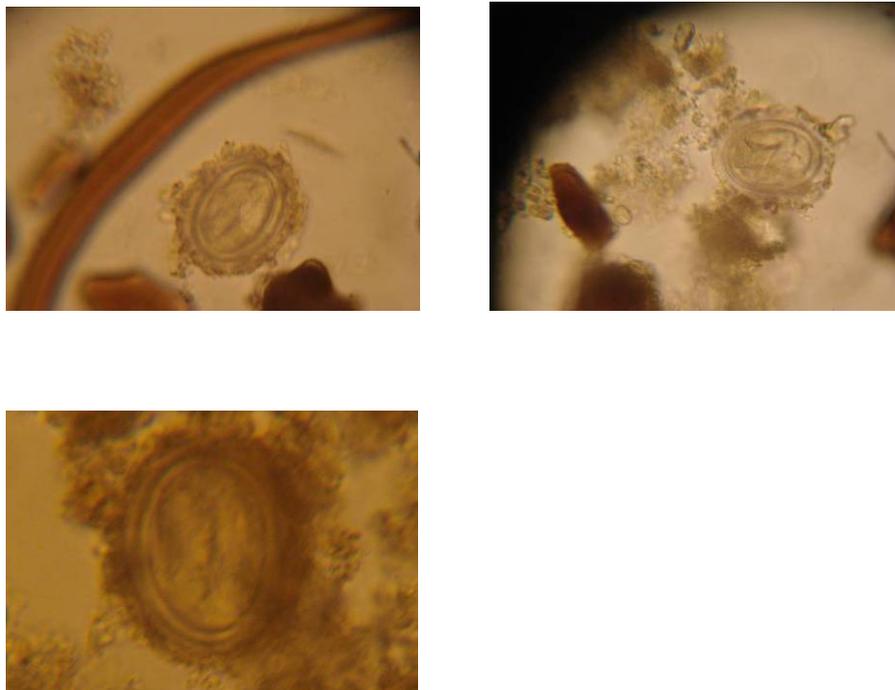


Figure 4.8: *Physaloptera* sp. eggs (X400) with roundish shaped and a mamillated outer layer surrounding a coiled larva.

4.4.1.3 *Streptopharagus* sp.

Family: Spiruroidea

Phylum: Nematoda

Genus: *Streptopharagus* Blanc, 1912

Nematode eggs with a smooth double shell surrounding clear unsegmented contents were diagnosed among baboons at Gombe and identified as *Streptopharagus* based on Müller-Graf (1994). These eggs were ovoid with fairly pointed ends and size length of 37.5 - 40.0µm and a width range of 20.0 - 22.5µm (Table 4.3). Each egg had a smooth double shell surrounding undeveloped larva (Figure 4.9). Eggs with similar features identified as *Streptopharagus* have been reported from wild baboons at Amboseli and Mpala Research Centre in Kenya (Hahn *et al.*, 2003), West Bugwe Forest Reserve, Uganda (Ocaido *et al.*, 2003) and Gombe National Park, Tanzania (McGrew *et al.*, 1989; Müller-Graf, 1994; Murray *et al.*, 2000).

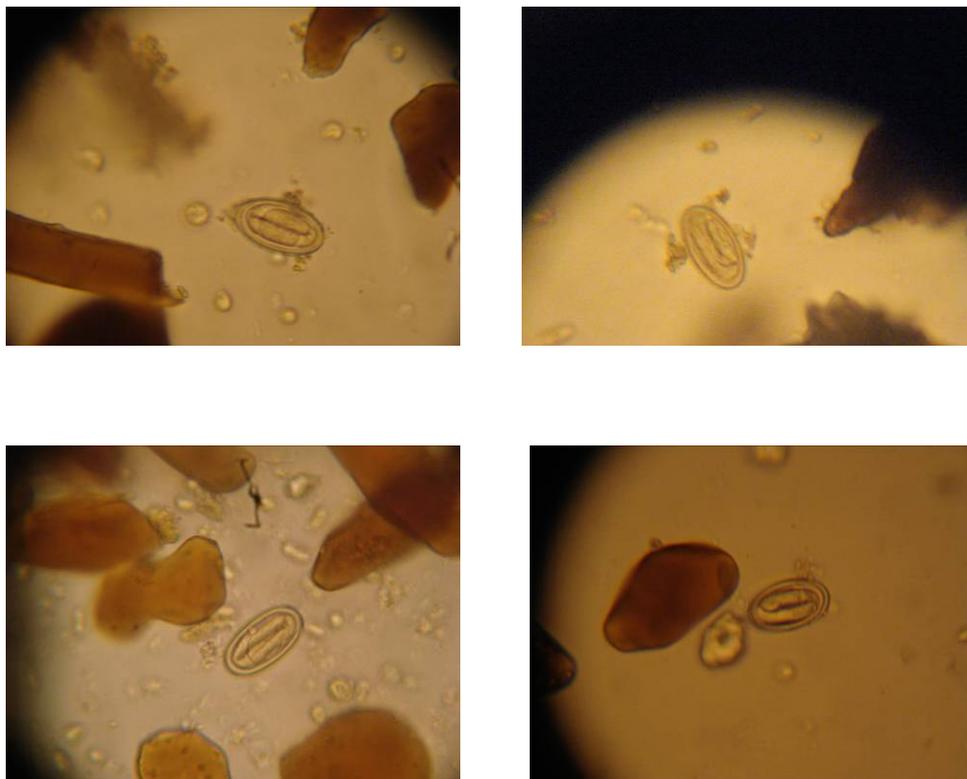


Figure 4.9: *Streptopharagus* sp. eggs (X400) with ovoid shape pointed and a smooth double shell surrounding undeveloped larva.

4.4.1.4 Hookworms

Phylum: Nematoda

Family: Ancylostomatidae

Common name: Hookworms

Description: Ovoid nematode eggs with size length ranging from 40.0 - 75.0 μ m and width range of 25.0 - 52.5 μ m were recorded in baboons (Table 4.3). The eggs had thin and smooth walls surrounding transparent, bulbous contents (Figure 4.10). These eggs belonged to hookworm parasites such as *Necator* spp. and or *Ancylostoma* spp. (Healy and

Myers, 1973; Cheesbrough, 1998; Ash and Orihel, 2007), but due to close similarities between the eggs of these species it was not possible to identify them to species level. They were instead classified simply as hookworms. Hookworms are commonly known to occur in baboons in Gombe and other sites and are considered part of the natural baboon parasite fauna (Myers and Kuntz, 1972; File *et al.*, 1976; Nutter, 1993; Lilly *et al.*, 2002).

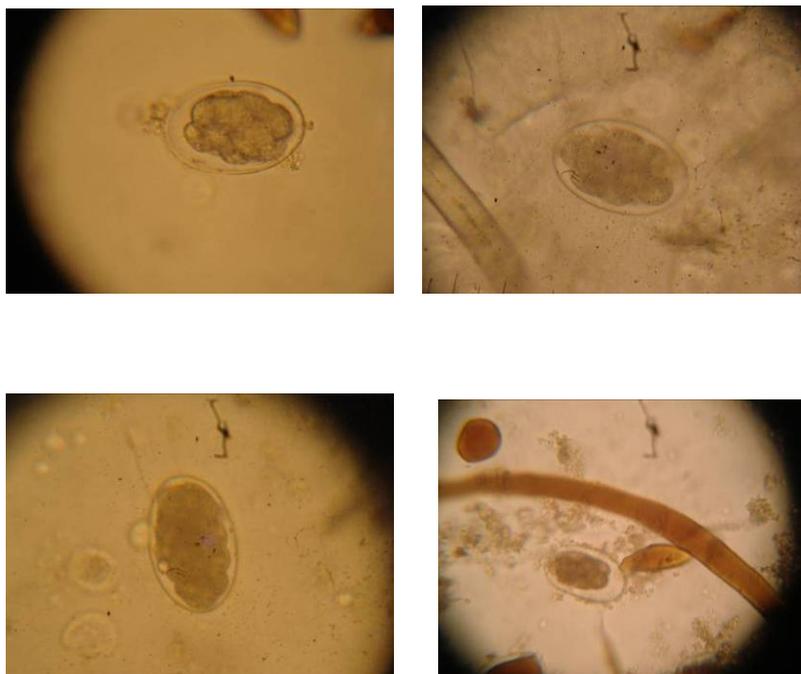


Figure 4.10: Eggs of hookworm parasites (X400) reported from baboons at Gombe National Park.

The eggs are characterized by thin and smooth shells surrounding transparent, bulbous contents.

4.4.1.5 Unidentified nematode eggs and larvae

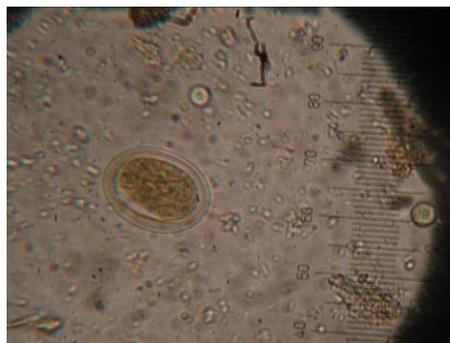
Phylum: Nematoda

Unidentified helminth eggs diagnosed in baboons were similar to nematode parasites previously reported in wild baboons. Some of the eggs were round in shape, with size ranging from 27.5-137.5 μm in length and 21.3-87.5 μm in width (Table 4.3). Other eggs were elongated in shape with smooth thin shells surrounding partially segmented contents that looked like *Strongyloides* eggs (Figure 4. 11A). Although *Strongyloides* parasites are known to infect baboons in the wild and have also been reported at Gombe (McGrew *et al.*, 1989; Müller-Graf, 1994; Murray *et al.*, 2000), the present eggs could not be identified to genus level. *Strongyloides* are also commonly identified as larvae in stools rather than eggs; thus, this made it difficult to make a clear conclusion on their identification. Other eggs had round shapes with thick shells surrounding unsegmented contents (Figure 4.11B), but given the lack of robust identification keys, it was not possible to confirm their genus.

In addition to nematode eggs, unidentified nematode larvae measuring between 187.5-475.5 μm long by 10.0 – 42.5 μm width were also observed in baboon stools (Table 4.3). The characteristic mouth openings that are used to identify nematode larvae in faecal samples, particularly the rhabditiform stages of *Strongyloides* (Soulsby, 1968; Spencer and Monroe, 1982; Cheesbrough, 1998), were not clearly seen due to poor quality and resolution of the images (Figure 4.11C and D). Based on their size and morphology, together with previously reported infections in baboons (File *et al.*, 1976; Nutter, 1993; Ashford *et al.*, 2000; Murray *et al.*, 2000), the larvae were probably rhabditiform stages of *Strongyloides stercoralis*. There is no possibility that there were fly larvae or other foreign materials because the faecal samples were picked up immediately after void from the animals and were collected while taking caution to exclude soil and other contaminants.



A



B



C



D

Figure 4.11: Unidentified nematode eggs (A and B; X400) observed from baboon stools at Gombe National Park.

The eggs, which had an average length of $51.7 \pm 5.2 \mu\text{m}$ and width $32.6 \pm 3.3 \mu\text{m}$, were distinguished by a range of features, including elongated shapes (A) and smooth thin shells surrounding partially segmented contents (B). Several types of larvae were also observed, with body characteristics such as tapering ends (C and D; X400) and an average length 363.9 ± 40.5 and width of 22.5 ± 4.8 . Distinguishing characteristics for nematode larvae such as mouth openings were not clear due to poor quality and resolution of the images. Thus the nematodes were reported as unidentified nematodes.

4.4.1.6 *Paragonimus* sp.

Phylum: Platyhelminthes

Class: Trematoda

Family: Troglotrematidae

Genus: *Paragonimus*

Common names: Trematodes, pulmonary or lung flukes

Unembryonated ovoid parasite eggs with an operculum were diagnosed in baboons (Figure 4.12). The eggs were characteristic of *Paragonimus* due to their golden brown colour, thick shells with prominent opercula and a thickening on the abopercular end (WHO, 1994; Ash and Orihel, 2007). The sizes of these eggs, which ranged from 67.5-90.0µm long by 35.0-55.0µm wide (Table 4.3) were smaller than 80 µm by 45µm, which is the lower size range reported for Asian *Paragonimus* i.e *P. westermani* (Ash and Orihel, 2007). The size of the present *Paragonimus* eggs was within the size range of African *Paragonimus* eggs, reported to be 50-125µm long by 35-60µm wide (Ash and Orihel, 2007). Their size was close to that of *P. uterobilateralis* (50-95µm long by 35-55µm wide), which has been reported in humans in West Africa (Ash and Orihel, 2007; WHO, 1994).

The eggs of *Paragonimus* can be confused with the fish tapeworm eggs (*Diphyllobothrium latum*), which are nonetheless smaller (58-75µm long by 40-50µm wide) and have a characteristic knob on their shell at the end opposite to the operculum (abopercular) (Ash and Orihel, 2007; WHO, 1994). On the other hand, *Paragonimus* eggs are distinguished by their size, which is larger than *D. latum* as well as their prominently thickened shell at the

abopercular end (Ash and Oriel, 2007). The eggs observed in the present study did not have an abopercular knob but instead had a considerably thickened shell at the end opposite to the operculum (Ash and Oriel, 2007). This classification level was supported by the Center for Disease Control and Prevention (CDC), who confirmed the parasite to be *Paragonimus* sp. through identification of the parasite's image sent to them (CLIA ID: 11D0668319). However, the eggs of *Paragonimus* species are difficult to distinguish from those of other trematodes (A. Blair, *personal communication*) and so the current identification of this parasite to genus level as *Paragonimus* spp. is tentative, pending further studies.



Figure 4.12: *Paragonimus* sp. eggs (X400) with their characteristic golden brown colour. The eggs were also distinguished by thick shells and a prominent operculum and a shell thickening on the end opposite the operculum (abopercular).

4.4.1.7 *Schistosoma mansoni*

Phylum: Platyhelminthes

Class: *Digenea*

Family:

Genus: *Schistosoma*

Species: *Schistosoma mansoni* Sambon, 1907

Common names: Trematodes, blood flukes

Trematode eggs identified as *S. mansoni* were diagnosed in baboons stool samples (Figure 4.13). The eggs, whose size was 145.0-175.0µm long and 65.0-70.0µm wide (Table 4.3), had smooth shells with lateral spines, which is a distinguishing feature for *S. mansoni* eggs (WHO, 1994; Ash and Orihel, 2007). The presence of a lateral spine makes *S. mansoni* eggs easily identified and cannot be confused with other schistosomes or eggs of other helminths (Cheesbrough, 1998; WHO, 1991, 1994). *S. mansoni* eggs have been reported in wild baboon populations in various locations, including Al-Baha, Saudi Arabia (Zahed *et al.*, 1996), four localities in Ethiopia (Legesse and Erko, 2004), Kibwezi, Kenya (Miller, 1960), various localities in Uganda (Miller, 1960; Nelson, 1960) and in Manyara and Gombe National Parks in Tanzania (Fenwick, 1969; Müller-Graf *et al.*, 1997; Murray *et al.*, 2000).

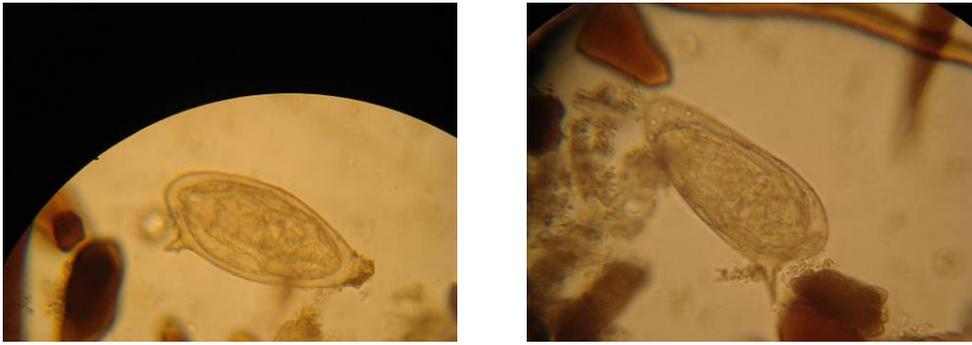


Figure 4.13: *Schistosoma mansoni* eggs (X400), with characteristic lateral spine.

The spine is important for the parasite identification while it is also said to aid in the movement of the egg across tissues in the definitive host.

Table 4.3: Mean length and width of parasite taxa observed in the baboons of Gombe National Park.

The table indicates parasite taxon identified and the mean of measured lengths and widths for eggs (or larvae) used to confirm their respective sizes and the number (n) of parasite individuals for each taxon from which the measurements were obtained. Numbers in brackets indicate the standard error (SE) for mean length and width.

Parasite taxon	Mean length (μm)	Mean width (μm)	n
<i>Trichuris</i> spp.	54.40(\pm 0.80)	25.20(\pm 0.40)	12
<i>Physaloptera</i> spp.	56.25(\pm 8.75)	43.125(\pm 6.86)	2
<i>Paragonimus</i> spp.	80.00(\pm 3.04)	47.86(\pm 2.64)	7
<i>Schistosoma mansoni</i>	160.00(\pm 6.21)	67.50(\pm 1.02)	4
<i>Streptopharagus</i> spp.	38.75(\pm 0.72)	21.88(\pm 0.63)	4
Hookworms	59.43(\pm 2.15)	40.47(\pm 1.97)	24
Unidentified nematode eggs	51.73(\pm 5.16)	32.62(\pm 3.29)	21
Unidentified nematode larvae	363.94(\pm 40.48)	22.50(\pm 4.75)	8

4.4.2 Confirmation of trematode species identity using DNA sequencing

DNA was extracted from parasite eggs isolated from the faeces of five baboons (Amara, Akita, Arancin, Altodi, and Smoky) showing evidence of schistosome infection based on microscopy and two individuals diagnosed with having *Paragonimus* (Adivil, Akarura). For the nested PCR of the 18S rRNA region (see Chapter 2), positive amplification of the 721 bp fragment expected was obtained from all individuals. However, the quality was only high enough to sequence products from two individuals. One individual had been identified as having been infected with schistosomes (Akita) and the other with *Paragonimus* (Akarura). The overall quality of sequences was low but a readable portion of the sequence (146 bp out of the 721 bp fragment) was obtained from Akita; using BLAST, this was found to be 100% identical to *S. mansoni* sequences isolated from humans. The sample from Akarura appeared to consist of more than one amplification product (i.e. it was a mixture) and so no identification could be made using BLAST. Detailed methods and results on the microsatellite analysis of humans and baboon schistosome DNA were shown in Appendix 2 in respect to chapter two.

4.4.3 Retrospective analysis of age among schistosome infected baboons

Retrospective age analysis of the current habituated baboon population in the park indicated that most individual baboons currently infected with schistosomiasis in Gombe National Park were born after the previous observation of the disease in the area in 1991-1992 (Table 4.4; Müller-Graf, 1994). There were also a few individual baboons that could not be assigned an age, as they immigrated from unknown baboon troops in the Park (Table 4.4). Literally, this also means that the animals were not in the troop at the time of the study by Müller-Graf *et al* (1994) in 1991/92.

Table 4.4: Retrospective analysis of the age of baboons currently infected with schistosomiasis at Gombe National Park.

The tables also gives the group membership, sex and date of birth of these baboons, and indicating that most of them were born after Müller-Graf's study in 1992. The age of individuals marked "Adult immigrant" and the only juvenile baboon (Sex: unknown) from BB troop could not be determined.

Baboon name	Group	Sex	Date of birth*
Acapulca	BA	Female	08-Feb-04
Akarura	BA	Female	11-Apr-97
Akita	BA	Female	07-Sep-01
Altodi	DA	Female	30-Aug-99
Amara	BA	Female	12-Nov-92
Antigua	BA	Male	09-Jan-04
Anzanza	BB	Female	02-Jun-92
Araceae	BB	Female	29-Oct-93
Arancin	DA	Female	07-Aug-95
Aruba	BB	Unknown	Juvenile
Basela	BB	Male	Adult immigrant
Mikania	BA	Female	25-May-93
Mzee	AC	Male	Adult immigrant
Poe	DA	Male	Adult immigrant
SFI	BA	Male	06-Apr-98
Smoky	DA	Female	24-Jun-96
Whitelown	BA	Female	24-Apr-92

* = Extracted from research archive at Gombe with permission from Gombe Stream Research Centre.

4.4.4 General characteristics of parasite infections in habituated baboons

The overall prevalence of infection for each parasite taxon is shown in Figure 4.14A. *Trichuris* spp. was the most prevalent parasite at 35%, followed by hookworms and unidentified nematodes at 27% and 25% prevalence, respectively. Although the prevalence of *Paragonimus* sp. was very low, its mean intensity was the highest (47 ± 21.69 epg; range: 1-737 epg), followed by the intensities of *Streptopharagus* sp. and *Physaloptera* sp., respectively. The lowest intensity of infection was exhibited by *S. mansoni* at 4 ± 1.06 epg while that of *Paragonimus* sp. was the highest at 40 epg (Figure 4.14B). The frequency distributions of the egg counts of *Trichuris* sp. and *Physaloptera* sp. are shown in Figure 4.15 as representative examples of the observed negative binomial patterns for parasite intensity. The total parasite load in infected individuals ranged from 1 to 745 epg, while the Shannon-Wiener index of species diversity ranged from 0.02 to 0.72 (Figure 4.16). For parasite richness, the median number of parasite taxa was two while the maximum was five, which came from one adult male baboon (Figure 4.17).

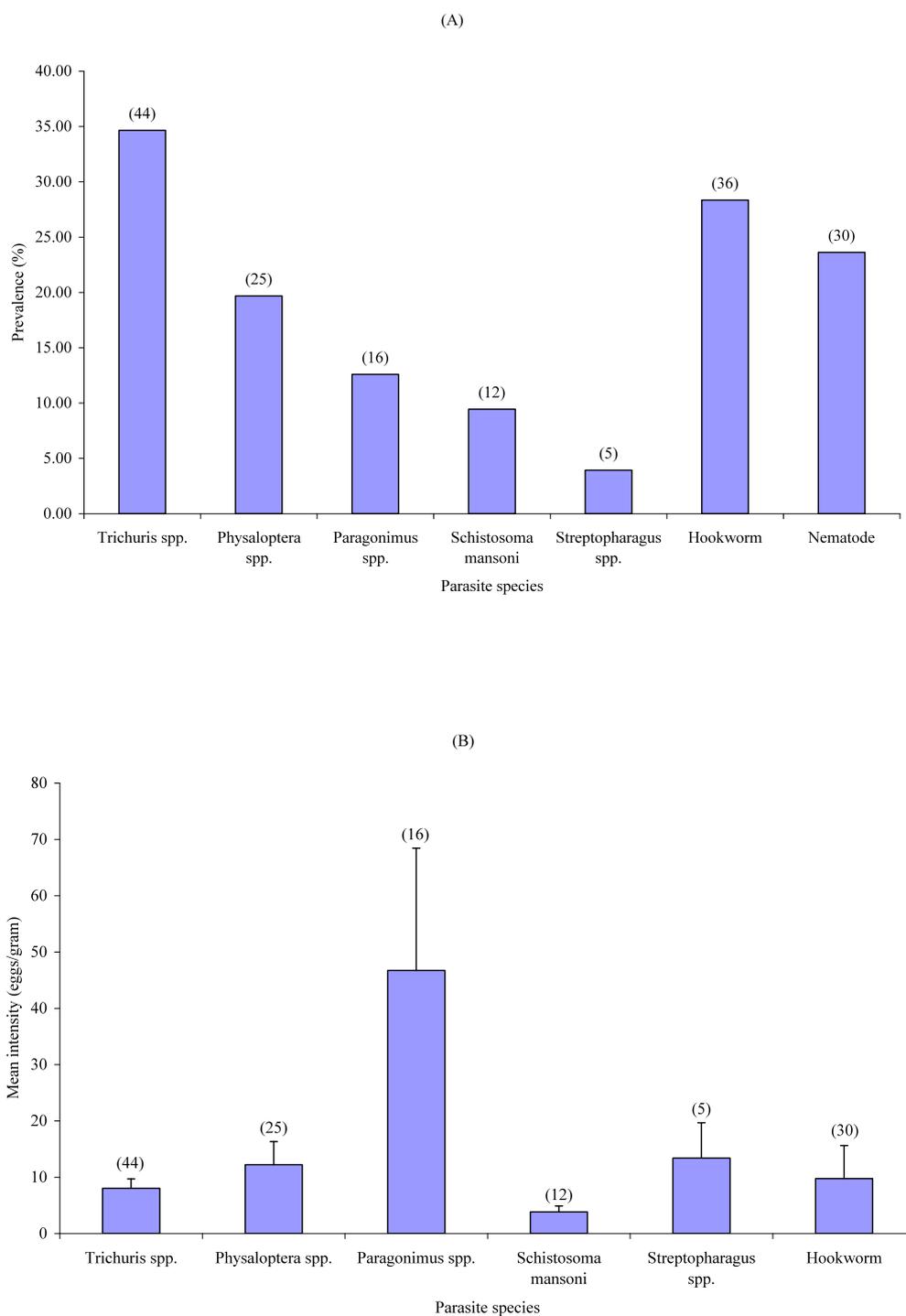


Figure 4.14: Overall prevalence (%) of individuals infected (A) and intensity of infection measured in eggs per gram of faeces (B).

The measures of infection were recorded for various parasite taxa diagnosed in habituated baboons at Gombe National Park. Bars on top of each histogram show standard errors while figures on top of each bar indicate the number of infected individuals for each parasite taxon (N = 126).

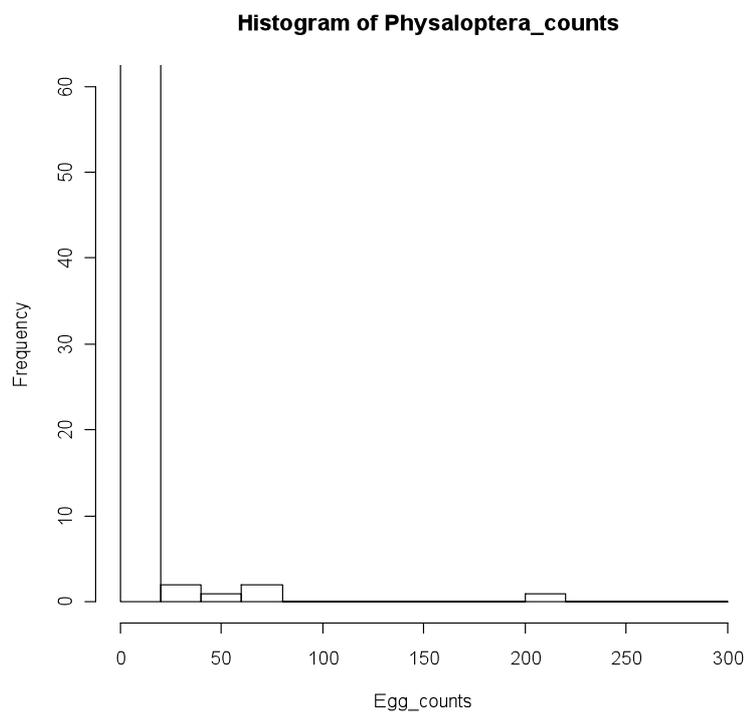
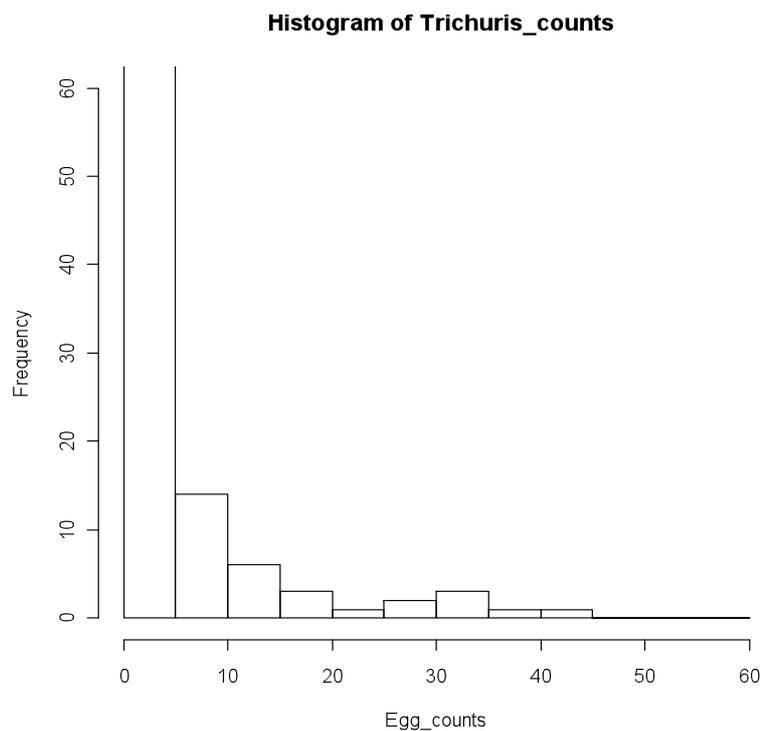


Figure 4.15: Frequency distribution of egg counts of *Trichuris* sp. and *Physaloptera* sp. The figure indicates an overdispersed pattern of distribution with excess zeros. All other parasites taxa showed the same type of distribution.

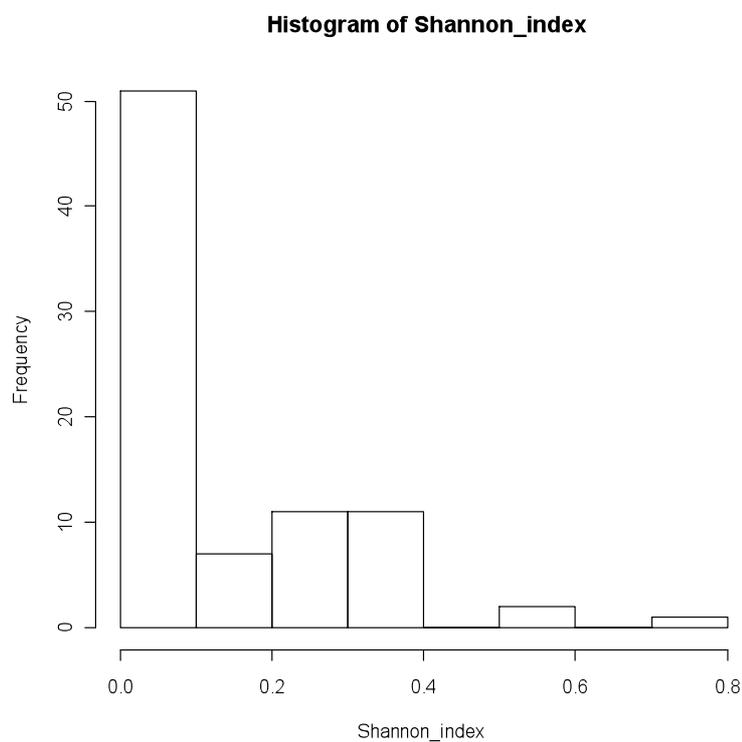
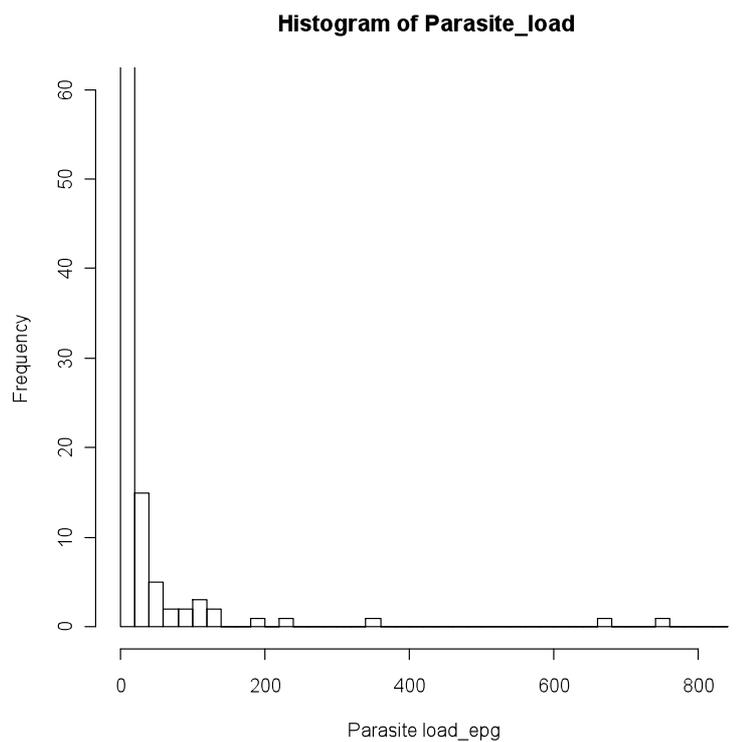


Figure 4.16: Frequency distribution of parasite load and species diversity (Shannon-Wiener Index) for parasite taxa in baboons.

The figure indicates an overdispersion and zero inflation that suggest a negative binomial distribution.

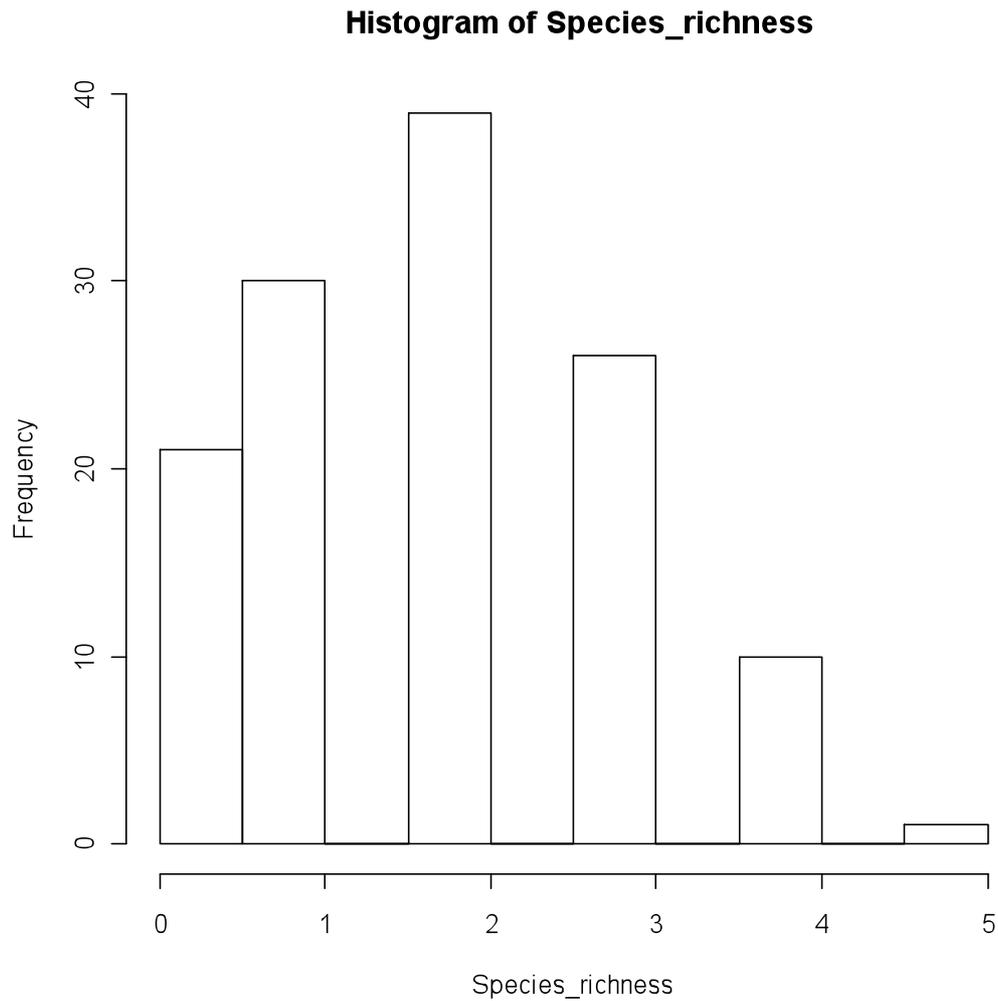


Figure 4.17: Frequency distribution of species richness indicating a relatively normal distribution pattern for species richness in baboons.

The distribution is characterized by a symmetric spread of the number of species per individual host (x-axis) with a single peak in the middle (y-axis).

4.4.5 Variation of parasite infection with baboon troop membership and season in habituated baboons

Levels of infection were only sufficient to statistically assess whether there were differences in prevalence and intensity in relation to habituated baboon troops and season for *Trichuris*, hookworms and nematodes. However, there were no general trends across parasite species. A summary of prevalence and the mean intensity for each parasite taxon in the wet and dry season for each habituated baboon troop is given in Table 4.5. GLM analysis with a ZINB distribution for *Trichuris* sp. egg counts indicated a significant interaction between baboon troop and season (Table 4.6). However, this was only true for the count part of the model (i.e. the negative binomial component assessing variation in the overdispersed count data) but not the zero-inflated part (i.e. assessing variation in the excess zeros), where there was also no effect of season or troop on their own. The prevalence of *Trichuris* sp. did not vary significantly between seasons and across troops although it was higher in the dry season than wet season in troops AC, BA, BB and DA, while it was higher in the wet than the dry season in individuals from DB (Figure 4.18A). In general, there was little variation among troops in intensity of infection in the dry season but substantial variation in the wet season, which likely drove the significance of the interaction (Figure 4.18B). In the wet season, the highest levels of infection were seen in the BA and DB troops and the lowest in AC and BB troops, with an intermediate level in DA troop.

Table 4.5: A summary of prevalence (%) and mean intensity (epg) for each parasite taxon, sampled in the wet (A) and dry season (B).

Numbers in brackets after troop names indicate the numbers of individuals examined (sample size) for each habituated baboon troop at Gombe National Park. For logistical reasons, no stool samples were collected from baboons in troop DC in the dry season and this is represented in the table as NA (Not Applicable).

		<i>Trichuris</i>	<i>Hookworms</i>	<i>Paragonimus</i>	<i>Physaloptera</i>	<i>Nematodes</i>	<i>Streptopharagus</i>	<i>Schistosoma</i>
AC(18)	Prevalence	38.9	5.6	20.0	0.0	11.1	0.0	5.6
	Intensity	2.3	4.0	46.0	0.0	6.0	0	2
BA(28)	Prevalence	14.3	28.6	5.6	0.0	25.0	3.6	14.3
	Intensity	11.0	32.1	3.0	0.0	2.7	1	4
BB(10)	Prevalence	30.0	60.0	83.3	0.0	50.0	20.0	30.0
	Intensity	1.3	3.5	1.0	0.0	1.6	16.0	3.0
DA(22)	Prevalence	18.2	36.4	0.0	100.0	18.2	4.5	18.2
	Intensity	6.0	2.3	3.0	1.0	3.3	34	6
DB(21)	Prevalence	100.0	33.3	75.0	100.0	23.8	4.8	0.0
	Intensity	13.7	6.1	45.8	1.3	1.8	2	0
DC(22)	Prevalence	63.6	13.6	0.0	90.9	27.3	0.0	0.0
	Intensity	10.2	3.0	0.0	13.9	40.6	0.0	0.0

(B)

		<i>Trichuris</i>	<i>Hookworms</i>	<i>Paragonimus</i>	<i>Physaloptera</i>	<i>Nematodes</i>	<i>Streptopharagus</i>	<i>Schistosoma</i>
AC(10)	Prevalence	100.0	20.0	11.1	60.0	10.0	0.0	0.0
	Intensity	9.6	6.5	377	12.3	30	0.0	0.0
BA(18)	Prevalence	72.2	27.8	3.6	72.2	16.7	0.0	0.0
	Intensity	7.6	2.2	3	29.5	3.7	0.0	0.0
BB(6)	Prevalence	50.0	50.0	10.0	83.3	16.7	0.0	0.0
	Intensity	7	2.3	8	5.8	1	0.0	0.0
DA(16)	Prevalence	68.8	37.5	4.5	81.3	50.0	0.0	0.0
	Intensity	10	4.7	0	14.1	16.8	0.0	0.0
DB(12)	Prevalence	33.3	33.3	52.4	33.3	16.7	0.0	0.0
	Intensity	8.1	1.3	123.4	4.5	5.5	0.0	0.0
DC(0)	Prevalence	NA	NA	NA	NA	NA	NA	NA
	Intensity	NA	NA	NA	NA	NA	NA	NA

Table 4.6: Statistical results for ZINB analysis on the impact of season and baboon troop on the intensity egg counts of *Trichuris* sp.

The results indicate coefficient values for the count and zero-inflation model components. Parameter estimates indicate that season and baboon troop season interactions were significant predictors of *Trichuris* egg counts. The zero-inflation part of the data was not significant influenced by these factors.

Count model coefficients (negbin with log link):				
	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	2.2618	0.3781	5.981	2.21e-09 ***
SeasonWet	-2.1236	0.6646	-3.195	0.00140 **
GroupBA	-0.4392	0.5075	-0.865	0.38680
GroupBB	-0.5396	0.7931	-0.680	0.49627
GroupDA	-0.1229	0.5231	-0.235	0.81420
GroupDB	-0.2580	0.5145	-0.502	0.61599
SeasonWet:GroupBA	2.5479	1.0084	2.527	0.01152 *
SeasonWet:GroupBB	-0.5149	1.1511	-0.447	0.65467
SeasonWet:GroupDA	1.5193	0.9663	1.572	0.11588
SeasonWet:GroupDB	2.6130	0.8666	3.015	0.00257 **
Log(theta)	-0.2820	0.2451	-1.150	0.25004
Zero-inflation model coefficients (binomial with logit link):				
	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-20.2756	7994.5914	-0.003	0.998
SeasonWet	19.0436	7994.5916	0.002	0.998
GroupBA	18.1967	7994.5916	0.002	0.998
GroupBB	19.7633	7994.5915	0.002	0.998
GroupDA	18.8268	7994.5915	0.002	0.998
GroupDB	2.0547	8811.1827	0.000	1.000
SeasonWet:GroupBA	-15.0151	7994.5918	-0.002	0.999
SeasonWet:GroupBB	-34.5572	9480.6881	-0.004	0.997
SeasonWet:GroupDA	-16.4153	7994.5917	-0.002	0.998
SeasonWet:GroupDB	-0.3239	8811.1829	0.000	1.000

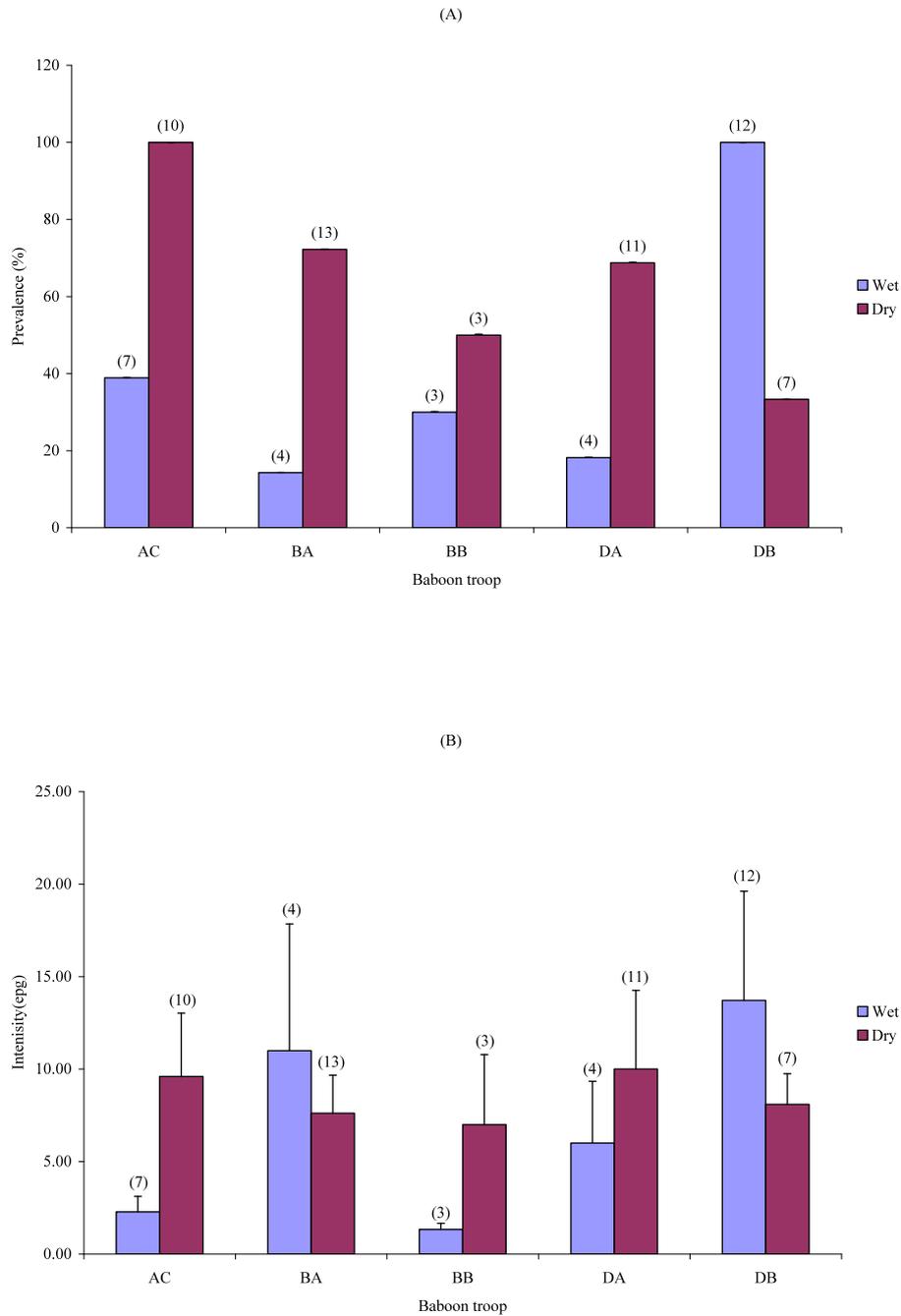


Figure 4.18: Variation of parasite prevalence (A) and intensity (B) of *Trichuris* sp. with season and baboon troop among baboons at Gombe National Park.

Troop DC which was sampled in the wet season only was excluded from this analysis. Bars on top of each histogram indicate standard errors while numbers on top bars indicate the number of infected individuals in each troop. The sample size for each troop in the wet season: $n = 18$ (AC); $n = 28$ (BA); $n = 10$ (BB); $n = 22$ (DA); $n = 21$ (DB) and in dry season: $n = 10$ (AC); $n = 18$ (BA); $n = 6$ (BB); $n = 16$ (DA); $n = 12$ (DB).

For hookworms, the prevalence did not vary across most baboon troops in the wet and dry season and although it was highest in BB troop and lowest in AC in the wet season (Figure 4.19A); this variation was not statistically significant. On the other hand, hookworms showed a much higher intensity of infection in the wet season than in the dry season (Figure 4.19B) although as with the variation in prevalence above, this difference was not statistically significant.

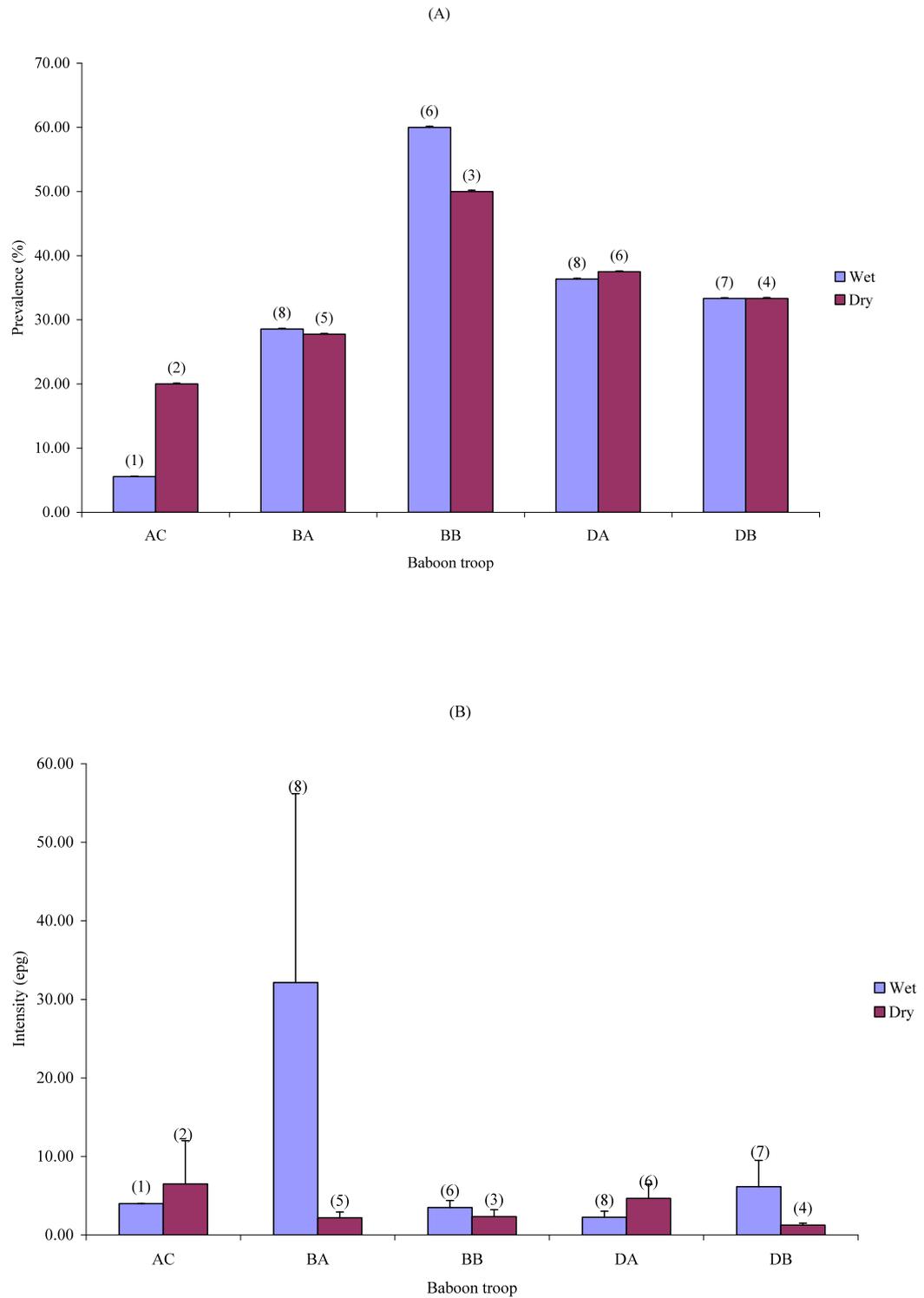


Figure 4.19: Variation of parasite prevalence (A) and intensity (B) of hookworms with season and baboon troop.

Standard errors are given by bars on top of histograms while the number of infected individual for each baboon troop (except DC which had wet season samples only) is shown on top of each error bar. The sample size for each troop in the wet season: $n = 18$ (AC); $n = 28$ (BA); $n = 10$ (BB); $n = 22$ (DA); $n = 21$ (DB) and in dry season: $n = 10$ (AC); $n = 18$ (BA); $n = 6$ (BB); $n = 16$ (DA); $n = 12$ (DB).

For *Paragonimus*, based on a GLM analysis under a binomial distribution, there was a significant effect of baboon troop but not season (Table 4.7). Troops BB and DB showed the highest prevalence, and this was particularly true in the dry season (Figure 4.20A). However, there was an overall trend for higher prevalence in the dry season at troops except DA, which also had very low prevalence in the wet season. The lack of significance could therefore be due to insufficient power to detect a difference. Levels of infection were too low to statistically assess intensity of infection but it showed a similar pattern in relation to season. However, only troops AC and DB showed substantial numbers of egg counts (Figure 4.20B).

Table 4.7: Results of GLM binomial analysis for the effect of season and baboon troop membership on the prevalence of *Paragonimus* sp. in habituated baboons at Gombe National Park.

Parameter estimates for the p value indicate that baboon troop membership was a significant predictor of *Paragonimus* prevalence.

Model Coefficients:				
	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-1.3863	0.7906	-1.754	0.0795.
SeasonWet	-0.6931	1.0897	-0.636	0.5247
GroupBA	-1.4469	1.2976	-1.115	0.2648
GroupBB	2.9957	1.3509	2.218	0.0266 *
GroupDA	-17.1798	1630.6598	-0.011	0.9916
GroupDB	2.4849	1.0341	2.403	0.0163 *
SeasonWet:GroupBA	0.2305	1.8120	0.127	0.8988
SeasonWet:GroupBB	-3.1135	1.8705	-1.665	0.0960
SeasonWet:GroupDA	16.2147	1630.6603	0.010	0.9921
SeasonWet:GroupDB	-0.3102	1.3501	-0.230	0.8183

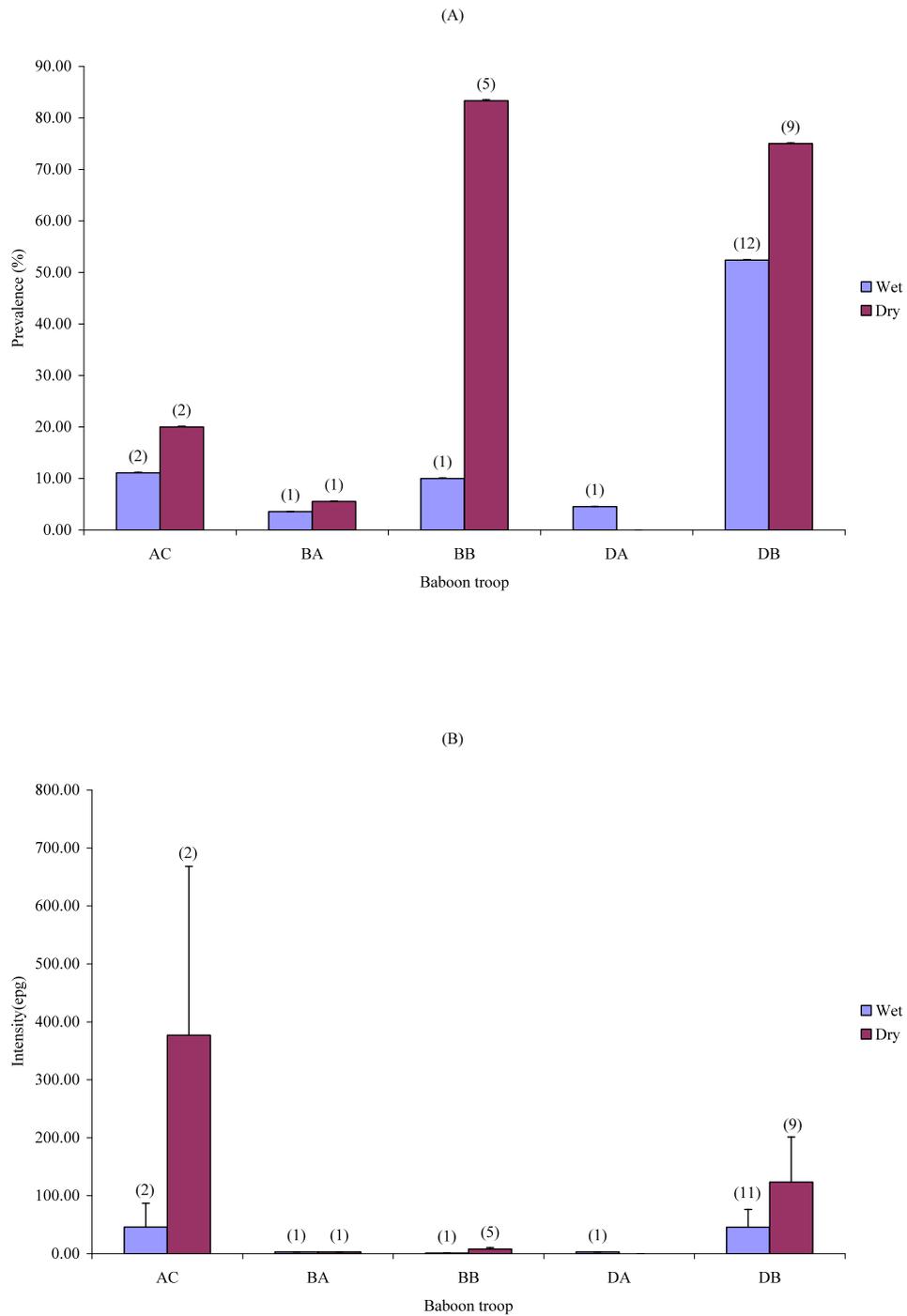


Figure 4.20: Variation of parasite prevalence (A) and intensity (B) of *Paragonimus* sp. with season and baboon troop among baboons.

Bars indicate standard errors while numbers on top of each bar indicate the number of infected individuals for each troop (except DC). The sample size for each troop in the wet season: n = 18 (AC); n = 28 (BA); n = 10 (BB); n = 22 (DA); n = 21 (DB) and in dry season: n = 10 (AC); n = 18 (BA); n = 6 (BB); n = 16 (DA); n = 12 (DB).

Physaloptera sp. was detected in all troops in the dry season but only in DA and DB in the wet season (Figure 4.21), where only one and two individuals from DA and DB respectively were diagnosed with the parasite (Figure 4.21). In the dry season, there was not much variation among troops in terms of prevalence, except for DB, which was lower than the rest (Figure 4.21A). Intensity was highest in BA and lowest in DB and BB (Figure 4.21B) but this variation was not statistically significant.

For unidentified nematodes, statistical results from GML binomials analysis on the effect of baboon troop membership and season on prevalence indicated that there was no significant impact. Similarly, ZINB results indicated that season and troop did not also have a significant impact on parasite egg counts. Graphical presentation of the prevalence and egg counts for these parasites showed that prevalence was highest in troop BB and lowest in AC the wet season while during the dry season it was highest in DA and also lowest in AC troop (Figure 4.22A). The intensity (egg counts) showed that there was a slight variation between dry and wet season in most troops except DA where it was exceptionally higher in the dry season than the wet season (Figure 4.22B)

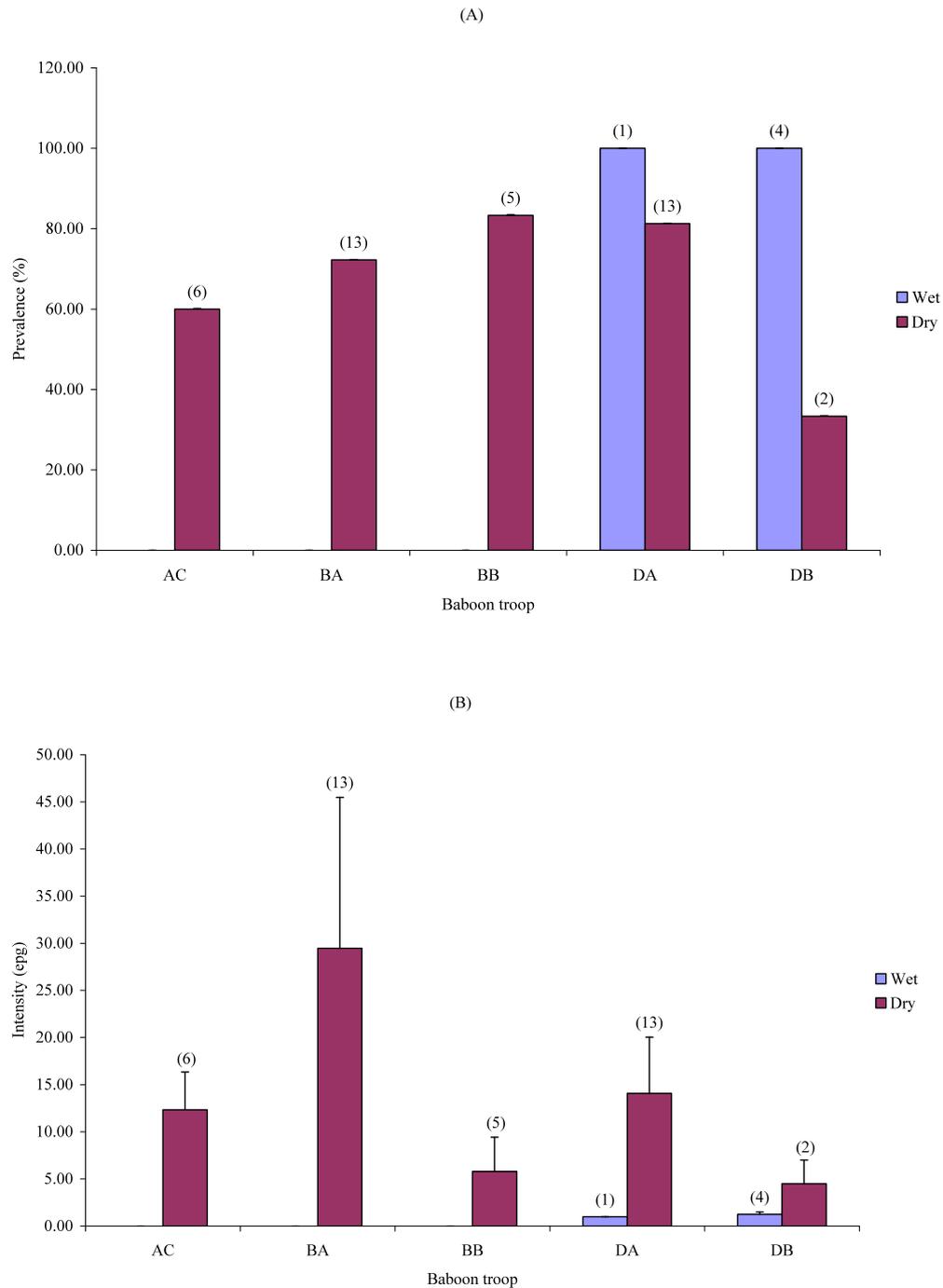


Figure 4.21: Variation of parasite prevalence (A) and intensity (B) of *Physaloptera* sp. with season and baboon troop among baboons.

Standard errors are given by bars on top of histograms while the number of infected individuals for each troop (except DC) is shown on top of each bar. The sample size for each troop in the wet season: $n = 18$ (AC); $n = 28$ (BA); $n = 10$ (BB); $n = 22$ (DA); $n = 21$ (DB) and in dry season: $n = 10$ (AC); $n = 18$ (BA); $n = 6$ (BB); $n = 16$ (DA); $n = 12$ (DB).

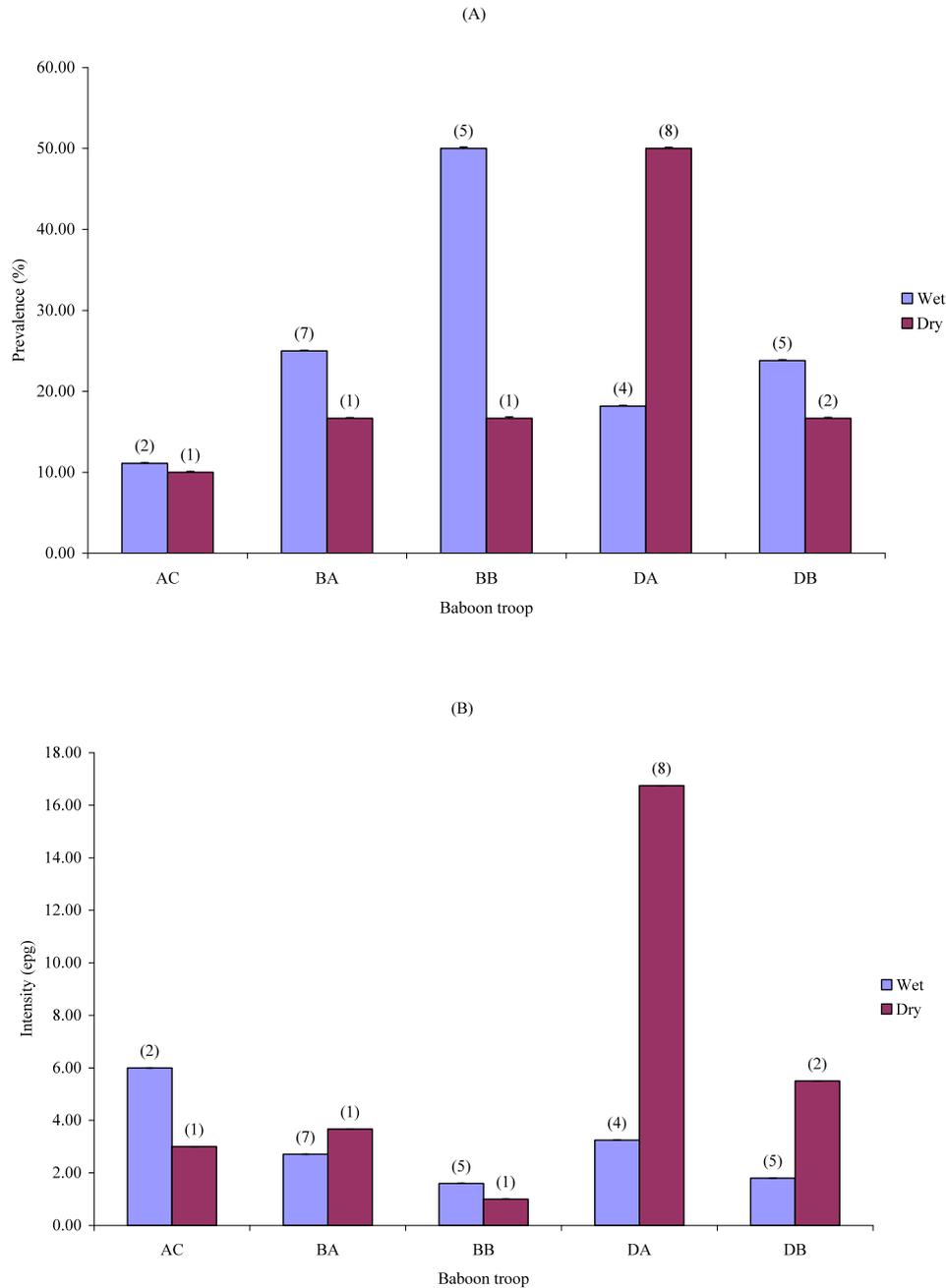


Figure 4.22: Variation of parasite prevalence (A) and intensity (B) of unidentified nematode with season and baboon troop.

Shown on the top of each histogram are standard errors while the numbers given on top of each bar signify the number of infected individuals in each baboon troop (except DC). The sample size for each troop in the wet season: $n = 18$ (AC); $n = 28$ (BA); $n = 10$ (BB); $n = 22$ (DA); $n = 21$ (DB) and in dry season: $n = 10$ (AC); $n = 18$ (BA); $n = 6$ (BB); $n = 16$ (DA); $n = 12$ (DB).

GLM analyses under a zero-inflated Poisson distribution to test the effect of season and baboon troop on parasite species richness and parasite load for habituated baboons also indicated a significant interaction between season and baboon troop (Tables 4.8 and 4.9). While species richness tended to be higher in the wet season in troops AC, BA, BB and DA, there was no seasonal difference for baboons in troop DB (Figure 4.23). Parasite load on the other hand showed marked variation seasonally, where it was higher in the dry season than the wet season in all troops except BA, and the highest loads were observed in troops AC and DB in the dry season. In the wet seasons, BA and DB showed the highest loads but they were much lower than AC and DB in the dry season (Figure 4.24A). Although there were some differences among troops, there was no clear trend in species diversity (Shannon-Wiener index) across seasons (Figures 4.24B) and this variation was not significant.

Table 4.8: GLM poisson results on the impact of season and baboon troop membership on parasite richness among habituated baboons in Gombe National Park. The p value estimates indicate that both season and troop and an interaction between them are significant predictors of season interactions

Coefficients:				
	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	-0.5108	0.4082	-1.251	0.21084
SeasonWet	1.2040	0.4655	2.587	0.00969 **
GroupBA	0.8293	0.4606	1.801	0.07177
GroupBB	0.8473	0.5563	1.523	0.12777
GroupDA	0.8293	0.4606	1.801	0.07177
GroupDB	1.2484	0.4584	2.723	0.00646 **
SeasonWet:GroupBA	-0.6751	0.5392	-1.252	0.21050
SeasonWet:GroupBB	-0.3878	0.6420	-0.604	0.54584
SeasonWet:GroupDA	-0.6751	0.5392	-1.252	0.21050
SeasonWet:GroupDB	-1.0943	0.5439	-2.012	0.04424 *

Table 4.9: Parameter estimates from GLM poisson analysis on the impact of season and baboon troop membership on total parasite load.

P values indicate that season and baboon troop and the interactions between the factors significantly influence parasite load in baboons at Gombe National Park.

Coefficients:				
	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	4.54116	0.03265	139.081	< 2e-16 ***
SeasonWet	-2.28987	0.10767	-21.268	< 2e-16 ***
GroupBA	-1.11527	0.05567	-20.035	< 2e-16 ***
GroupBB	-1.61800	0.10871	-14.883	< 2e-16 ***
GroupDA	-1.53301	0.06444	-23.790	< 2e-16 ***
GroupDB	0.15932	0.04350	3.662	0.00025 ***
SeasonWet:GroupBA	1.74238	0.13282	13.118	< 2e-16 ***
SeasonWet:GroupBB	1.40359	0.20996	6.685	2.31e-11 ***
SeasonWet:GroupDA	1.14838	0.15808	7.265	3.74e-13 ***
SeasonWet:GroupDB	0.51166	0.13001	3.935	8.30e-05 ***

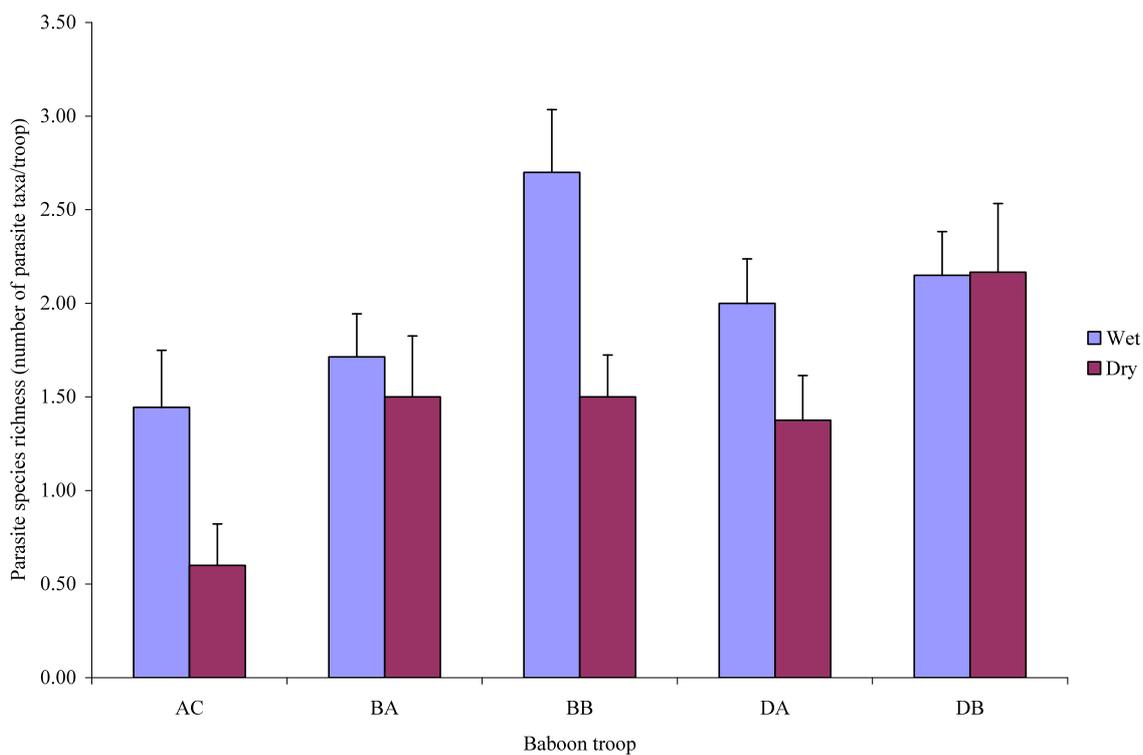


Figure 4.23: The influence of baboon troop on species richness in habituated baboon troops at Gombe.

Bars on top of histograms indicate standard errors. The sample size for each troop in the wet season: $n = 18$ (AC); $n = 28$ (BA); $n = 10$ (BB); $n = 22$ (DA); $n = 21$ (DB) and in dry season: $n = 10$ (AC); $n = 18$ (BA); $n = 6$ (BB); $n = 16$ (DA); $n = 12$ (DB).

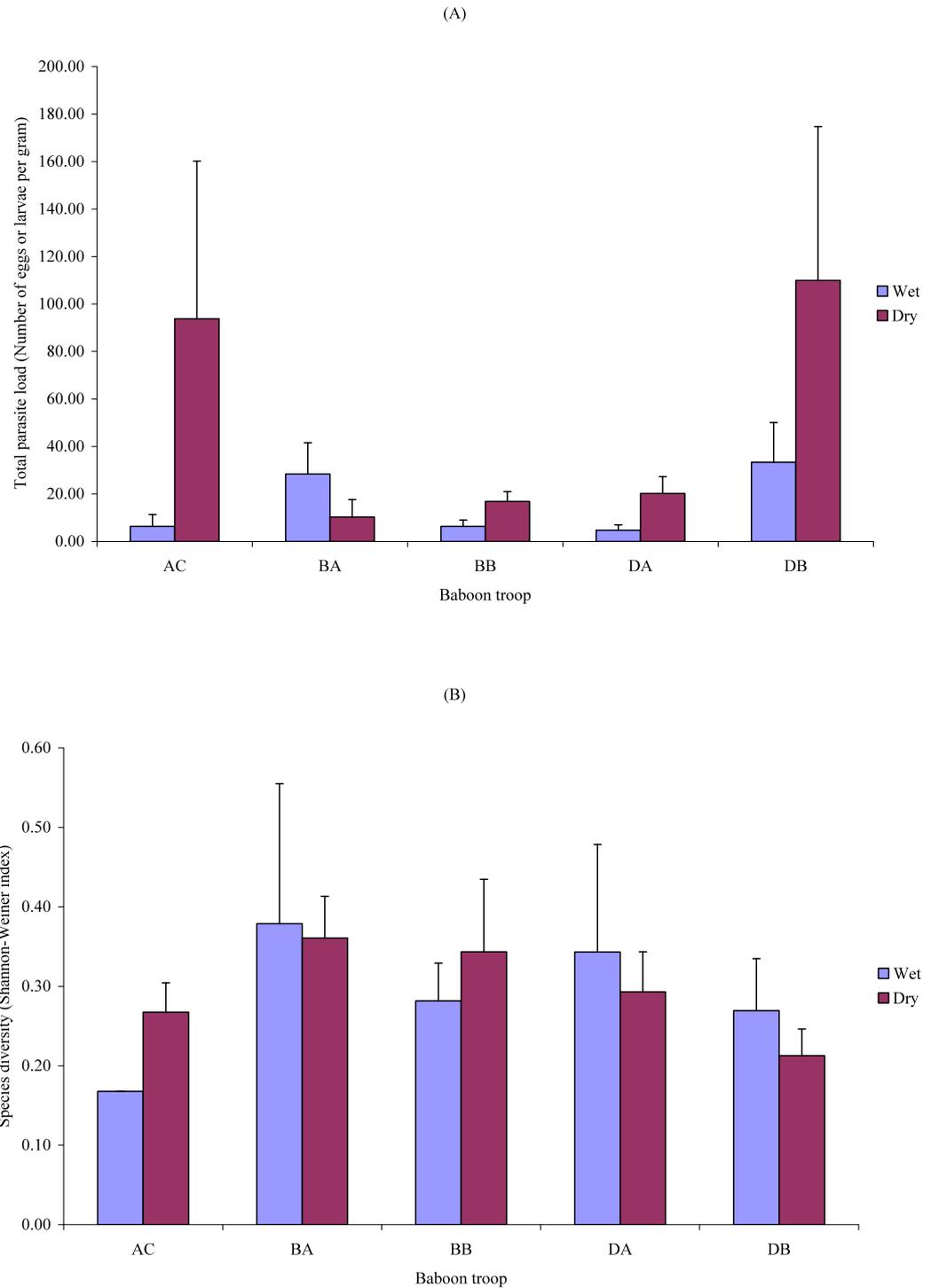


Figure 4.24: The influence of baboon troop and season on parasite load (A) and species diversity i.e. Shannon-Wiener index (B).

Standard errors are given by bars on top of histograms. The sample size for each troop in the wet season: $n = 18$ (AC); $n = 28$ (BA); $n = 10$ (BB); $n = 22$ (DA); $n = 21$ (DB) and in dry season: $n = 10$ (AC); $n = 18$ (BA); $n = 6$ (BB); $n = 16$ (DA); $n = 12$ (DB).

4.4.6 The impact of habituation on levels of parasite infections at Gombe among baboons at Gombe (for wet season samples only)

In the wet season, habituated baboons were infected with all of the five parasite taxa diagnosed while non-habituated baboon populations were found with all taxa except *Paragonimus spp.* and *Streptopharagus spp.* (Figure 4.25). GLM analysis under a binomial distribution indicated that the prevalence of unidentified nematodes was significantly higher among unhabituated than habituated baboons ($z = 2.922$, $p = 0.00348$). These results were also reflected in the prevalence values for nematodes, which were higher in unhabituated baboons than habituated ones (Figure 4.25A). The prevalence of *Physaloptera sp.* was also higher among non-habituated baboons than habituated populations (Figure 4.25A) although results of a GLM binomial analysis indicated that the differences were not significant. Similarly, the prevalence of other parasite taxa like *Trichuris sp.*, and hookworms was higher among habituated baboons than non-habituated populations, but the differences were not significant based on GLM binomial analysis results.

ZINB analysis on the effect of baboon habituation on the infection intensity (egg counts) of *Trichuris sp* showed no significant effect. Absolute intensity values of *Trichuris sp.* intensity shown in Figure 4.25B indicate that parasite's egg count was nonetheless higher in unhabituated baboons than habituated populations. Similarly, although the egg counts of *Physaloptera sp.* and hookworms were higher in habituated baboons compared to non-habituated populations (Figure 4.25B); the differences were not statistically significant. Habituation did not also have significant effect on the intensity of both hookworms and unidentified nematodes. Graphical display of the intensities of these parasites showed that hookworms were higher among habituated than non-habituated while the opposite trend was observed for unidentified nematodes (Figure 4.25B) but as hinted above, the variation

did not reach significance level. *Streptopharagus* sp. and *Paragonimus* sp. were only detected among habituated baboon populations. *S. mansoni* was predominantly found in habituated baboons with only one unhabituated individual being infected with the parasite (Figure 4.25). Due to the lack of sufficient data, a statistical comparison could not be made between habituated and unhabituated baboons for the three parasite taxa.

GLM analysis of parasite load (under a zero-inflated Poisson distribution) indicated no significant effect ($t = -0.433$, $p = 0.666$) of habituation, which is also reflected in Figure 4.26(A) where differences in parasite load between habituated and non-habituated animals were minimal. Species diversity and Shannon's index of diversity were higher in habituated than unhabituated baboon populations (Figure 4.26B), but the differences were not significant.

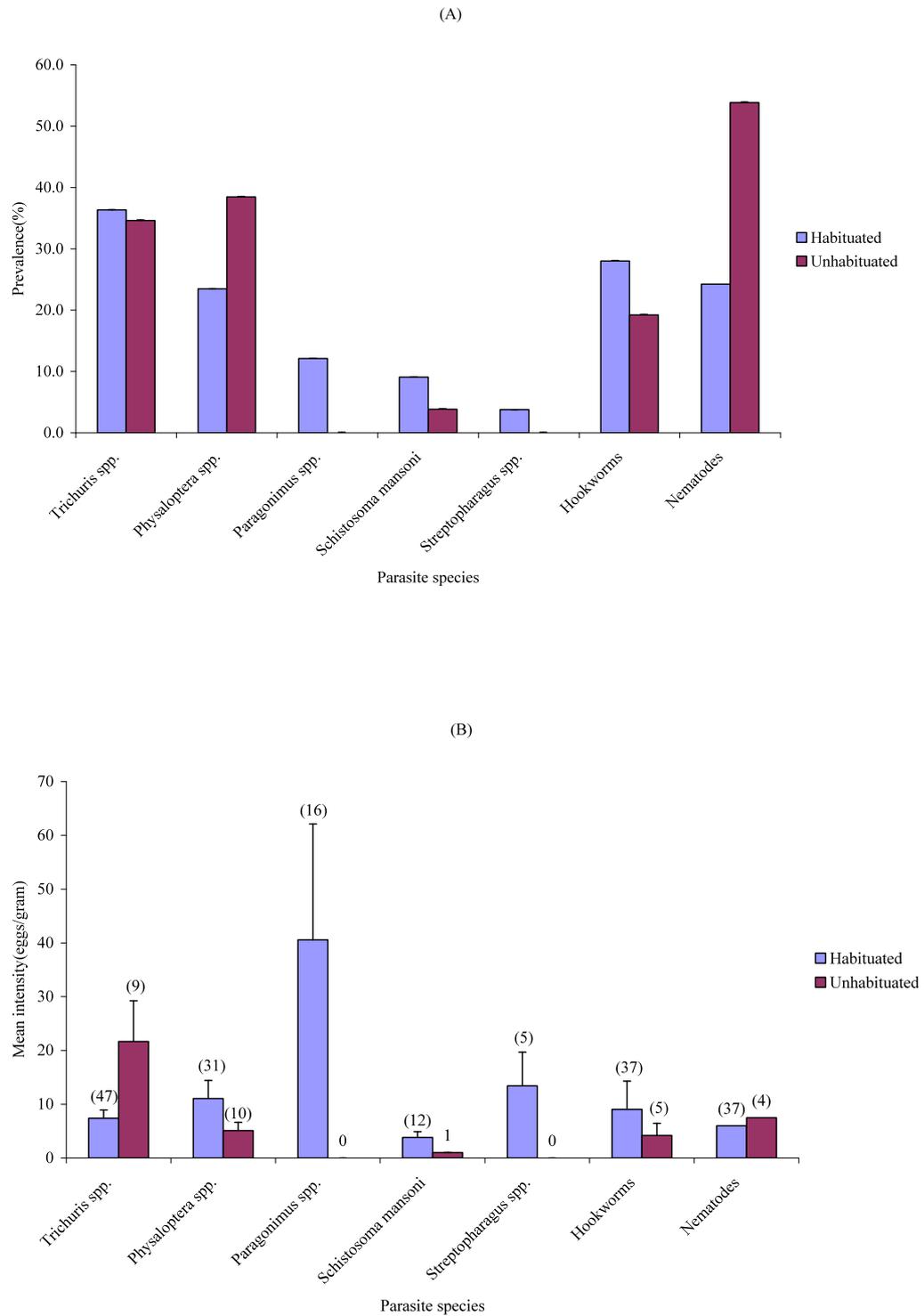


Figure 4.25: The impact of habituation on the prevalence (A) and intensity (B) of parasite infection in baboons at Gombe for wet season samples only.

Standard errors are given by bars on top of histograms while the number of baboons found infected by each parasite in each community is shown on top of bars ($n = 132$ for habituated while $n = 26$ for unhabituated baboons).

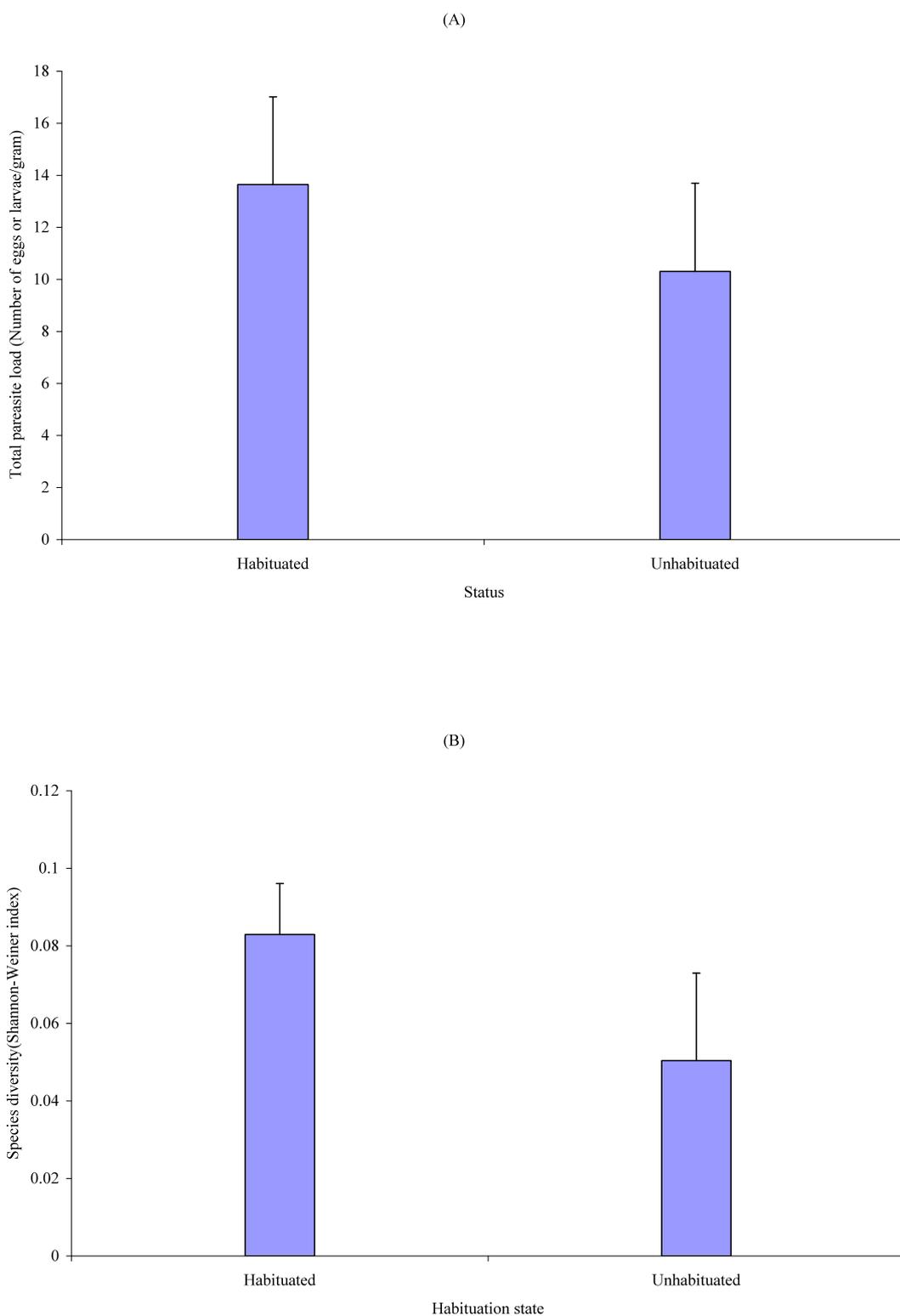


Figure 4.26: The influence of habituation on total parasite load (A) and Shannon Wiener index of diversity (B) among habituated and unhabituated baboons in Gombe National Park.

Bars on top of histograms indicate standard errors. The sample size (n) = 132 for habituated while n = 26 for unhabituated baboons.

4.4.7 Parasite taxa identified from vervet monkeys at Gombe

Four parasite taxa were identified in vervet monkeys at Gombe (Table 4.10; Appendix 3). All of the parasites that were found in the Gombe vervets have previously been reported in vervet monkeys at other sites (Sulaiman, 1986; Gillespie *et al.* 2004; Legesse and Erko, 2004). Two parasites were identified to genus level as *Physaloptera* sp. and *Trichuris* sp. (Appendix 3 A-D), respectively, based on their egg size and morphological features that were similar to those of baboon parasites above. The other parasites found were classified as hookworms (Appendix 3 E-F) or unidentified nematodes (Appendix 3 G-J). Those classified as hookworms were comparatively larger than those found in baboons (55.0 – 77.5µm in length by 42.5 – 45.0µm in width; Table 4.10) but were of similar size and morphology as has been recorded in vervets in the Barbados (Mutani *et al.* 2003). The unidentified nematode eggs and larvae were similar in shape and size to those found in baboons, with the egg size range of 37.50-80.0µm long by 22.5-47.5µm wide and the size range of the larvae 77.5-425.0µm long by 7.5-25.0µm wide, respectively.

Table 4.10: The mean length and width of parasite taxa identified from vervet monkeys in Gombe National Park.

The total number of host individuals infected by each parasite taxon (n)

Parasite species	Mean length (μm)	Mean width (μm)	Number (n)
<i>Trichuris</i> spp.	55.00 (\pm 0.68)	26.75 (\pm 0.94)	5
<i>Physaloptera</i> spp.	62.50	47.50	1
Hookworms	68.44 (\pm 3.53)	43.75 (\pm 0.47)	8
Unidentified nematode eggs	53.44 (\pm 4.84)	34.38 (\pm 2.82)	8
Unidentified nematode larvae	260.42 (\pm 49.11)	13.33 (\pm 2.47)	6

4.4.8 Patterns of parasite distribution in vervet monkeys at Gombe

Twelve independent samples were collected from vervet monkeys sampled in the dry season at the Gombe-Mwamgongo border area, while nine were obtained from those at the Mtanga park border area (Table 4.11). The prevalence of infection of Hookworms and nematode eggs and larvae was higher among vervets at Mwamgongo than Mtanga, while that of *Trichuris* sp. showed the opposite trend (Figure 4.27A). However, GML binomial analysis of the prevalence of three common parasite taxa showed that these differences were not statistically significant (*Trichuris* spp.: $z = -0.005$, $p = 0.996$; Hookworms: $z = 0.923$, $p = 0.356$; Nematode: $z = 0.759$, $p = 0.448$). *Physaloptera* sp. was only detected among Mwamgongo vervets and was not therefore included in this analysis.

Parasite intensities for all parasites except nematode larvae were higher among the Mwamgongo vervet community than that of Mtanga (Figure 4.27B). ZINB analysis results for the most common parasite indicated that these differences were statistically significant (*Trichuris* spp.: $z = 2.443$, $p = 0.0146$; Hookworms: $z = 2.084$, $p = 0.0371$; Nematode eggs: $z = 2.647$, $p = 0.00811$). For nematode larvae, intensity was higher in vervets at Mtanga compared to Mwamgongo, although this difference was not statistically significant (Figure 4.27B; $z = -1.322$, $p = 0.18622$).

Table 4.11 : The number of independent stool samples collected from the communities of unhabituated vervet monkeys at Gombe National park in the wet and dry season.

Due to the small size of the vervet troop, only five stool samples were obtained from the animals at Mwamgongo in the wet season while nine vervets were sampled from the Mtanga area in the wet season.

Community	Season	
	Wet	Dry
Mwamgongo	5	12
Mtanga	10	9

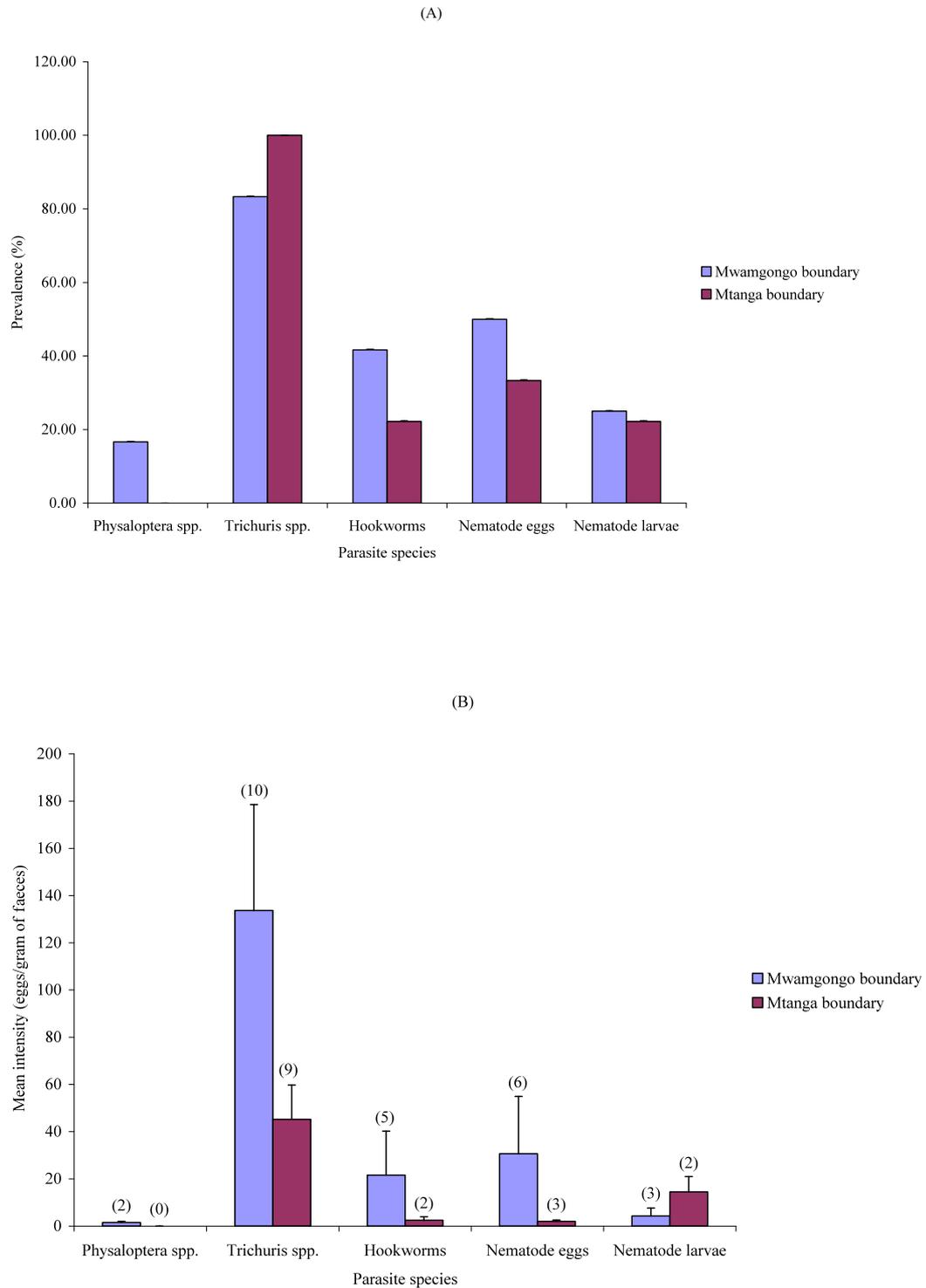


Figure 4.27: The influence of locality on the prevalence (A) and intensity (B) of parasite infection in vervet monkeys at Gombe for dry season samples only.

Bars indicate standard errors while numbers on top of each bar indicate the number of individuals that were found infected by each parasite taxon in each vervet community. The sample size (n) = 12 for Mwamgongo while n = 9 for Mtanga.

4.5 Discussion

4.5.1 Parasite taxa recorded in baboons: Observed temporal patterns

The present study identified seven parasite taxa among the Gombe baboons, all of which have previously been described in the animals, except for *Paragonimus* sp., which was named unidentified trematode. The presence of various parasite taxa among the baboons at Gombe shows that these primates harbour a variety of intestinal parasites, some of which occur at high prevalence. Some parasites such as *Physaloptera* spp., hookworms and unidentified nematodes were common among baboons, while *Paragonimus* and *S. mansoni* occurred at low prevalence (Figure 4.14B). The current findings bear some similarities and differences to previous reports of baboon parasites at Gombe National Park. The first study on the parasite fauna of baboons in the park in the 1970s by McGrew *et al.* (1989) identified seven parasite taxa, including three of the taxa found in this study (*Trichuris* spp., *Physaloptera* spp. and *Streptopharagus* spp.), plus *Strongyloides* spp., *Oesophagostomum* spp., unidentified trematode and *Balantidium coli*. There were parasite eggs in the present study that looked like those of *Strongyloides* spp. and *Oesophagostomum* but species identities were not confirmed. In 1989, Murray *et al.* (2000) found the same types of parasite species in these baboons (except *B. coli*) but also diagnosed *Schistosoma mansoni*, *Endamoeba coli* and an unidentified nematode (Murray *et al.*, 2000). This raised the total number of parasite taxa in baboons at Gombe to eleven. In 1991/92, Müller-Graf (1994) identified all of the parasites reported in the two previous studies (except for *Oesophagostomum* spp.) and recorded one new parasite taxon, an amoeba.

Temporal similarities and differences can also be found in the infection levels and distribution of parasites among individuals. For instance, while previous findings have reported infection by at least one parasite in all individual baboon hosts sampled (Müller-

Graf, 1994), the present study found that 17% of the sampled baboons were not infected with any faecal parasites. The prevalence of infection for the major parasite taxa has also fluctuated between studies. For instance, the prevalence of *Trichuris* spp. and *Streptopharagus* spp. increased from 35-42% in the 1970s (McGrew *et al.*, 1989) to 48-66% in the 1980s (Murray *et al.*, 2000) and then to 67-81% in the 1990s (Müller-Graf, 1994), which is similar to prevalence in the current study (68-80%). The observed temporal variation in the types and numbers of parasites recorded in baboons at Gombe from the 1970s (McGrew *et al.*, 1989) to the present could be a result of various factors, including the differences in sampling or diagnostic techniques applied and improved sanitation and hygiene in the park. Variation in the sensitivity of the different techniques used in studying parasites and possibly temporal changes in parasite infection among the baboons at Gombe may explain the discrepancy between the present results and previous surveys (Nutter, 1993; Murray *et al.*, 2000). For instance, Murray *et al.* (2000) used a smaller sample size than in their 1989 study but recorded more parasites than previous studies (McGrew *et al.*, 1989). It is also possible that recent health and sanitation measures initiated in the park, outlined in Pusey *et al.* (2008), may have reduced parasitic infections in animals through reduced reintroductions and less human-animal contact. However, this may not be a strong argument for the observed variations because the improved health and sanitation may apply to some parasites and not others.

The results from Gombe are also similar to other surveys in other baboon sites, such as Muriuki *et al.* (1998), who reported *Trichuris* spp. as one of the most common parasites of baboons from Amboseli National Park and Mpala Wildlife Research Centre, Kenya. The occurrence of common parasites among baboons in different sites is a probable indication of their host-parasite co-evolutionary relationships (Nunn and Altizer, 2006). Baboons have possibly evolved to host a range of parasites, particularly nematodes. The similarity in baboon parasite fauna across their range may also reflect their common risk behaviours,

such as omnivorous feeding (Hamilton *et al.*, 1978). This possibly exposes them to contaminated food, such as insects that may be parasite vectors. Their water contact habits and the tendency to adapt quickly to living near human settlements further predispose them to parasites and diseases, particularly those originating from humans (Appleton and Henzi, 1993; Huffman *et al.*, 1997). Some differences in the types or numbers of parasites have also been reported across baboon populations. For instance, protozoan parasites like *Entamoeba histolytica* that have been reported in baboons in Kenya (Muriuki *et al.*, 1998) and *Enterobius* (Hahn *et al.*, 2003) have not been found in baboons at Gombe during the current and previous studies. Local variation in baboon behaviour and the occurrence of unique environmental conditions, such as the presence or absence of relevant parasite vectors, are some of the factors that may account for variation in the types of parasite in different baboon populations. However, comparison of parasites and their infection rates between different sites is often compounded by many factors, including variation in the sampling techniques used by various authors. Thus, the use of standardized techniques would be required to make direct comparisons of epidemiological studies across time and space.

4.5.2 The detection of *Paragonimus* parasite in baboons at Gombe

Previous researchers such as Müller-Graf (1994), Müller-Graf *et al.* (1996) and Murray *et al.* (2000) reported the presence of an unidentified trematode in baboons at Gombe. The name *Paragonimus* was first suggested by Müller-Graf *et al.* (1996) to describe the unidentified trematode in baboons in the area. Based on the descriptions of the previously infected baboon troops and the parasite identification aided by photographs of its eggs, the parasite currently identified as *Paragonimus* is the same parasite as found in the Müller-Graf *et al.* (1996) study. The present study utilised the best currently available information from the literature and a picture of the parasite was sent to the Center for Disease Control

and Prevention (CDC) in Atlanta, Georgia USA to confirm that the parasite was indeed *Paragonimus* sp. (CLIA ID: 11D0668319). However, due to similarities among eggs of *Paragonimus* and those of other trematodes, it is difficult to confirm parasite identity just on the egg morphology only. Sequencing of the ITS region of rDNA performed in the present study to confirm the *Paragonimus* identification was not successful. The sequence from baboons that tested positive for *Paragonimus* appeared to be a mixture and so species identity could not be confirmed using BLAST. This mixture could suggest that more than one trematode might have amplified. A resolution on how to sequence high copy number regions from the single eggs or larvae of trematodes using another gene region (e.g. the nuclear ITS2 or mitochondrial cytochrome oxidase I gene) would be helpful in identifying the worms. Such studies would also help to determine whether there is sharing of this parasite between humans and non-human primates such as baboons.

Unlike other parasites in the present study, *Paragonimus* sp. was mostly found in resident baboons in troop DB and a few individuals in BA, BB and DA troops (Figure 4.20A and B). Baboons in DB troop in particular have been observed to actively feed on freshwater crabs from underneath rocks in the Kakombe stream, which could be the source of the infection (A. Collins, *personal communication*). Certain species of freshwater crabs transmit *Paragonimus* parasites in Africa, such as members of *Liberonautes* genus in Liberia and Ivory Coast and *Sudanonautes* genus in Cameroon and Nigeria (Aka *et al.*, 2008). In nature freshwater crabs serve as second intermediate hosts in the life cycle of *Paragonimus* and mammals acquire the parasites by feeding on crabs (Ash and Orihel, 2007). The baboons from troop DB in which most individuals were diagnosed with *Paragonimus* were emaciated and had lower reproductive output compared to other baboons in the park that do not feed on crabs (A. Collins, *personal communication*). Over the past 30 years, the population size of the crab-eating troop (DB) has remained the same size while its sister troops that do not eat the crabs have multiplied and divided repeatedly

to produce eight separate troops (Collins *et al.*, unpublished report). DB baboons moved recently into the Kakombe area, which was formerly occupied by the C troop. They shifted to feed on freshwater crabs and snails after the closure of fishing camps on the Gombe beach along Lake Tanganyika, where the animals had plenty of fish (A. Collins, *personal communication*). A more extensive survey in terms of both space and time might help to elucidate whether the shift to feeding on crabs has increased exposure to *Paragonimus* in this troop. Perhaps this could reveal a new phenomenon similar to what happened to the people of Eastern Nigeria in 1970s who, due to lack of food during the Biafran war in 1967-1970, resorted to feeding on freshwater crabs (Aka *et al.*, 2008). The majority of these people were later found to be infected with paragonimiasis, whose source was confirmed to be the crabs (Aka *et al.*, 2008).

In humans, paragonimiasis is acquired through consumption of raw or partially cooked crustaceans, such as crayfishes and freshwater crabs. The disease is mostly found in Southeast Asia, some parts of South America and to some extent in Western Africa (Aka *et al.* 2008). Although it mostly affects humans, paragonimiasis has also been detected in animals such as the Drill (*Mandrillus leucophaeus*) in Cameroon (Sachs and Voelker, 1975) and the crab-eating monkeys in Malaysia (Kim, 1978). If confirmed as *Paragonimus*, this will be the first time this parasite has been reported in wild Olive baboons (*Papio anubis*) and in areas east of the Congo basin in Africa.

4.5.3 Variation of parasite infection with season and baboon troop membership

Although not consistent for all parasites, there was a seasonal effect on parasite infection among baboons, which varied with baboon troop membership. It is not possible to explain the effect without temperature or rainfall records, but given that the seasonal effect varied with locality, a general discussion on the implications of these results can be made. More

parasites were commonly recorded among baboons during the dry season than the wet season, with a few exceptions. *Paragonimus* sp. was almost absent in most troops except for BB and DB, where it occurred at high prevalence while *Physaloptera* sp. was only detected in the dry season in troops AC, BA and BB (Figures 4.20 and 4.21, respectively). The prevalence and intensity of *Paragonimus* sp. were higher in the dry season than wet season for all baboon troops where it was present, although the variation of prevalence was not statistically significant, possibly due to insufficient power, given the low levels of infection. Similarly, the prevalence and intensity of *Trichuris* sp., unidentified nematodes and *Physaloptera* sp. were also higher in the dry season than wet season in most baboon troops, although the differences were not always significant. Although a power analysis was not conducted, in general, the low numbers and prevalence of most of the parasite species precluded rigorous statistical testing so definitive conclusions about the relationship between season and troops can not be drawn.

There also could be a sampling bias that might have affected comparisons between seasons. Sampling was not consistent between seasons across baboon troops, as more samples were obtained during the wet season than the dry season in all troops. However, to control for this bias, only baboons that were examined in the wet and dry season in each troop were included in the analysis on the effect of season on parasite infection. For instance, baboons in troop DC, which were only sampled in the wet season, were excluded from the seasonal analysis. The dry season in Gombe National Park is marked by food shortages and loss of weight in animals, including baboons and chimpanzees (Goodall, 1986). The lack of sufficient food leads to reduced body immunity and hence susceptibility to parasitic infection including parasites (Harrington, 2010). There is also a lack of water, as many streams dry out during the dry season, causing animals to congregate in few sites with available water, which enhances parasite transmission among them. Primate species in other sites have also been diagnosed with higher parasite infection during the dry season

than in the wet season (van Geldorp and van Veen, 1976; Appleton and Henzi, 1993). For instance, the South African Chacma baboons (*Papio ursinus*) had higher parasite prevalence in the dry season than the wet season, which was linked to lowered immunity in the dry season (Appleton and Henzi, 1993). However, other studies such as Kawabata and Nishida (1991) and Huffman *et al.* (1997) have reported higher parasite loads for *Oesophagostomum* sp. and *Strongyloides fülleborni* among chimpanzees during the wet than the dry season. Since moist conditions are more favourable for parasite survival than dry climates (Stuart *et al.*, 1998), higher levels in the wet season might be expected. It is also possible that the onset of dry season prompts the parasites to delay egg production until conditions improve in the wet season (Fritsche *et al.*, 1993). However, only longitudinal studies on parasite infections among the Gombe baboons would produce a more comprehensive picture of the factors affecting seasonal differences in parasite loads.

Variation in baboon troops could be attributed to troop spatial locality. Among the habituated baboons, the current findings did not suggest that living close to humans affects the dynamics of parasite infections in baboons at Gombe. Nonetheless, the prevalence and intensity of unidentified nematodes were highest among baboons in DA troop (Figure 4.22), whose range covers the village camp in the Kasekela area (Fig. 4.2). There are also more people living as researchers and national park staff and their families at Kasekela than other areas of Gombe, and many more tourists visit Kasekela than Mitumba (Nutter, 1993) to the north of the park. It is possible that increased human activities may be most implicated in the dynamics of parasite transmission in the Kasekela area. Elsewhere, humans and their activities have been found to influence the levels of parasite infections among the primates (Woodford *et al.*, 2002; Ocaido *et al.* (2003). Ocaido *et al.* (2003) for instance reported similar parasites between baboons and humans living in close proximity to each other. Humans living or working with wild primates may introduce new pathogens into non-human primate habitats or may create environments conducive to parasite growth,

through activities such as waste disposal (Woodford *et al.*, 2002). This, however, needs further investigation including using molecular methods to delineate the parasite species infecting humans and non-human primates in shared habitats.

There were some trends in parasite infection across troops which suggested the possible effect of a large troop size. For instance, the prevalence and mean intensity of *Physaloptera* sp. and unidentified nematodes were higher among baboons from the large troops DA and BA (over 50 individuals) than in the small AC and DB troops (about 20 individuals). In contrast, the prevalence of *Trichuris* and *Paragonimus* sp. was higher in baboons from small troops of AC and DB than in the larger BA and DA troops. Parasite loads were also higher in small AC and DB troops than in the large BA and DA troops. Previous studies, have reported that mammals living in large groups are infected with higher parasite load and prevalence than those found in smaller groups but this could vary with the parasite species or other features of the host populations (Freeland, 1979; McGrew *et al.*, 1989; Nunn *et al.*, 2008). Living in larger groups causes overcrowding, which in turn leads to repeated use of sleeping nest/areas and other gathering sites than would be the case for smaller groups (Freeland, 1979; Vitone *et al.*, 2004; Nunn *et al.*, 2008). This in turn can increase the chances for parasite re-infection and cross-infection, especially when the parasites involved are soil transmitted, such as nematodes (Wilkinson 1985; Shields and Crook 1987; Nunn *et al.*, 2008). Other factors, such as heterogeneities in the habitats and individual baboon genetics, could account for the observed variation in parasite infection across troops. Other studies have however not established any trend on the relationship between group size and parasite infection in animal populations (Ezenwa *et al.*, 2006; Snaith *et al.*, 2008). On the other hand, more comprehensive studies would be needed to address this argument.

4.5.4 Influence of habituation on parasite infection in baboons

Although there were differences in prevalence and intensity of particular parasites between habituated baboons in the central part of Gombe National Park and non-habituated baboons living on the park borders, there was no clear trend that habituated animals had higher loads of individual parasite species than non-habituated. Overall, habituated baboons had higher parasite loads and species diversity (Shannon-Wiener index) than non-habituated baboons (Figure 4.26), but the differences were not significant. It was also observed that some parasites were more prevalent among non-habituated baboons. For example, the prevalence of unidentified nematodes was significantly higher among non-habituated baboons than habituated populations. The Oxford Online Dictionary (World English) defines habituation as “the diminishing of an innate response to a frequently repeated stimulus”. The repeated stimulus or exposure in this context involves the prolonged (deliberate) encounter of researchers with primates in the wild. With time, the animals lose fear and this enables the researchers to follow them around, stand or sit close to them and study their behaviour in the field. Although the process allows researchers some access to the animals, it also has some drawbacks, including the possibility of disease transmission (Wallis and Lee, 1999). Studies on primate pathogens have shown that most parasites infecting baboons and other primates are probably of the same type or are closely related to those found in humans, particularly in sympatric areas (Healy and Myers, 1973; Nutter, 1993; Ashford *et al.*, 1990; Lilly *et al.*, 2002). Due to the close evolutionary similarities between humans and baboons, it is possible that they could share the same parasites.

The high number of people in Gombe, including both visitors and residents means that humans are likely to share parasites and other pathogens with animals, particularly in habituated populations. Like chimpanzees, baboons in Gombe National Park have been under constant observations of their behaviour since the 1960s (Goodall, 1986; Inskipp,

2005). They have been habituated and as a result these animals have no fear of humans and tend to spend time around human settlements. This puts them at the risk of not only being killed or poisoned, but also acquiring parasitic infections as reported among gorillas in Uganda (Nizeyi *et al.*, 1999). As observed for other non-human primates such as chimpanzees (Homsy, 1999), baboons are vulnerable to human infections because they may not develop resistance to human diseases. Animals in Gombe National Park drink untreated water from the same streams as humans and they also have constant contact with tourists and research and park staff. This scenario can have significant implications by increasing the potential risk for parasite infection to chimpanzees and a threat to human health too. A report by Nasher (1988), showed that sacred baboons (*Papio hamadryas*) living near human residences in Asir Saudi Arabia were more infected with parasitic worms than those living in isolated habitats. Similarly, it was therefore concluded that the animals might be exchanging parasites with humans in the shared habitats as they both harboured the same parasite taxa. However, the variation in the infection levels observed between the habituated and non-habituated baboon populations in the current study could also be caused by local variation in topography and microhabitats between the populations. As recommended above, more comprehensive studies would be required to investigate the factors affecting parasite dynamics and interactions between humans and nonhuman primates.

4.5.5 Implications of the confirmation of *S. mansoni* infection in baboons in Gombe

The current confirmation of the presence of *S. mansoni* in baboons at Gombe is of paramount significance because of the importance of the disease to human health in the area and its potential implications to animal health in the park. Previous studies at Gombe reported the highest levels of infection with schistosomiasis in baboons that had high rates of contact with humans (Müller-Graf *et al.*, 1997). Results from the present study seem to

suggest the contrary, as the disease was recorded only in the baboon groups with low human contact. The parasites were found among BA and BB troops (B-group in Müller-Graf, 1994) and DA troop (D-group in Müller-Graf, 1994) which, based on their current home ranges, should have less access to human settlements at Gombe compared to baboons in DC troop (Figure 4.2). No schistosome parasites were found in baboons from DC, whose home ranges include the camp village where research and national park staff live (Figure 4.2). However, only dry season samples were collected from this troop. So, the findings could be a result of seasonal variation. However, baboons in the BA and BB troops surveyed at the same time as DC and were found to be infected with the disease. Baboons in these two troops are genealogically related and it is possible that they were infected before they split from the former B-group (Nash, 1976; A. Collins, *personal communication*). Although the other infected troop (DA) is not genetically related to these troops, its home range overlaps with that of BA and BB (Figure 4.2). It is therefore possible that baboons in the three troops are exposed to an infectious site within their home ranges, especially in areas where they all intersect. A more thorough understanding of habitats in the home range of the infected group would be useful to identify the source of infections. Future work should follow the infected individuals closely to trace the sites from which these animals get infected. In addition, detailed surveys of prevalence of infection in local snails (see chapter 5) could help to provide insights on the transmission dynamics within the park.

It is possible that the transmission of schistosomiasis infection among baboons in Gombe National Park has continued since it was first reported in the area in 1982-3 (A. Collins, *personal communication*). Most individual baboons currently infected with schistosomiasis in Gombe were born after Müller-Graf's study in 1992 (Table 4.4; Müller-Graf, 1994). This means that the animals were infected after the study that was conducted between June – November, 1991 and May – June, 1992 (Müller-Graf, 1994). This could

suggest that there is a continuity of transmission of schistosomiasis among the Gombe baboons, as shown by these new infections. It also suggests that human re-introduction of the disease has continued and that baboons are still infected by introduced schistosomes. There were also a few individual baboons that immigrated from unknown baboon troops uphill in the Park (Table 4.4), which means that they were not in the troop at the time of the study. There is thus the possibility that they brought the parasites with them but this cannot be distinguished from infection after arrival, based on the data currently available. This contradicts the long held view that schistosomiasis infection in wild baboons is an accidental event and that the disease cannot be maintained in the animal population for much longer, even if suitable snail hosts were available (Ouma and Fenwick, 1991; A. Fenwick, *personal communication*). The prevalence of *S. mansoni* in this area also appears to have increased and spread in the baboon population since its first diagnosis in Gombe in the 1980s (Müller-Graf *et al.*, 1996; Murray *et al.*, 2000). Schistosomiasis was first reported in humans in the area in 1983 (Müller-Graf *et al.*, 1997), then in one baboon in 1989 (Murray *et al.*, 2000) and then in two chimpanzees in 1991 (Nutter, 1993). The disease may have been introduced into Gombe by research and park staff, who spend their annual holidays in other schistosome-endemic areas in Tanzania (Müller-Graf *et al.*, 1997). It is thought that as people travel between Gombe and other endemic areas in Tanzania, they may have introduced the parasite into Gombe, where it then crossed over to the baboon population (Müller-Graf *et al.*, 1996). Gombe was also initially inhabited by humans in the 1940s, before it was declared a National Park in 1960, following which all conventional human settlement and activities such as cultivation were banned (Goodall, 1986). In more recent years, people have re-settled back in the park as research and national park staff and their families. Currently the people living in the area use the same habitat and other resources with the animals such as the forests, swamps and water bodies, including the Dell site where schistosome-transmitting snails have been reported (Müller-Graf *et al.*, 1997) and were found during the present study.

Schistosomes have also been found in baboons in Manyara National Park in northern Tanzania (Fenwick, 1969). The baboons of Manyara were thought to be the main reservoir of schistosome infection in the area, due to isolation of the study area from regular human contact (Fenwick, 1969). *S. mansoni* have also been reported in other baboon populations across Africa, including Kibwezi, Kenya (Nelson, 1960) and Omo National Park in Ethiopia (Fuller *et al.* 1979). The present observation also supports the suggestion that wild baboons might be able to maintain the transmission of intestinal schistosomiasis in nature. Schistosomiasis due to *S. mansoni* has been described as anthroponotic rather than zoonotic because is thought to be primarily a human disease (Nelson 1960). Baboons and vervets could be more susceptible to this disease than other primates because of their close association with water and human settlements but it is not known whether they can act as primary hosts for the parasites (Ouma and Fenwick, 1991; Nunn and Altizer, 2006). Field studies have shown that baboons are capable of maintaining schistosome infection on their own for a long time even in the absence of humans and that they can also transmit it to humans (Zahed *et al.*, 1996). Zahed *et al.* (1996) reported that *S. mansoni* identified in Hamadryas baboons *Papio hamadryas* in Saudi Arabia were viable and could infect snails, leading to suggestions that they could also infect humans. However, the genetic relationships among the population(s) of *S. mansoni* in humans and baboons sharing the same habitat in the wild environment have not been established (Ouma and Fenwick, 1991; Müller-Graf *et al.*, 1997). It is thus not clear to what extent non-human primates such as baboons and vervets harbouring *S. mansoni* parasites could possibly serve as reservoirs for human infections in shared habitats. Although the region of DNA sequenced in this study was not variable enough to determine whether humans and baboons might share the same genotypes of parasites, the rRNA regions sequenced were identical to *S. mansoni* sampled from humans. This could reflect sharing of schistosomiasis between humans and baboons at Gombe, due to high rates of interaction between them in the park (Wallis and Lee. 1999).

4.5.6 Patterns of parasite infection in vervet monkeys

The four parasite taxa presently identified in vervet monkeys at Gombe have previously been reported in vervet monkeys in other sites, such as in various localities in Ethiopia (Legesse and Erko 2004), Blue Nile Province in Sudan (Sulaiman, 1986), and Lake Saka in Uganda (Gillespie, *et al.* 2004). An investigation by Mutani *et al.* (2003) on the parasite fauna of vervet monkeys in Barbados that had been imported into the country from West Africa reported the presence of the same parasites as found in the Gombe monkeys, but also others that were not detected such as *Strongyloides* sp., *Oesophagostomum* sp., *Trichostrongylus* sp. and *Ascaris* sp. The small sample size of vervet monkeys examined at Gombe (19 independent samples) could not account for the smaller numbers of parasites species found at Gombe. Nevertheless, analysis of dry season samples was performed to determine variation of parasite infection between the two communities of vervets at Gombe. The results indicated that infections were generally higher among the vervets in the northern park boundary near Mwamgongo village than the southern community at Mtanga (Figure 4.27). This was the case for all parasites except for the prevalence of *Trichuris* spp. and the intensity of unidentified nematode larvae, respectively, which were higher among the southern Mtanga community. Except for *Physaloptera*, all of the parasites observed in the vervet monkeys can also infect humans (Cheesbrough, 1998; Ash and Orihel, 2007). Since vervet monkeys from the most infected Mwamgongo population visit human houses nearby (Figure 4.5), it is possible that these monkeys could serve as reservoirs for human parasites. However, due to the small sample size involved, the present findings may not reflect the true picture on the ground. Nonetheless, the present results corroborate a report by Legesse and Erko (2004) who found that vervet monkeys living close to human settlements in Ethiopia harboured protozoan and helminth parasites that were similar to those of humans. The authors concluded therefore that the monkeys posed a high risk to public health in the area (Legesse and Erko, 2004). Similarly, Mutani

et al. (2003) also reported that vervet monkeys in shared habitats with humans were infected with parasite species that also infect humans and observed that these primates can play a role as reservoir hosts for human parasites.

The paucity of parasite taxa observed in the vervet monkeys in the present study echoes the results reported by Gillespie *et al.* (2004) in the Ugandan monkeys. Only three parasite taxa namely *Strongyloides* sp., hookworms (*Necator* sp.) and Physaloptera sp. were found among these monkeys. It was concluded that the low number of parasite taxa detected in the monkeys was possibly due to the small sample size of the host that was sampled. Only twelve individual vervet monkeys had been sampled and as highlighted by other worker (Gregory and Blackburn, 1991), results obtained from lower sample sizes may not reflect the real picture of parasitic infections in the host populations. It is therefore likely the present findings do not provide a definite representation of parasite infection among vervet monkeys at Gombe.

4.5.7 Conclusions

In the present study, all vervet monkeys and most baboons were infected with one or more parasite taxa and this is perhaps an indication of the importance of parasites in the ecology and health of these animals. It would thus be useful for future studies to examine the specific roles these parasites might have on the dynamics of the baboon and vervet populations at Gombe. There was significant variation in parasite infections between baboon and vervet groups, which was possibly a result of variation in group size and other local environmental factors. Infections for some parasites were significantly higher among baboons in the dry season than the wet season, probably due to low body immunity in the host resulting from lack of sufficient food and other dry season related stresses. Differences in parasite infections between habituated and non-habituated baboons were not

significant although the former had proportionately higher prevalence for some parasite taxa. This was probably caused by introduction of parasites from humans to baboons in the habituated populations. Although parasite infection in vervet monkeys varied between Mwamgongo and Mtanga areas, no conclusions could be made on this relationship due to lack of sufficient data. As the parasites observed in the present study (with the exception of *Physaloptera*) all are capable of infecting humans, baboons and monkeys in the Gombe area could potentially serve as reservoirs of human gastrointestinal helminths. Effective measures aimed at controlling the infections should therefore take into consideration the human-baboon contact in the areas investigated.

Chapter 5: Comparison of PCR and Microscopy-based Assessment of the Prevalence of *S. mansoni* from Snails in the Gombe Ecosystem, Tanzania

5.1 Abstract

Snails are essential for the transmission and maintenance of schistosomiasis in endemic areas, as they serve as intermediate hosts for schistosome parasites. A clear understanding of the snail species present in an area, their local distribution and infection status is therefore a prerequisite for effective control of schistosomiasis. The identity and infection status of snails transmitting intestinal schistosomiasis in areas along the shores of Lake Tanganyika in western Tanzania is not known. The purpose of this study was to evaluate how infection status of snails in the region varies by location and season, using microscopy-based and molecular methods. Snails were collected from 12 sites along one stream located close to human settlements in Gombe National Park as well as in streams in the surrounding villages (Mtanga, Mwamgongo, Bugamba and Kiziba) and in the largest nearby city (Kigoma). Snails ($n = 240$) were individually exposed to light to induce shedding of schistosome larvae and examined under a dissecting microscope. They were then dissected and their soft tissues preserved in RNA-later solution until required for molecular analysis. The ITS region of the ribosomal DNA was PCR amplified and sequenced to confirm the species identification of the snail and to determine infection status with trematodes. Snails were present in all streams except at Mtanga and all were morphologically identified as *Biomphalaria pfeifferi* based on previous sightings and their blackish sinistral coiled shells. Based on microscopy examination, none of the snails from Gombe or Bugamba shed schistosome larvae, while larvae were shed from 10.6% of individuals from Mwamgongo, 22.6% from Kiziba and 26.62% from Kigoma town. Overall, an infection prevalence of 11% was observed in snails based on experimental shedding and microscopy while 45% of the snails were infected with trematodes using the PCR-based technique. However, sequencing indicated that trematodes other than schistosomes were also present at some sites and so the PCR method is likely an overestimate of schistosome infection levels. The PCR also indicated that not all snails were

Biomphalaria pfeifferi. Both methods consistently indicated that the Gombe snails had no schistosome parasites while snails from all other sites were infected. Microscopy analysis indicated higher infection in the wet season than dry season while molecular analysis results indicated the opposite. The results support the previous assumption that *Biomphalaria pfeifferi* snails transmit schistosomiasis in the area but suggest that the community structure of both snails and trematodes may be more complicated than previously thought. The implication of these infections is discussed in the context of the observed spatial and seasonal variation in snail infections.

5.2 Introduction

Studies on schistosomiasis (caused by the trematode *Schistosoma mansoni*, Schistosomatidae) transmission in humans have shown that the disease can only be acquired in areas where people come in contact with a water body containing infected snails (Appleton, 1978). Without suitable snail hosts, the transmission of schistosomiasis cannot occur and parasites shed by infected people will not reach infective stages. If this continues for long periods, the infection may cease to exist in such areas (Southgate and Rollinson, 1987). Hence, knowledge of snail distribution and habitat preference is a crucial tool in understanding the epidemiology and control of schistosomiasis (Utzing and Tanner, 2000). As discussed in chapter one, three snail species of the genus *Biomphalaria* are known to transmit *S. mansoni* in Tanzania. These include *B. sudanica*, which is confined to the northern part of the country, *B. choanophala* occurring along the shores of Lake Victoria and *B. pfeifferi*, which is thought to occur throughout the country (Magendantz, 1972; Doumenge *et al.*, 1987). In their reviews, Doumenge *et al.* (1987) and Rugemalira (1991) have noted that *B. pfeifferi* is possibly present in areas along the shores of Lake Tanganyika. However, the distribution patterns, habitat preferences and infection status of schistosome-transmitting snails in the area have not been investigated (Rugemalira, 1991). The expansive shores along Lake Tanganyika are rocky and constantly agitated by heavy waves and may thus not be suitable for snail survival (Nutter, 1993; Müller-Graf *et al.*, 1997) but the lake is fed by multiple mountain streams, which could provide suitable snail habitat. A study by Müller-Graf *et al.* (1997) reported the presence of *Biomphalaria* snails in an inland marshy area in Gombe National Park but the species of the snails was not confirmed. Thus, it is still unclear what species or sub-species of *Biomphalaria* are responsible for transmitting *S. mansoni* especially in these western parts of Tanzania. Physical barriers to snail dispersal in the area caused by mountains and valleys (refer section 1.14 in Chapter 1) may have led to low or no migration of snails

between villages. This migration restriction of snails might result in variation between sites in the presence or absence of snails and their schistosome infection status and so it is important to investigate prevalence not only in Gombe but also in the surrounding villages.

The objective of this chapter is to determine the role of snails in the transmission of schistosomiasis in selected localities along the shores of Lake Tanganyika, by identifying the snail species responsible for transmitting *S. mansoni* and determining their infection status in relation to spatial distribution and season. Specifically, the purpose of the study was to evaluate the effectiveness of microscopy and molecular techniques in assessing schistosomiasis infection in snails, in order to make recommendations for surveillance programmes to monitor transmission dynamics of the disease in snails, humans and other potential reservoir hosts in the area such as non human primates and rodents.

5.3 Materials and Methods

5.3.1 Study area

Detailed information on the study area is given in chapter 1 (section 1.14 and Fig. 1.5)

5.3.2 Study planning and sampling design for snail collection

Snail sampling followed the protocol described in Madsen (1995). Initially, the study area was surveyed to identify streams and possible sampling sites in each village stream. Streams were chosen based on a strategic location and with maximum influence from human activities. A stream was chosen for sampling if it was located close to human settlements or if it passes in the middle of the village, to increase the chances of finding infected snails, as recommended in Madsen (1995). Sampling sites on each stream were selected by pacing out the length of the river, with each site being located at least 200m apart. A total of 12 sites were selected and sampled along each stream with half of them located outside the village residential areas. Snails were systematically searched for in each site for a period of 15 minutes and the snails found were counted and collected. Snails were sampled using a scoop (30 by 30 cm) covered with a 2x2 mm size mesh wire (Figure 5.1). Snail sampling for each site was conducted twice per season, separated by two weeks in each case. In areas where it was not possible to use scoops, such in shallow stony spots, snails were picked by hand using a pair of long forceps, as explained in Ouma *et al.* (1989). To maintain consistency, the same person (J. Bakuza) searched for the snails throughout the study period. Snails from each site were placed in separate plastic vials (120ml) containing mud and water from the site. The containers were labelled with site name (locality) and date and wrapped with aluminum foil (except the top parts) to prevent the snails from shedding cercaria larvae before reaching the laboratory.



Figure 5.1: A 30x30cm scoop (attached to a wooden handle) that was used to collect snails from stream water.

Collection of snails sometimes involved wading in deep water while wearing full protective gear against schistosomiasis contraction, including wearing whole body clothing, gumboots and long arm gloves. A local woman can be seen in the background washing up utensils in this stream at Mwamgongo village, which was found to contain snails infected with schistosomes.

5.3.3 Shedding of parasite larvae (cercaria) from snails

In the laboratory, each snail was individually washed using distilled water (or filtered stream water) and transferred into a well of a 12 or 24 well plate containing filtered stream water. The plates were then exposed to light (60W bulb) to enable the snails to shed cercaria larvae, following guidelines in Wolmarans *et al.* (2002), with slight modifications (Figure 5.2). After a period of 6 to 12 hours, each snail was observed under a dissecting microscope to determine if they were shedding schistosome larvae. Each snail was then dissected and its tissue preserved in a 1:3 volume ratio of tissue to RNA-later at room temperature until required for DNA extraction. The RNA-later preservative was chosen over others because it is safe for handling and shipping and is effective in preserving schistosome eggs and larvae for molecular analysis (Webster, 2009).



Figure 5.2: Shedding of parasite larvae (cercaria) from snails through exposure to artificial (electric) light.

The illumination was performed under a 60W bulb powered by solar panels in a baboon research room at Gombe Stream Research Centre in Gombe National Park.

5.3.4 DNA extraction and PCR amplification of snail tissues

Snail tissues (240 independent samples) were chopped into small pieces using scissors and DNA was extracted in the Molecular Ecology laboratory in Glasgow using DNeasy® Blood and Tissue Kits and the procedures described in Jannotti-Passos *et al.* (2006) and chapter 2. Extractions were performed in sets of 24 and always included a negative extraction control to ensure that there was no contamination. Where evidence of contamination was observed (based on amplification of a PCR product), the entire set were re-extracted.

Initially, the primers 16 Sab and 16 Sar were used to amplify a region of the mitochondrial 16s ribosomal RNA (rRNA) region in the snails as described in Jannotti-Passos *et al.* (2006), while Schfo17 and Schfo19 primers were used to detect *S. mansoni* parasites in the snails based on the small subunit (18S) of the nuclear rRNA array, according to a nested primer approach described in Melo *et al.* (2006). Therefore, the results on snail species and schistosome prevalence presented here are based on a single amplification approach to sequencing the ITS region of the rRNA, where it was possible to amplify both snail and parasite DNA using the same primers, which amplified reliably in all samples. The results described were obtained from an Honour's project performed by Robert Gillespie (under my supervision), using the same samples as used for microscopy.

The primers used were ETTS2 (5' TAA CAA GGT TTC CGT AGG TGA A 3') and ETTS17 (5' CGA GCC GGA TGA TCC ACC GC 3'). The 20µl PCR reactions combined 2µl of 10x reaction buffer, 2µl of 10mM dNTPs, 1µl of 50mM MgCl₂, 0.2µl of 10mM forward (ETTS2) and reverse (ETTS17) primers, 0.2µl of 5U/µl Taq DNA polymerase (Invitrogen Inc., Paisley UK), and an appropriate volume (1-2µl) of DNA template, with double distilled H₂O added to bring the volume to 20µl. The following PCR cycle was

used: initial denaturing for 3 minutes at 95°C, 34 cycles at 95°C for 45 seconds, 55°C for 1 minutes (annealing), and 72°C for 1 minutes (primer extension), followed by a final extension of 5 minutes at 72°C. For each set of reactions, a negative PCR control (i.e. only containing water), the negative extraction control from the extraction set, and a positive control (DNA template from previously identified *B. pfeifferi*) snails were used. Amplification products were visualised by running them through a 2% agarose gel and visualizing them on a Bio Rad Gel Doc machine, using Quantity One Software. In cases where there was evidence of contamination in the negative extraction control, the entire set of DNA extractions was repeated and PCR performed on the new extractions.

Initial screening revealed that multiple size bands were present and so bands were gel purified using QIAquick Gel Extraction Kits, using the manufacturer's instructions (Qiagen Ltd, Crawley – West Sussex, UK). Initial sequencing revealed that the snail band was approximately 600 bp and the *S. mansoni* band was approximately 500 bp. In addition, a larger band was present (1000 bp) in some individuals; a BLAST search of Genbank revealed that the closest match to this was another trematode that was not *S. mansoni*. Presence of the 500 bp band was therefore used to assess prevalence of *S. mansoni* within snails and presence of the 1000 bp band was used to assess prevalence of the unknown trematode.

To ensure that lack of amplification of parasites was not due to poor DNA quality, two approaches were used. Firstly, only samples for which the snail band amplified cleanly were used for assessment of prevalence; in cases where this was not true, PCR was repeated using new DNA extractions. Secondly, the DNA quality and quantity (in ng/ul) was assessed using a Nanodrop® ND-1000 UV-Vis Spectrophotometer and Nanodrop ND-1000 (V3.3.0) software. DNA quality was assessed based on the ratio between the absorbance values at 260nm and 280nm (260/280). For DNA samples, a 260/280 reading

of 1.8-2.0 indicated that the sample was of high quality Hoisington *et al.* (1994). Values below this can be indicative of contaminants such as proteins, and higher values can indicate contamination with chloroform or phenol (Hoisington *et al.*, 1994). In such cases, DNA extractions were repeated and PCR redone.

Samples were sequenced at the GenePool (University of Edinburgh) using cycle sequencing with Big Dye and an ABI 3730 sequencer. The ETTS17 primer yielded cleaner sequences and so it was used for most of the sequencing.

5.4 Results

5.4.1 Prevalence of schistosomes based on microscopy

Snails were found at few sites in all streams except in Mtanga villages where no snails were present. The snails were morphologically identified as *Biomphalaria pfeifferi* based on their blackish sinistral (left) coiled shell (Figure 5.3) while following snail identification guidelines in Brown (1994) and descriptions from previous reports on snail sightings in the area (Müller-Graf *et al.*, 1997; Tanzania 2005-Schistosomiasis, unpublished report; West *et al.*, 2003). Experimentally-induced shedding indicated there was considerable variation in prevalence of schistosomes among snails from different villages (Table 5.1; Fig. 5.4). No infected snails were found from Gombe or Bugamba. Although sample sizes were small, there was also variation between seasons, with more shedding in the wet than the dry season (Fig. 5.5).



Figure 5.3: Some of the snails obtained from the Dell site near Kakombe stream in Gombe National Park.

The snails were identified as *Biomphalaria pfeifferi* based on previous sighting reports and also on their left coiled shells (snistral) as the snails faced the observer. Sequencing of the ITS region confirmed the species identification.

Table 5.1: Comparison of prevalence of *S. mansoni* (Schistosomatoidea) in snails collected from Gombe National Park and neighbouring villages.

The results were obtained using PCR and microscopy-based methods. Also indicated are the % individuals that were infected with another trematode, *C. australis* (Echinostomatidae). The data shown below are combined for both wet and dry season.

Site	N Snails	% infected <i>S. mansoni</i> (PCR)	Snails <i>S. mansoni</i> (PCR)	% infected <i>S. mansoni</i> (Microscopy)	Snails <i>C. australis</i> (PCR)	% Snails Infected <i>C. australis</i>
Bugamba	23	30.4	0	0	26.1	
Gombe	47	0	0	0	0	
Kigoma	27	55.6	30	30	0	
Kiziba	27	63	22.2	22.2	0	
Mwangongo	96	57.3	12.5	12.5	18.8	
Total/Average	220	42.7	11.8	11.8	10.9	

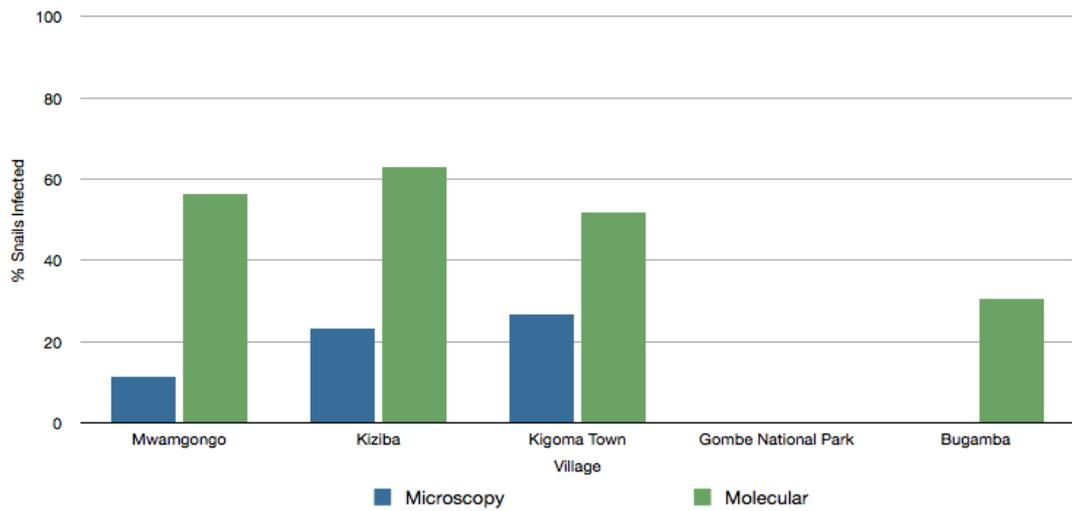


Figure 5.4: Seasonal variation of *S. mansoni* infection in snails based on shedding (microscopy) and PCR-based (molecular) screening in villages and Gombe National Park.

The results indicate the consistent lack of schistosome infection among Gombe snails based on both techniques. The figure also shows the ability of the DNA analysis technique to reveal infection in Bugamba snails, which were not shedding through shedding-based examination and microscopy.

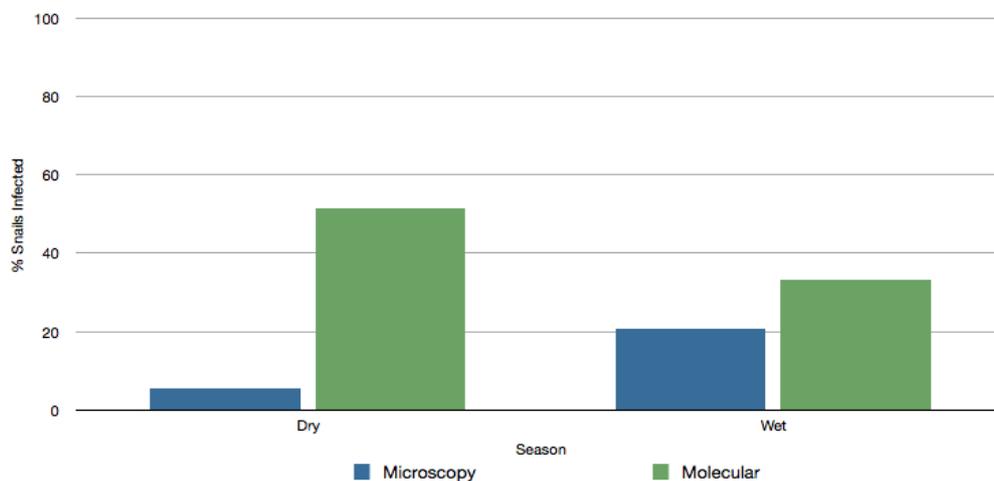


Figure 5.5: Seasonal variation of *S. mansoni* infection in snails based on shedding (microscopy) and PCR-based (molecular) screening methods.

The results indicate that higher prevalence of schistosome infection in snail during the dry season than the wet season using molecular technique while microscopy analysis results show the reverse.

5.4.2 Prevalence of schistosomes based on PCR

Based on prevalence of the expected band size for *S. mansoni*, the PCR technique was more sensitive than experimental shedding with schistosome infection detected in snails at an overall prevalence of 45% compared to 11% observed through microscopy (Table 5.1). Both methods indicated that none of the Gombe snails had schistosome parasites, while Bugamba snails that did not shed parasites through microscopy were found to be infected through molecular analysis (Table 5.1; Fig. 5.4). All parasite sequences obtained from this site were confirmed to be *S. mansoni*. Snails from Kibirizi site in Kigoma Township and those from the villages of Mwangongo and Kiziba were also confirmed to be infected with a trematode through both microscopy and molecular methods. However, data from Kigoma should be interpreted with caution, because *B. pfeifferi* was not found in this population and the only parasite sequences obtained were from an unidentified trematode. The presence of *S. mansoni* was confirmed by sequencing in Bugamba, Mwangongo and Kiziba. Based on presence of the 1000 bp band, *Curtuteria australis* was present only in Bugamba and Mwangongo (where it was confirmed by sequencing).

There were similar patterns of variation across villages, with both microscopy and molecular results indicating that the prevalence of infected snails was higher in snails from Kigoma and Kiziba than from the other sites (Fig.5.4). The lowest prevalence of snail infection through microscopy was Mwangongo, while Bugamba snails exhibited the lowest prevalence based on PCR results (Fig. 5.4). Microscopy results indicated that snails were more infected in the wet season than the dry season while results from molecular analysis showed an opposite trend (Fig.5.5).

5.4.3 PCR-based species identification of schistosomes and snails

Sequencing of the three main band sizes suggested that, while species identification could usually be predicted based on the size fragment, there were some exceptions. Sequences were obtained from 14 individual snails for the 500 bp band (predicted to be *S. mansoni*; Figure 5.6) for 11 of these (from one from Kiziba, two from Bugamba, and eight from Mwangongo, all sampled in the dry season) identical sequences were obtained that matched *S. mansoni* sequences available in Genbank (accession number HE601628, from the genome sequenced strain from Puerto Rico). However, for three individuals sampled from Kigoma in the wet season, although the band size was similar to that expected for *S. mansoni*, an unknown trematode was amplified instead (Figure 5.6); the sequence was not similar to *S. mansoni* and the next closest trematode sequence was identified as *Macroderoides typicus* (accession number HQ680849.1) but only showed 79% similarity.

The 600 bp band (predicted to be *B. pfeifferi*; Figure 5.6) was sequenced for 16 individual snails; for 13 of these (three from Gombe in the wet season, four from Bugamba in the dry season, and five from Mwangongo in the dry season and one in the wet season), *B. pfeifferi* was confirmed using BLAST (Figure 5.6). However, there were two different sequence variants, with a single base pair fixed difference between them; one type was found at all three sites; the second was found in two individuals from Bugamba and two from Mwangongo (one from each season). A completely different sequence was found in individuals sampled from Kigoma (two individuals) and Gombe (one individual), all in the wet season. The closest matches in BLAST (99% similar in each case) were for *B. smithi* (AY030373), *B. alexandrina* (AY030372) and *B. sudanica* (AY030369). Since only the latter is known to occur in Tanzania, this was tentatively identified as the species.

The 1000 bp band (predicted to be an unknown trematode; Figure 5.6) was sequenced for four snails from Mwangongo (in the dry season); all were confirmed to be 100% identical to one another and the closest match in Genbank was to *Curturteria australis* (FJ396161), but there was only a 79% similarity over 143 bp so sequence identity remains unclear. Although sequences were not obtained from this band from snails from Bugamba, the band was also present in that population and so it was assumed that it was the same species (Figure 5.6).

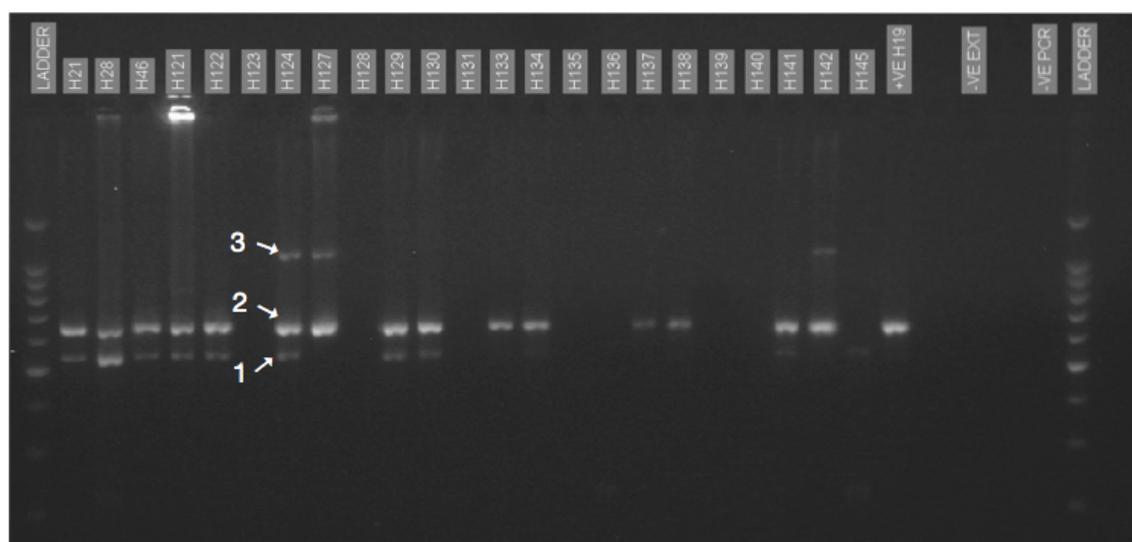


Figure 5.6: Gel electrophoresis of PCR products of snail DNA with multiple bands.

In lane 8, the bands for the species detected are represented by numbers: 1 stands for *S. mansoni* (500bp); 2 for *B. pfeifferi* (600bp) while 3 shows a trematode belonging to genus *Curturteria australis* (1000bp). Labels on top across the gel (left to right) indicate the DNA ladder used, sample codes and PCR positive and negative controls. The last lane on the right indicates a second DNA ladder that was used. The DNA ladders shown, designate bands at 100bp intervals.

5.5 Discussion

5.5.1 Performance of PCR and microscopy techniques in snail screening for schistosomes

Molecular analysis proved to be more sensitive than microscopy in detecting schistosome infections in snails. For instance, the prevalence of infected snails screened through the PCR technique was higher than that obtained through microscopic examination of shedding larvae (Fig. 5.4). Snails that could not shed schistosome larvae, possibly due to inadequate light exposure, were found to be infected using molecular analysis. Detection of pre-patency and patency infections of schistosomiasis in snails has proved to be of major significance in monitoring the disease transmission for control purposes (King *et al.*, 2006). Despite the apparent sensitivity of PCR, sequencing was necessary to resolve the identities of trematodes and intermediate hosts present; other organisms that could not be detected through microscopy were observed through molecular analysis (Table 5.1; Fig. 5.6). Although some species of trematodes had distinctive band sizes that could be used for diagnosis (e.g. the unknown trematode), others produced an amplification product of a similar size as that expected for *S. mansoni*, which could be misleading in prevalence assays based only on PCR. This was also true for different species of snails. Hence, PCR methods can be useful in improving the identification and understanding of trematode infections but it is critical to sequence amplification products to ensure species identity. Unfortunately, there was an unintentional bias in which snails and parasites used for sequencing were collected at different sites; *B. smithi* and the unknown trematode were only found in samples from the wet season, while *S. mansoni* sequences were only confirmed from the dry season and *B. pfeifferi* sequences were also predominantly from the dry season (with one from the wet season). So, additional sequencing would be required to confirm whether there is variation in the prevalence of different host species and different

trematodes by season or by site. This emphasises that, despite their high costs, molecular methods are useful in revealing microscopically undetectable schistosome infections in intermediate hosts such as patent infections in snails.

5.5.2 Implications of snail infection to schistosome transmission

Snails obtained from most of the populations sampled along the shores of Lake Tanganyika in Tanzania in the present study have been identified as *Biomphalaria pfeifferi* based on both morphology and molecular analysis. This confirmation corroborates previous reports that *B. pfeifferi* might occur in the area (Magendantz, 1972; Doumenge *et al.*, 1987). This species has been implicated as the specific intermediate host for schistosome parasites and is therefore necessary for the transmission of schistosomiasis (Southgate and Rollinson, 1987). This observation confirms the results from chapters 2 and that schistosomiasis could be a substantial problem in the Gombe region. However, in Kigoma town, a different species of snail was found (*B. sudanica*) based on sequencing, which was morphologically similar to *B. pfeifferi*. This may not be surprising as the shells of different snail species in genus *Biomphalaria* are difficult to separate based on morphology alone (Brown, 1994). Since sequencing confirmed that a different species of trematode was also found in Kigoma, it is not clear whether *B. sudanica* is able to transmit *S. mansoni*, or whether it is restricted to transmission of the unknown trematode that was found in snails at this site. Additional sequencing of snails and parasites is required to assess this. This emphasises the importance of confirming both the species of potential intermediate hosts and the species of parasite carried when assessing the transmission dynamics of parasites, either using molecular techniques or detailed morphometric analyses.

People living in areas where suitable snail hosts of schistosomes do not exist, cannot get infected with schistosomiasis (Wilkins, 1987). Even if schistosome parasites exist in infected individuals in such areas, the infection will not be transmitted to non-infected people. The presence of cercariae-shedding snails in this area is therefore a key observation that confirms the area as endemic to schistosomiasis. There was considerable variation in infection status of the snails between sites, with those from Gombe for instance showing no signs of infection either through molecular or microscopy-based surveys (Fig. 5.4). There were also no snails in Mtanga village; as discussed in previous chapters, schistosome infections in humans in the village were also low in this area. The presence of snails in Gombe National Park and their absence in Mtanga village signify the focal nature of snail distribution but the lack of shedding observed in Gombe suggests that schistosomes do not infect the snails observed in this area. In Gombe National Park, snails were only found at the Dell area near Kakombe stream, where Müller-Graf *et al* (1997) also reported them in the 1990s. An extensive survey for snails during a pilot study did not reveal the presence of snails in any other streams in Gombe. Given that snails from Gombe were not infected with schistosomes while baboons were, it remains a puzzle as to where exactly the baboons get infected from. That would be the main focus of future studies to establish the transmission foci within the park and also to conduct an extensive and sustained snail search.

5.5.3 Spatial and seasonal variation of schistosome infection in snails

The infection status of snails also varied between seasons, as well as sites (Figs. 5.4 and 5.5). In the former category molecular results showed that snails were more infected in the dry season than the wet season. In contrast, analysis of microscopy-based data showed that snails in the wet season showed a higher prevalence of schistosome infection than those collected in the dry season. It isn't clear why the results differed by method, but it

could be due to the small sample size of snails collected in the wet season. As stated by Woolhouse (1995), snail infection status is likely to vary between seasons and sites and a good understanding of the causes of this variation is essential for schistosomiasis control. Human contacts with water and temperature changes have been reported as the major causes of seasonal and spatial variation in schistosome prevalence in snail populations (Woolhouse, 1995). As also noted by Woolhouse and Chandiwana (1989), variability in temperature and rainfall are therefore likely to result in different transmission patterns both temporally and spatially and can be better described by year-long sampling. These variables were not measured during this study so it is not possible to give a true account of the infection dynamics. The study was also conducted for a short period of time, which was not sufficient for monitoring the development and emergence of schistosome from snails in field conditions. Only general remarks can be made based on geographical characteristics of the current study sites.

Fluctuations in temperatures affect the duration of growth and development of schistosomes in snails (prepatent period) with moderate temperatures causing shorter prepatency than low temperatures (Woolhouse, 1995). Variation in the number of infected snails with seasons suggests that schistosomiasis transmission is low during periods with a low number of infected snails. Shiff *et al.* (1975) and Chandiwana (1987) separately agreed that there is a coincidence between infection of *S. mansoni* in snails and human transmission. Although the region of the ITS sequenced was not variable enough to draw conclusions about sharing of schistosome strains, sequencing of schistosomes from humans and baboons in the Gombe region, confirmed that they carried that same sequence of *S. mansoni* as detected in the snails (Chapter 2). Shiff *et al.* (1975) showed that high cercarial production in infected snails is usually observed during hot and dry periods of the year, which is by coincidence the time when people are feeling hot and sweaty. People would therefore seek water to cool themselves and in the process they can get infected with

schistosomiasis (Chandiwana, 1987). This is also perhaps why in the current study, more snails were detected with schistosomes in the dry season than the wet using molecular analysis techniques (Fig. 5.5).

Snails were absent in many study sites and even in areas where they existed, few of them were shedding schistosome larvae. There is a possible link between the observed patterns of schistosomiasis infection in humans among study sites discussed in chapter two and the distribution and infection status of snails in these areas. As observed by Wilkins (1987), the abundance of infected snails in an area is an important aspect that indicates their ability to influence the transmission and infection outcome of schistosomiasis. Based on molecular analysis results, the most infected snails were obtained from Kiziba village followed by Mwamgongo village (Fig. 5.4). As discussed in chapter two, these villages (particularly Mwamgongo) had the highest prevalence and egg counts of schistosomes. The prevalence and egg counts of schistosomiasis were also lowest at Mtanga village, which also lacked the snails that transmit the disease. Lower levels of schistosomiasis infection were also recorded at Gombe, where the snails collected from the Dell area in the park were not shedding any schistosome larvae. Further analysis using DNA techniques also revealed these snails were not infected with schistosomes (Table 5.1). Schistosome-infected snails were also found in Bugamba village, where the prevalence and egg counts of schistosomiasis were slightly higher than in Gombe and Mtanga. These findings suggest that schistosomiasis transmission is closely related to the presence and infection status of specific intermediate host snails in these areas. The observations are consistent with results from other studies, which have associated the focal distribution of schistosomiasis to localized distribution and infection status of snails (Goll and Wilkins, 1984).

The patchy distribution of snails has been suggested as one of the major determinants of the patchiness of schistosomiasis distribution (Wilkins, 1987). Schistosomiasis infection patterns are widely known to be focal, with considerable variation in the levels of infection over relatively short distances (Scott *et al.*, 1982). It has been found that the greater the abundance of schistosome-infected snails in an area, the greater the probability of humans coming into contact with parasites, and hence the greater the infection rates of schistosomiasis (Southgate and Rollinson, 1987). Although the variation between sites in snail infection observed in the present study can not be properly explained due to lack of sufficient data, such observations have been replicated elsewhere. For instance, significant variation in the prevalence and intensity of schistosomiasis infection between adjacent villages and huts have been reported in areas around Lake Victoria in north western Tanzania (Forsyth and Bradley, 1966) and other endemic areas (Scott *et al.*, 1982). Availability of suitable habitats for snails and the individual characteristics of these habitats and the location of the habitats from human settlements are key determinants of variations in schistosome infection status between sites. However, the variation in the prevalence of snail infection between study sites shown in Table 5.1 is a combination of wet and season data. As discussed above season is a key factor that can influence the shedding of schistosome larvae in snails. Thus the lack of separation between seasons may have influenced the observed patterns and hence obscured the true spatial variation.

5.5.4 Conclusion

The study has confirmed the presence of schistosome-infected snails in areas along the shores of Lake Tanganyika and identified most of them as *Biomphalaria pfeifferi*. Results from the study have also indicated variation in the levels of snail infection between sites and seasons, which suggest similar transmission trends as observed for schistosomiasis among humans in the area. Given their infection status, snails are thus crucial in the transmission of schistosomiasis in this area and any control measures for the disease should include snail control in the programmes. The detection of other snails in samples from the area and the presence of other parasite species in snails detected through molecular analysis indicate the importance of confirming species identities through sequencing when monitoring for control programmes for schistosomiasis and other infections.

Chapter 6: General Discussion, Conclusions and Recommendations

6.1 Major Determinants of Schistosomiasis Transmission in Endemic areas

The disease of schistosomiasis has many facets that make it difficult to define it precisely or explain its existence in a given area. However, many social and development activists, including the World Health Organization, describe schistosomiasis as a disease of the poor. This is because the existence of schistosomiasis and other related infections, such as soil-transmitted helminthiasis, is linked to poverty, poor personal and environmental hygiene, and impoverished health services (WHO, 2002). To some extent, this description is reasonable because schistosomiasis mainly affects people in rural and peri-urban areas of developing countries, where poverty is also rampant (Fenwick, 2011). Coincidentally, the poor areas of developing countries where schistosomiasis is common happen also to be in the tropical or sub-tropical areas of the world. Possibly as a reference to that, scientists and other researchers define schistosomiasis as a tropical infectious disease, whose distribution is limited to the tropical areas of Africa, Middle East, South East Asia, the Caribbean and South America (WHO, 1985; Morgan *et al.*, 2001; Gill and Beeching, 2009). The warm temperature and adequate humidity in tropical areas facilitate the existence of snails that are responsible for transmitting schistosomiasis. These snails cannot thrive well in temperate or high altitude areas, hence endemic schistosomiasis is absent in these zones (Southgate and Rollinson, 1987; Poulin, 2006).

Ecological studies on schistosomiasis transmission have grouped the factors determining its existence and persistence into three major components, namely: (1) characteristics and behaviour of humans and other potential or reservoir hosts; (2) the existence of suitable snails; and (3) a conducive environment for the snails and parasites to survive (Despommier *et al.*, 2005). The life cycle of schistosomiasis described in section 1.3 in chapter one of this thesis can be used as a guide to evaluate the importance of these determinants. It can be seen from the life cycle that human behaviour and other related

characteristics are as important for the existence of schistosomiasis in the environment as the presence of snails and their favourable environment. Schistosomiasis transmission starts when people who are already infected with the disease have to defaecate or urinate in water and pass out the parasite eggs. Perhaps one could ask, why in this 21st century should someone sane defaecate or urinate in a stream that is used by their community as a source of domestic water? This is partly a result of ignorance of the consequences for doing that or possibly due to some elements of irresponsibility for the person doing that. To a large extent, for many people, this habit is influenced by the lack of home or public lavatories.

The lack of toilets in domestic and public places is common in many areas of developing countries. This forces many people in such areas to defaecate or urinate on land surfaces or in streams that may be passing near their residence. That was the case in Mwamgongo village where, during this study, human faeces were scattered along the banks of the river that passes through the village. Many people in the village do not own a toilet and public toilets are scarce (*personal observation*). This also relates directly to poverty because poor people live in unhygienic environments with no established sewage system for disposing of human excreta that may contain schistosome eggs. These people often lack basic education and awareness on the transmission or existence of schistosomiasis in their areas and as a result, they are partly victims of their own actions. If people were to refrain from polluting the water with faeces or urine, schistosomiasis would cease to exist. When schistosome eggs in human stool get in contact with water, they release immature larvae known as miracidia. The larvae then enter snail tissues and after undergoing some development and growth in the snails, the parasites come out as mature larvae (cercariae) that are capable of piercing and penetrating human skin. Without this developmental stage in the snails, the schistosome larvae emerging directly from human stools cannot infect

humans (Ash and Orihel, 2007; Fenwick, 2011). This indicates how important snails are in the transmission of this disease.

Since the parasites leaving the snails will only penetrate the skin of humans coming in contact with water, it also shows how important water is to the transmission process. Water is essential for schistosomiasis transmission because eggs from human stools cannot hatch unless they come in contact with water. The hatched miracidia and cercariae larvae also need water to locate snail and human hosts, respectively. Moreover, snails cannot survive in unsuitable water bodies such as marine water or water with extreme temperature. All of this emphasizes why environment is key for the existence and maintenance of schistosomiasis.

People become infected as they wade into water, which in theory is contaminated with their own or someone else's faeces or urine. If these people were aware that the polluted water (with faeces or urine) is potentially infectious with schistosomiasis, they would not wade into it. So, lack of education and awareness here plays a major role. Sometimes people have no alternative source of water, as it may be the only pond or stream in the village, as was the case in Mwamgongo, where the only stream in the village was the major source of water for all domestic uses. If the people coming into contact with infested water were protected say by protective clothing or other materials that prevent the snails from entering their skins, they would be safe. However, quite often people living in these areas can barely afford walking shoes *let alone* protective gear such as gumboots. They therefore walk into the water with bare feet, as was observed in the present study. They also do so not because they do not have shoes, but because they are working in the water, either washing up clothes or utensil, or bathing or swimming (particularly children).

Once in the human body, the schistosome larvae migrate through various organs and finally settle either in the intestines (intestinal schistosomes) or in the blood vessels of the bladder (urinary schistosomes). One powerful weapon for killing the parasites at this stage is chemotherapy through drugs such as praziquantel. Unfortunately, again due to poverty, most people infected with the parasites cannot afford the price of the drugs at the market price (Fenwick, 2011). The drugs have been subsidised and the prices are supposed to be affordable by most people. However, the long supply chain for the drugs from the manufacturer to the end user living in rural Africa means that the prices are still too high for most people to afford them. That is why most people volunteered in the present study because treatment was being provided by the project as an incentive. Some of the participants revealed that to the research team that they would not have been able to pay for the disease test and treatment in public or private health facilities. Unfortunately, even in areas where praziquantel is available to the majority, the drug does not prevent re-infection with schistosomiasis. So, quite often, in many endemic areas, infection levels disappear after treatment, only to reappear at the same or higher levels than pre-treatment levels a few years later. For instance, in the study area, most people who were found infected with schistosomiasis had previously been treated with praziquantel. Re-infection of schistosomiasis in most endemic areas is aggravated by the lack of public awareness on how the disease is transmitted, as has been reported among communities in Dudicha and Shesha Keke, Ethiopia (Mengistu *et al.*, 2009) and in Bananal (Sao Paulo State) Brazil (Da Silva *et al.*, 2002). Thus, behavioural change in human-water contact habits is potentially capable of reversing schistosomiasis transmission in many endemic areas. This study was fortunate enough to have a Scottish Hollywood actor, Brian Cox, visiting the study area in Tanzania to produce a film on schistosomiasis as a neglected tropical disease. The film covered a range of activities such as the local people's use of village streams, their attitudes and understanding of schistosomiasis, together with aspirations and hope for the future. During the filming a broad range of people were interviewed, from a common

person to senior medical experts in Tanzania. It was clear from the interviews representing views of different backgrounds that the lack of basic education and awareness on schistosomiasis, particularly among the common people on how the disease is transmitted and spread, was the major cause of its continued existence.

Either out of negligence or lack of policies, water-related projects in schistosome endemic countries often do not include the component of schistosomiasis transmission. As a result, water retention for Hydro-electric power (HEP) or irrigation purposes have fuelled schistosomiasis spread (Steinmann *et al.*, 2006; Fenwick, 2011). For instance, Fenwick (2011) reported that, water-related projects such as the construction of the Aswan dam (Egypt), the Blue Nile irrigation scheme (Sudan), and the Volta dam in Ghana are good examples of places where schistosomiasis was introduced in the areas following these human activities. Therefore it is not surprising that schistosomiasis is still expanding and spreading to new areas in some countries after the construction of water dams for HEP, such as in Mtera and Kidatu Tanzania (Rugemalira, 1991) and irrigation in Mashonaland East Province Zimbabwe (Chimbari *et al.*, 2004). While such projects create settlement sites and economic opportunities for people, they also make the areas habitable for snails, thereby encouraging schistosomiasis transmission. As people move in from other areas, they also come with schistosome parasites in their bodies. They then start using the water for various domestic purposes but also polluting it through defaecation and urination. Parasite larvae emerging from the eggs end up in the snails and then into humans again and the life cycle and transmission continues.

It can therefore be concluded that schistosomiasis is a tropical disease although its existence and persistence are facilitated by poverty and other related factors. For instance, schistosomiasis was once a major health problem in countries like Japan and other countries in the Middle East such as Iran but it has been eliminated in these countries as

civilization and economic development increased (WHO, 2002; Fenwick, 2011). Schistosomiasis also has been drastically reduced in middle income or fast growing economies such as Brazil, China and to some extent Egypt (Fenwick, 2011). These trends indicate the relationship between the decreases in schistosome infections with increasing development. So, unless the vicious cycle of poverty and ignorance in poor endemic countries are tackled, schistosomiasis transmission in these countries will remain a scourge to public health for many years to come.

One of the solutions, therefore, would be improved sanitation to prevent the contamination of the water with human waste. However, since this may not be possible in the near future in all areas, controlling snails without harming other biodiversity and the environment would offer some respite. The use of biological agents such as ducks and fish that feed on snails, have shown promising results in a few places but its large scale application may not be feasible (Fenwick, 2011). The use of well-designed water retention projects that incorporate measures to reduce snail survival should be encouraged in schistosome-endemic countries. The improvement in sanitation and the design of snail control strategies should go hand in hand with chemotherapy through large scale distribution of praziquantel to kill the parasites and to reduce suffering while long-term solutions are being tried. Another potential solution would involve providing education and awareness on schistosomiasis transmission so as to keep people away from being infected. However, any success in persuading local people to change their behaviour on water contact in areas with snails and infested water would result in substantial reductions in schistosomiasis transmission. Continued research for the development and use of vaccines against schistosomiasis would be another fruitful area. More importantly, however, is the establishment of economic relief and empowerment to people in endemic countries. Deliberate economic assistance through microfinance loans for poor people to invest in small businesses and agriculture, added to fair market for these products, and would make

a major difference to these efforts. This would help to raise the standard of living, which would in the end help to reduce the transmission of schistosomiasis and other poverty related diseases that blight much of the developing world.

6.2 General Implications of this Study's findings

It is now known that intestinal schistosomiasis in areas along the shores of Lake Tanganyika is caused by *S. mansoni* schistosomes that are transmitted mainly by *B. pfeifferi* snails. The snails were present in all sites, except at Mtanga village. Snails from all sites were infected with *S. mansoni* parasites except those collected from Gombe National Park. Since infected snails were identified during the current survey, snail control programmes would have a major impact on the efforts aimed to control schistosomiasis. As discussed above, successful projects that have attempted to eliminate schistosomiasis through the use of molluscicides to kill snails were implemented in the 1990s in Egypt and Sudan (Fenwick, 2011). Results of these attempts were dramatic and schistosomiasis transmission was significantly reduced in those countries. However, the continued use of chemicals to kill snails in water bodies has been discouraged because these chemicals harm the environment. Perhaps snail control through environmentally friendly methods such the draining of water bodies adjacent to human settlements would discourage snail existence (Fenwick, 2011). Another observation made during the present study was that the infection of schistosomiasis was very focal, with significant variation between study sites and host age groups, which was also related to the presence or absence of snails and their infection status. The levels of infection were high particularly among children; at Bugamba and Mwangongo, for example, infection in school children reached 68% and 80%, respectively. These sites were also some of the places where snails infected with schistosomes were found in the streams. The site with the least number of infected residents was Mtanga, where the schistosome-transmitting snails were also absent. The study concludes therefore that intestinal schistosomiasis in Tanzania is more widespread in the county than earlier thought. The western areas of Tanzania, which include the current study area, were formerly thought to be lightly infected with schistosomiasis or were free of the disease (Brooker *et al.*, 2009).

The study has also demonstrated that both host and environmental variables influence parasite prevalence in humans in the Gombe ecosystem. For instance, the possibility for children getting infected with schistosomiasis was highest at Mwamgongo, while it was lowest in Mtanga and Gombe National Park. Since schistosomiasis infections tend to vary even between adjacent sites, control strategies should therefore be planned and implemented at the local rather than regional or national levels. *S. mansoni* parasites were also detected among baboons in Gombe National Park, which perhaps indicates the ability of these animals to maintain the life cycle of these parasites in nature. The sharing of habitat between non-human primates and the local human population offers favourable conditions for zoonotic transmission of schistosomiasis in the area. Schistosome eggs from baboons that were subjected to molecular analysis confirmed that *S. mansoni* exists in humans and baboons but the gene region sequenced was not variable enough to assess whether they shared the same strain. Microsatellites analyses, which should have had more potential to determine this, were not conclusive due to amplification of multiple bands per individual for most loci. The results were, however, consistent with sharing of parasites between humans and baboons since there were no unique alleles in the baboons that they would indicate that they have their own parasite population that is distinct from that of humans. Therefore, it is possible that baboons at Gombe are infected with human schistosomes although it is still not known whether such parasites originating from baboons are capable of infecting humans.

6.3 Recommendations for Future work

The present study has produced baseline data on the infection of schistosomiasis in western Tanzania, which is useful for the planning and implementation of control measures for the disease. It has also supported previous assumptions that *Biomphalaria pfeifferi* snails transmit intestinal schistosomiasis in this area. The study has also produced an up-to-date situation of schistosomiasis and other helminths in baboons at Gombe National Park that is useful for the management of wildlife and diseases in the park. Nonetheless, there are other interesting research questions that are worthy of being investigated but which this study could not undertake due to resource limitations.

One area requiring immediate attention is the understanding of social and economic factors driving the transmission of schistosomiasis in humans in areas along the shores of Lake Tanganyika. For instance, the lack of public awareness on how schistosomiasis is transmitted seems to fuel the transmission and re-infection of the disease in the area. It is not understood what cultural or social factors force people to keep using the streams, although most of them have been told that these are the source of the schistosomiasis in their area. Also related to that would be an understanding of how the density of the disease is related to housing patterns and distance from the village streams. Currently, the patterns and distance of settlement are different between some villages and the levels of schistosomiasis infection in these villages seem to correspond to this variation in settlement arrangement (see chapter two). In Mwamgongo village for instance, it was noticed during the present study that houses were more concentrated close to the stream than in other villages and it was speculated that this may encourage parasite transmission. Other ecological factors that may influence the infection of schistosomiasis in the area include water quality variables such as flow rate, water hardness, temperature, pH, nitrate, and calcium concentrations. These variables form a major component to the environment in which the parasites and their snail hosts live. They are therefore likely to influence the

distribution and abundance of snails and are thus worth being investigated. Alongside that longitudinal studies on the variation of schistosome infection with season, detailing monthly changes in temperature and rainfall for each site and relating these to schistosome infection dynamics in the area, would help to elucidate the site-specific factors influencing the infection of the parasite. The findings from such studies would also help to indicate the highest risk period for schistosomiasis transmission in the area.

Since *S. mansoni* can infect humans and other host mammals such as rodents, vervets and baboons, these animals may therefore serve as reservoir hosts for human schistosomes. Given that humans and primates and possibly rodents interact regularly in the Gombe area it is important that infections in these potential reservoir hosts are updated and monitored. In addition to humans and baboons, future studies should strive to determine the types of schistosome species in other wild and domestic mammals (e.g. rats, chimpanzees, goats, dogs, cats and domestic fowls) in the area and establish levels of infection of the disease in these hosts. The degree of hybridization of schistosome parasites in this area is also wanting, given the high propensity for schistosome species to hybridize in nature, particularly in their molluscan phases (Steinauer *et al.*, 2008b; Moné *et al.*, 2012).

Another area requiring more data is whether human and non-human primates in the area share the same or different strains of parasites. Although humans and baboons in this area have been diagnosed with similar parasites, it has not been established whether the parasite strains found in humans, baboons and vervet monkeys are the same or different. It is therefore recommended to expand on the present findings by initiating a study to investigate the possibility of parasites crossing over from baboons to humans and vice versa in this shared environment. The study would apply molecular techniques to identify and determine the types and genetic structure of parasite strains present in humans and baboons in the Gombe area.

It is also important to understand that, although the presence of schistosomiasis has been confirmed in both humans and baboons at Gombe, intermediate host snails from the area were not infected with the disease. It has also not been possible to establish transmission points or foci for schistosome infection in baboons in the park. It thus remains a puzzle as to the source of infection of the disease, particularly among the baboons in the middle of the park, as the movement of these animals outside the park is limited or non-existent. However, seasonal variation in infection could be the reason behind this observation. The objective here would be to establish the foci of schistosomiasis transmission in Gombe National Park. Future studies should aim to establish the areas where animals congregate such as water ponds, swamps and streams and how human movements may interact with them. The use of microsatellites would help to resolve whether gene flow occurs among the parasites of these hosts. Molecular techniques have previously been used to implicate bovines as reservoir hosts for human schistosomiasis (*S. japonicum*) in the Philippines (Rudge *et al.*, 2008). The techniques have also ruled out the existence of gene flow of *Ascaris* between humans and pigs in Guatemala in shared environments (Anderson *et al.*, 1993).

It is also not known whether *S. mansoni* strains in allopathic baboon populations are taxonomically related. Since schistosomes have widely been reported among wild baboons in Africa (Miller, 1960; Nelson, 1960; Fenwick, 1969; Else *et al.*, 1982; Erko *et al.*, 2001; Legesse and Erko, 2004), it is important to determine the evolutionary and taxonomic affinities of *S. mansoni* strains from disparate populations of wild baboons. This will help to ascertain the local adaptations of schistosomes in local hosts (sympatric baboons) as opposed to distant (allopatric) hosts. The study would also determine if there are any localized compatibilities between schistosomes and baboons; i.e. adaptive differentiation given the lack of host mobility. Results from the study would help to determine the adaptive strategies of schistosomes in new hosts.

Another particular aspect worthy of being investigated at Gombe would be the epidemiology of *Paragonimus* in baboons at Gombe National Park, Tanzania. Paragonimiasis is mainly a human disease acquired through consumption of raw or partially cooked crustaceans, such as crayfishes and freshwater crabs (Ash and Orihel, 2007). Although it mostly affects humans, the disease has been detected in non-human primates such as the Drill *Mandrillus leucophaeus* in Cameroon (Sachs and Voelker, 1975) and the crab-eating monkeys in Malaysia (Kim, 1978). At Gombe National Park in western Tanzania, baboons are known to feed on crabs (A. Collins, *personal communication*). These baboons are emaciated and have lower reproductive output compared to other baboons in the park that do not feed on crabs. An assessment of the prevalence, intensity, characteristics and consequences of the parasite's infection to individual animals and populations would be necessary. This study would use molecular techniques to confirm the species of *Paragonimus* present in the area and study the ecology of its first and second intermediate hosts (snails and crabs, respectively). Information obtained will help to ascertain whether the parasite is a human strain that might have crossed over into baboons. It could also be a different species or strain of *Paragonimus* that may or may not infect people and this needs to be known. The findings will also be useful in planning the conservation of wildlife at Gombe. If confirmed as *Paragonimus*, this will be the first time this parasite has been reported in wild baboons in areas east of the Congo basin.

Finally, for snails in the study area, an understanding of the ecology of their dispersal patterns in areas along the shores of Lake Tanganyika is wanting. Snails are critical for the life cycle and transmission of schistosomiasis, as they serve as the indispensable intermediate host for schistosomes to complete their life cycles. Further molecular analyses should be conducted to assess whether the same genotypes are shared with humans between neighbouring villages and to investigate the role that snails play in cross-species transmission. Apparently, Lake Tanganyika does not harbour schistosome parasites

but a more detailed survey of snails along the lake's shoreline would help to understand the fate of snails that are flushed off into the lake from adjoining streams during floods.

Appendices

Appendix 1: A model of the World Health Organization Helminthiasis and Schistosomiasis School Survey form (Annex 4) that was used to register demographic information (age, sex, occupation, health status and area of residence) for participants during stool collection in humans. The form model form has been slightly modified with permission from the World Health Organization to reflect the real form that was used.

ANNEX
4 Child form/*Adult form*

SOIL-TRANSMITTED HELMINTHIASIS AND SCHISTOSOMIASIS SCHOOL SURVEY CHILD FORM/<i>Adult form</i> PARASITOLOGICAL/NUTRITIONAL DATA to be completed by the survey team					
<i>Occupation:</i> Personal data		<i>Study Year:</i>		Date ___/___/___	
ID Number _____	School (or village) _____		Sex M <input type="checkbox"/> F <input type="checkbox"/>		
Name _____	Age _____ years _____				
Nutritional data		<i>Health status:</i>			
Weight _____ kg	Height _____ cm	Hb _____ g/dl			
Anaemia (Hb < 11 g/dl) Yes <input type="checkbox"/> No <input type="checkbox"/>		Severe anaemia (Hb < 7 g/dl) Yes <input type="checkbox"/> No <input type="checkbox"/>			
<i>Macroscopical examination (stool):</i> Parasitological data					
(a) Stool examination	eggs/slide	eggs/gram (epg)	Moderate/heavy-intensity threshold	Moderate/heavy-intensity infection	
				Yes	No
<i>Ascaris lumbricoides</i>			≥5000 epg		
<i>Trichuris trichiura</i>			≥1000 epg		
Hookworms			≥2000 epg		
<i>Schistosoma mansoni</i>			≥100 epg		
<i>S. japonicum</i>			See note		
Other parasites identified					
(b) Urine <i>Stool</i> visual examination				Present	
				Yes	No
Visible haematuria					
Microhaematuria (using reagent strips)					
(c) Urine, examination by microscope	eggs/10 ml of urine	Heavy-intensity threshold	Heavy-intensity infection		
			Yes	No	
<i>Schistosoma haematobium</i> (filtration)			≥50 eggs/10 ml		

Note: For *S. japonicum*, any intensity of infection is considered to be heavy.

Appendix 2: Preliminary microsatellite analyses on schistosome eggs sampled from human and baboon faecal samples

Methods:

Sampling and DNA extractions:

Faecal samples were collected from humans and baboons, and DNA extracted (in Dar Es Salaam) as described in Chapter 2. DNA was extracted from snails (in Glasgow) as described in chapter 2. Based on reliable amplification the ITS loci sequenced in chapters 2 and 5, 41 humans and 9 baboons (Table 1) were selected for microsatellite genotyping.

Microsatellite genotyping:

Eight microsatellite loci previously used by Steinauer *et al.* (2008a); AF325697, A1067617, M5305, L46951, M85304, L25065, BH795456 were screened (Table 2). The forward primer of each pair was labelled with Eurofins MWG Operon fluorescent dyes 6-FAM (blue), HEX (green) and ATTO550 (yellow). Products were amplified by multiplex polymerase chain reaction (PCR), using the default reagent concentration recommended by the kit instruction manual (QIAGEN Multiplex PCR Kit, QIAGEN Ltd). Thermocycling was performed on a PTC- 0220 DNA Engine Dyad (MJ research) machine using the following programme 95°C for 15min followed by 12 cycles of 94°C for 30s, 60°C (drop by 1°C per cycle), 72°C for 60s, followed by 33 cycles of 89°C for 30s, 55°C for 90s, 72°C for 60s and a final 60°C for 30min. Multiplex products (1:160) were genotyped using an ABI 3730 sequencer (by The Sequencing Service, University of Dundee). Genotypes were analysed using GeneMapper 4.0 (Applied Biosystems) and corrected manually.

Table 1: Sample names of individuals used for microsatellite genotyping.

Baboons	Humans	Snails
AMARA	HB41CHWA	H27
AKITA	HB17ADWA	H29
ARANCIN	HB2CHWA	H45
ALTODI	HB18CHWA	H900
SMOKY	HB36CHWA	H909
ADIVIL	HB3CHWA	H905
AKARURA	HB16CHWA	H906
BURIMA	HB4CHWA	H910
ARACEA	HB34CHWA	H911
	HB32CHWA	H912
	HB49CHWA	H1801
	HB28ADWA	H1802
	HB35CHWA	H1809
	HB47CHWA	H1810
	HB14CHWA	H1812
	HB33CHWA	H1814
	HB11CHWA	H1817
	HB39CHWA	H1825
	HB17CHWA	H130
	HB21CHWA	H21
	HB30CHWA	H28
	HB79WC	H46
	HB49WB4	H22
	HB82WC	H23
	HB71WC	H24
	HB74WC	H25
	HB72WC	H26
	HB47WB	H901
	HB66WB	H908
	HB24WB	H917
	HB33WB	H918
	HB30WB4	H919
	HB46WB	H920
	HB53WB	H921
	HB80WB	H922
	HB81WB	H1805
	HB51WB1	H1807
	HB43WB4	H1811
	HB35WB1	H1815
	HB34WB4	H1819
	HB52WB3	H1821
		H1824
		H13
		H14
		H15

Table 2: Microsatellite loci used to screen schistosome eggs, indicating the locus name, primer sequence and the fluorescent dye used to label the primer, the allele size range, the total number of alleles found (N alleles), and the maximum (max) number of alleles found per individual.

Locus	Primer Sequence	Dye	Allele Size range	N alleles	Max
AF325697 ¹	CCCACCACAATTTATTGA TCTC	6-Fam	121-187	17	8
	GGGTCCTCCATTCCACTG				
A1067617	GAAGGTCATTATATTCGT C	6-Fam	212-231	7	3
	GTTGAAATCTATACAG				
M85305 ²	TTCTACAATAATACCATC AAC	6-Fam	263-306	11	7
	TTTTTTCTCACTCATATAC AC				
L46951	CAAACATATACATTGAAT ACAG	Hex	156-220	22	14
	TGAATTGATGAATGATTG AAG				
M85304 ³	CATTTCCCATCTTCAAC CTAAAGCTGGGCACC	Hex	260-302	14	6
L25065 ⁴	GAATTACTGTCCCTTTATC TC	Hex			
	AAACTATTCATTACTGTC GGG				
BH795456	AATCACCAATGGCAACAA TCTG	ATTO550	146-194	17	14
	CGTCATCACCTTAAACAT GAAC				
AF325694	CACCCATTGTCTTAAAAC C	ATTO550	213-234	12	7
	GATGTCACACCCTC				

¹ this locus only worked for 1 baboon, 20 snails and 5 humans

² this locus only amplified for 7 snails and 9 humans

³ this locus only amplified for 1 baboon, 7 snails and 11 humans

⁴ this locus did not amplify reliably for any taxonomic group and so was excluded

Data Analysis

Because more than two fragment sizes were found per individual for most loci (Table 2), it was assumed that more than one type of organism was amplified from the faecal samples. This meant that it was not possible to perform standard population genetics analyses or statistics to determine whether baboons, humans and snails shared the same schistosome genotypes. Instead, analyses were based on presence or absence of particular size fragments for each locus and the number of alleles shared and number of alleles and unique to each species were counted. The data therefore only provide a very crude assessment of whether baboons host their own parasites: 1) if unique bands were found in baboons compared to humans, this would have provided some evidence that baboons host their own genotypes; 2) if baboons shared a subset of the bands in humans (which were more widely sampled), this would have been consistent with sharing of parasites between humans and baboons but it could also just mean that the microsatellites used were not variable enough to demonstrate distinctive differences among genotypes.

Results and Discussion:

Only four loci amplified reliably across a majority of individuals sampled and one locus consistently failed to amplify (Table 2). However, all loci showed more than 2 clear microsatellite peaks in some individuals, with up to 14 fragments sizes apparent in some individuals and there was a large size range of amplified products amplified by single sets of primers. This could suggest nonspecific amplification, contamination of samples, or the presence of multiple species that are recognised by the same microsatellite primers. Reliability of amplification was particularly poor among baboons, so it was not possible to interpret results based on microscopy and ITS screening (using sequences from the ETTS17 primer) in relation to microsatellites (Table 3). The maximum number of loci that amplified for individual baboons was four but different sets of loci amplified for different individuals and many individuals showed no amplification or amplification of only a single locus. This could suggest poor quality of the DNA samples from baboons. Data are therefore not very reliable or informative but are included here for reference on the method used and the degree of variation seen.

However, some general patterns are notable (Table 4). Amplification tended to most reliable in snails and there tended to more size fragments found among the snails than humans. The majority of alleles found in baboons were shared with both humans and snails, although some were shared only with humans and only a single allele was shared with snails but not humans. In no cases were unique alleles found in baboons, whereas an equal number of alleles were unique to either snails or humans. The largest number of alleles was shared between humans and snails, but they also had the largest sample sizes.

Together, these results are consistent with sharing of parasites among baboons and humans, with snails acting as the intermediary. However, the multiple amplification

products, poor amplification in baboons, and variable amplification across loci does not allow this possibility to be distinguished from insufficient resolution of the microsatellite loci used.

Table 3: Baboon samples used for the microsatellite analysis, showing the number of loci that amplified for each individual (No loci amplified), the Troop from which the baboons were sampled, which trematode parasites were observed through microscopy, and what the results of the ITS sequencing showed (ETTS17 screening).

Baboons	N loci amplified	Troop	Parasites observed through microscopy	ETTS17 screening
Amara	0	BA	Schistosoma mansoni ova	PCR but failed sequence
Adivil	0	DB	Paragonimus sp. ova	mixture sequence
Akarura	1	BA	Paragonimus sp. ova	mixture sequence, no match in BLAST
Akita	4	BA	Schistosoma mansoni ova	sequenced; 100% match to S. mansoni
Altodi	1	DA	Schistosoma mansoni ova	PCR but too weak to sequence
Aracea	1	BB	Schistosoma mansoni ova	not screened
Arancin	4	DA	Schistosoma mansoni ova	sequenced; 100% match to S. mansoni
Smoky	3	DA	Schistosoma mansoni ova	not screened
Burima	0	DB	Paragonimus sp. ova	not screened

Table 4: Comparison of fragments (alleles) amplified across 7 microsatellite loci in baboon, snail and human samples, indicating the number of individuals for which reliable amplification was obtained (N), the total number of alleles found at a particular locus for each species (N Alleles), the number of alleles shared between various combinations of species (N Alleles Shared), and the number of alleles that were unique to a single species (N Alleles Unique).

Locus	N			N Alleles			N Alleles Shared			N Alleles Unique		
	Baboon	Snail	Human	Baboon	Snail	Human	Baboon Snail, Human	Snail, Human	Snail Baboon	Baboon	Snail	Human
A106761 7	1	19	5	1	7	4	1	3	0	0	3	0
AF32569 4	2	30	25	6	9	9	6	0	0	0	3	3
AF32569 7	3	34	30	3	17	15	2	13	1	0	1	0
BH79545 6	4	38	25	9	16	14	9	4	0	0	3	1
L46951	3	27	30	8	18	22	8	10	0	0	0	4
M85304	1	7	11	1	11	10	1	6	0	0	4	3
M85305	0	7	9	0	6	9	0	4	0	0	2	5
Total	9	45	41	28	84	83	27	40	1	0	16	16

Appendix 3: Types of parasites reported from vervet monkeys at Gombe National Park.



A

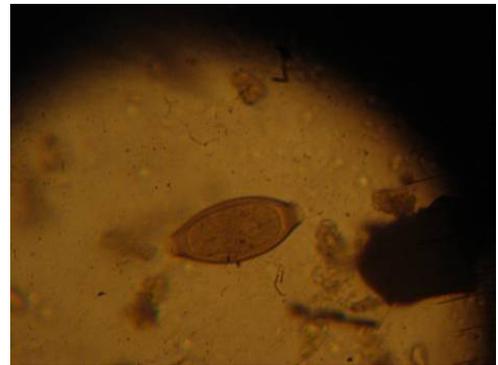


B

Physaloptera spp. eggs, A - B (X400)

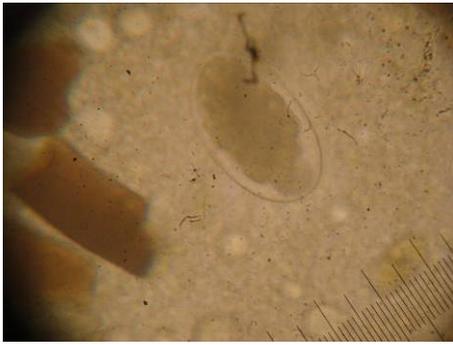


C

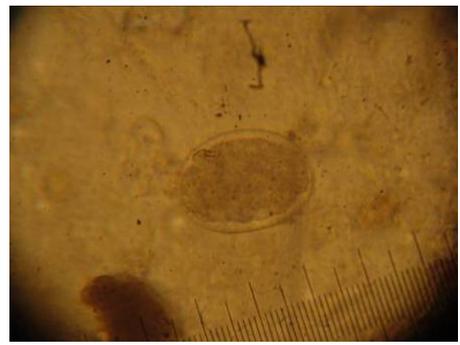


D

Trichuris spp. eggs, C-D (X400)



E



F

Hookworms eggs, E-F (X400)



G



H



I



J

Unidentified nematode eggs, G-H and larvae I-J (X400). It is possible that the unidentified egg in G above could be *Strongyloides* sp. due to its characteristic shell surrounding an 8-shaped larva.

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