EPIDEMIOLOGICAL STUDY OF MULTIPLE PARASITIC INFECTIONS AMONG FIVE RURAL COMMUNITIES IN KANO STATE, NIGERIA

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FACULTY OF MEDICINE
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ABSTRACT

Multiple parasitic infections or polyparasitism is the concurrent presence of different parasitic species in a single host. Humans are often infected with more than one species of parasite, especially in developing countries, where parasitism is endemic and widely distributed. This study aimed to investigate the epidemiology of polyparasitism based on a single collection of faecal, urine and blood samples among five rural communities in Kano state, Northern Nigeria. Of the 551 participants, prevalence of parasitism was 84% comprising of single (39.1%) and multiple (60.9%) infections. A total of 15 parasitic species were identified, among which *Plasmodium* (60%), *Blastocystis* sp. (29.2%), *Entamoeba* (16.3%) and hookworms (15.4%) were most prevalent. Concurrently, up to eight parasitic species / genus were detected in a single host. Factors such as an infected family member and not wearing shoes outside home were associated with the increased risk of having polyparasitism. *Plasmodium falciparum* was detected as the highest prevalent and its risk factors were associated with younger age group, lower family monthly income and not using bed net. Despite more than 70% knew about malaria infection, it's cause, symptoms and own bed net however majority do not use the bed nets. As for schistosomiasis, risk of infection relates to younger age group, male, farming as occupation, presence of infected family member and previous history of infection. Respondents were aware of schistosomiasis (74%) and recognised that polluted water body is the source of infection and yet 50.9% still had contact with the stagnant water mostly for domestic purposes (68.1%). As for *Blastocystis* species four subtypes were recovered [ST1 (39.2%), ST3 (33.3%), ST4 (13.7%) and ST2 (7.8%)] and is the first report from Northern Nigerian communities.

ABSTRAK

Jangkitan pelbagaian parasit atau poliparasit adalah dirujuk kepada kehadhiran beberapa spesies parasit yang berbeza didalam satu perumah. Manusia sering dijangkiti lebih dari satu spesies parasit terutamanya dinegara membangun dimana jangkitan parasit adalah endemik dan tersebar luas. Kajian ini bertujuan untuk mengkaji epidemiologi jangkitan poliparasit berdasarkan kutipan tunggal sampel tinja, air kencing dan darah dari lima komuniti pendalaman di negeri Kano, Nigeria. Dari 551 sampel, prevalens keseluruhan jangkitan parasit ialah 84% meliputi jangkitan tunggal (39.1%) dan jangkitan berbilang (60.9%). Sejumlah 15 spesies parasit dikesan, dimana *Plasmodium* (60%), *Blastocystis* sp. (29.2%), *Entamoeba* (16.3%) dan cacing kait (15.4%) adalah yang paling prevalen. Jangkitan poliparasit sehingga lapan jenis parasit dikesan dalam satu perumah. Peningkatan poliparasit usus berhubung rapat dengan adanya ahli keluarga yang terjangkit dan tidak memakai kasut diluar rumah. *Plasmodium falciparum* telah didapati mempunyai prevalen yang paling tinggi dan faktor risiko nya berkait rapat dengan golongan muda, pendapatan bulanan keluarga yang rendah dan tidak menggunakan kelambu. Walaupun lebih daripada 70% responden mempunyai pengetahuan mengenai jangkitan malaria, sebab, tanda dan simptom dan mempunyai kelambu tetapi kebanyakannya tidak menggunakan kelambu di rumah. Disamping itu jangkitan *Shistosoma* species pula berkait-rapat dengan golongan muda, lelaki, berkerja sebagai peladang, terdapat ahli keluarga yang terjangkit dan mempunyai sejarah jangkitan dimasa lampau. Responden sedar tentang skistomiasis (74%) dan tahu bahawa pencemaran air adalah punca jangkitan tetapi 50.9% daripada mereka masih menyentuh air tenang yang tidak mengalir untuk tujuan domestik (68.1%). Untuk *Blastocystis* spesies, empat subtip [ST1 (39.2%), ST3 (33.3%), ST4 (13.7%) dan ST2 (7.8%)] telah dikesan dan ini adalah laporan pertama dari komuniti di utara Nigeria.

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LIST OF SYMBOLS AND ABBREVIATIONS

X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside
AIDS	Acquired immune deficiency syndrome
bp	Base pair
CI	Confidence interval
°C	Degree Celcius
DNA	Deoxyribonucleic acid
DALY	Disability-adjusted life year
EDTA	Ethylenediaminetetraacetic acid
EPG	Egg per gram
FCT	Federal capital territory
g	Gram
HIV	Human immune deficiency virus
IPI	Intestinal parasitic infection
IPTG	Isopropylthiogalactoside
IRS	Insecticide residual spray
ITN	Insecticide treated net
KAP	Knowledge, attitude and practice
LAC	Latin American countries
LB	Liquid broth
LGA	Local government area
LLIN	Long lasting insecticide nets
$MgCl_2$	Magnesium chloride
μL	Microlitre
mL	Millilitre

NJ	Neighbor-joining
NNPC	Nigerian national population commission
NGN	Nigerian naira
NTD	Neglected tropical disease
BLASTn	Nucleotide Basic local alignment search tool
OR	Odd ratio
%	Percentage
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PHC	Primary health care
qPCR	Real-time PCR assay
RBM	Roll back malaria
RDT	Rapid diagnostic test
rRNA	Ribosomal ribonucleic acid
sp.	Species
SPSS	Statistical package for social sciences
ST	Subtype
STH	Soil transmitted helminth
TAE	Tris-Acetate EDTA
TB	Tuberculosis
UNICEF	United Nations children's emergency fund
UV	Ultraviolet
US$	US Dollar
WHO	World health organisation
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside

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CHAPTER 1: INTRODUCTION

1.1 Background

Multiple parasitic infections or polyparasitism is regarded as the concurrent presence of different parasitic species in a single host (Steinmann *et al.,* 2008a). Polyparasitism is always associated with high prevalence and overlapping endemicity of parasitic infections in selected communities, ubiquitous worldwide implying public health significance for many decades (Stoll, 1947; Bhattacharya *et al*., 2010). Polyparasitism is usually ignored in epidemiological surveys, and the findings are presented only on per-species basis (Cox, 2001). However, recent studies have highlighted the public health importance of multiple parasitic infections. As such clinical entities in developing countries demonstrated that even low-grade infections with multiple helminths results in significant morbidity and increased nutritional and organ pathology (Pullan & Brooker, 2008; Ezeamama *et al*., 2008; 2005a). Polyparasitism may also increase susceptibility to other infections by driving the immune response towards non-protective, non-cytophilic antibody formation (Elias *et al*., 2007; Druilhe *et al*., 2005). It was reported to be associated with chronic infections especially in children who suffer deficits in physical growth, physical fitness, cognition, school attendance & performance and micronutrient status (such as iron and vitamin A) (Papier *et al*., 2014; Terer *et al*., 2013; Ahmed *et al*., 2012; Bustinduy *et al*., 2011; King *et al*., 2006). Moreover, higher risk of maternal and neonatal mortality was reported in pregnant women (Christian *et al*., 2004).

In Africa, parasitic infections were commonly reported with high prevalence across the regions (Florey, 2009; Onabamiro, 1957; Ahmed & Uraka, 2011; Humphries *et al*., 2013; Damen *et al*., 2011; Mazigo *et al.*, 2010; Midzi *et al*., 2008; Bogoch *et al*., 2006; Anosike *et al.*, 2004; Tchuem Tchuente *et al.*, 2003; Gundiri *et al*., 2001). In Ghana, a cross sectional study documented 37% of Ghanaian children were infected with

intestinal parasites especially hookworms and the infections or re-infection were associated with poor nutritional status (Humphries *et al*., 2013). Beside, schistosomiasis was also widespread and heterogeneous (Magalhães *et al*., 2011). In Tanzania, parasitic infections were endemic with 54.5% prevalence and 29% had more than one parasitic infection (Mazigo *et al*., 2010). In Cameroon, 98% of febrile children were infected with malaria and 11.9% were coinfected with intestinal parasitic infections (IPIs) especially protozoa (Njunda *et al*., 2015). In East Africa (Lake Victoria), approximately 90% of the study subjects were infected with intestinal parasites (Barda *et al*., 2014). In Kenya, urinary schistosomiasis (26%) and hookworm (21.4%) infections were commonly found in both single and multiple infections (Bisanzio *et al*., 2014). In Zimbabwe, soil transmitted helminth (STH) infections were reported overlapping with schistosomiasis with prevalence of 5.5% and 22.7%, respectively (Midzi *et al*., 2014).

Neglected tropical diseases (NTD) occur wherever there is poverty including Asia, Latin America and the arctic regions (Hotez *et al*., 2014). Asian countries, particularly Japan and Korea reported significant decline in prevalence and intensity of parasitic infections especially STH infections (Hong *et al*., 2006; Hara *et al*., 2001) although, parasitic infections are still a serious public health importance in disadvantaged communities of Asia (Ahmed *et al*., 2011). In Philippines, more than half the population were infected with intestinal helminths (WHO, 2008a). A national survey in Myanmar found 57.5%, 48.5% and 6.5% were infected with trichuriasis, ascariasis and hookworms, respectively (Montresor *et al*., 2004). In Thailand, parasitic infections were found up to 60.0% and 70.8% of schoolchildren and community members, respectively (Waikagul *et al*., 2008). In Malaysia, more than 90% of participating schoolchildren had parasitic infections with trichuriasis being most prevalent (Al Delaimy *et al*., 2014; Ahmed *et al*., 2011).

In Latin American countries (LAC), more than 200 million people were infected with parasitic infections (Hotez *et al*., 2008). The predominant infections were trypanosomiasis followed by intestinal parasitic infections (Hotez *et al*., 2014; Murray *et al*., 2013). In Mexico, more than 50% of school children were infected with intestinal helminths (Quihui-Cota *et al*., 2004). In Brazil, the study subjects were infected with hookworms (71%), *Schistosoma* spp. (50%) and 41% were infected with a mixture of these helminths (Pullan *et al*., 2008). In Peru, 94% of children were infected with parasite species and *Ascaris lumbricoides* was the most dominant (Gyorkos *et al*., 2011). Furthermore, in the arctic regions, most of the reported parasitic infections were zoonotic foodborne such as trichinellosis (caused by the unique species *Trichinella native*) (Hotez, 2010), diphyllobothriasis (Scholz *et al*., 2009), echinococcosis (Rausch, 2003), toxoplasmosis (Messier *et al*., 2009) and giardiasis (Hotez, 2010).

Nigeria is reported to have the highest prevalence of many parasitic infections including malaria, schistosomiasis, hookworm infections, ascariasis, trichuriasis, onchocerciasis and lymphatic filariasis (WHO, 2015; Hotez & Kamath 2009). A survey across five states in South Eastern Nigeria showed high (42.4%) prevalence of parasitic infections (Gundiri *et al*., 2001). The existence of malaria, mansonelliasis, loasis and trypanosomiasis were probably due to a consequence of the peculiar environment that favours breeding of the respective arthropod vectors. Subsequently, up to 52% of the study subjects had intestinal parasitic infections in Sokoto state northwestern Nigeria (Muhammed *et al*., 2015). The Nigerian children were the most vulnerable group to parasitic infections due to continuous exposure with contaminated environment (Amuta *et al*., 2009). However, intestinal parasitic infections among schoolchildren was reported to have decreased in 2015, which paralleled the government efforts to reduce infant mortality and improve environmental sanitation (Abah & Arene, 2015).

Several factors such as low level of education, occupations like farming, fishing or launderette (washing clothes) pose a higher risk of infection. The climatic condition (Ekundayo *et al*., 2007), low socioeconomic status, lack of safe water supply, lack of environmental/personal hygiene, improper sewage disposal, non-functional drainage system and abundance of arthropod vectors (Obiukwu *et al*., 2008) were considered as contributing factors to the endemicity of parasitic infections. Furthermore, the absence of extensive deworming programmes, periodic drug distribution, and increase development of urban slums due to over growth of Nigerian cities aggravate the situation (Barda *et al*., 2014).

However, the presence of concurrent multiple parasitic infections were subjective to human perception, attitude and social behaviours/practices (Ekwunife *et al*., 2004). Epidemiological studies were imperatively used to explore the people's knowledge, attitude and practices regarding parasites' transmission, prevention, treatment impact and control (Lu *et al*., 1988). Factors such as ignorance, lack of awareness about parasites' transmission mode entails the promotion of infections and diseases. For instance, in a survey by Van Herck *et al.* (2004) about 83% of travellers (from Europe to developing countries) did not sought for any health advice before travelling thus consequently increasing the potential of importing infectious diseases. Better understanding of social, cultural, behavioural and community awareness affects the epidemiology and control of parasitic infections consequently aid in designing effective control strategies of the diseases (Nyantekyi *et al.*, 2010). Besides, successful control programmes should include community participation and be viewed as a mutual learning process where obstacles are identified, discussed and solutions shared among community members and project staff.

1.2 Statement of Research Problem

Parasitic infections are the most prevalent among the poorest of rural communities in developing countries (Drake & Bundy, 2001). In spite of the high population in Nigeria approximately 182,000,000 [Nigerian National Population Commission (NNPC), 2017], the bulk live below the poverty line and highly exposed to many parasitic infections, yet multiple parasitic infections were seldom reported. Majority of previous studies reported on per species prevalence from the various communities in different regions (Anosike *et al.*, 2004). Focusing mainly on specific groups such as school-aged children, women of childbearing age, HIV infected, and mostly hospital-based which do not reflect the general population. Many of the debilitating diseases in Nigeria were directly or indirectly attributed to parasitic infections (Ugbomoiko *et al*., 2012). Nigerian children from low socioeconomic families have been found to be anaemic, stunted with retarded growth and underweight due to malnutrition (Onadeko & Ladipo, 1989; Ogbuagu *et al*., 2010). Few studies reported significant association of these deficits with parasitic infections but did not consider multiple parasitic infections. Furthermore, control of these parasitic infections is trifling, not only due to absences of infrastructure but also due to ignorance and some cultural beliefs. Information about parasitic infections and the possibility of controlling and preventing these infections amongst Nigerians has not received the justified consideration, although the negative impact of parasitic infections on individuals' health and nutritional status (especially in children) were well documented worldwide.

Nigeria is the world's most endemic country for malaria where it was found to have varying intensity throughout the nation but there is scarcity of information from Kano State. Likewise, several studies on schistosomiasis were conducted in several different states on schoolchildren and communities but again lacking enough data from Kano State. Furthermore, data on knowledge, attitudes and practices (KAP) toward malaria as

well as schistosomiasis in Kano has not been documented. There were few studies on intestinal protozoan infections reported from Nigeria and in general no molecular studies were reported.

1.3 Objectives of the Study

1.3.1 General Objective

To investigate the epidemiology of multiple parasitic infections in five rural communities in Kano State and to investigate the molecular characterization of the most prevalent intestinal protozoa.

1.3.2 Specific Objectives

1. To determine the prevalence and distribution of multiple parasitic infections in five communities in Kano State.
2. To identify the significant risk factors associated with multiple parasitic infections among the study population.
3. To investigate the current prevalence of malaria and schistosomiasis (the most important public health problems in Nigeria) and the risk factors associated with these infections.
4. To evaluate the knowledge, attitudes and practices (KAP) of the study population towards malaria and schistosomiasis.
5. To identify the genotypes of the highest prevalent intestinal protozoan by DNA sequencing.

1.4 Hypothesis of the study

1. Multiple parasitic infections are prevalent and associated with some characteristics namely demographic, socioeconomic and environmental factors among the five communities.
2. The prevalences of malaria and schistosomiasis are high in the study population.
3. The level of knowledge about parasitic infections is low among the participants.
4. The prevalence of intestinal parasites infections could be high among the participants.

CHAPTER 2: LITERATURE REVIEW

2.1 Prevalence of multiple parasitic infections

Multiple parasitic infections or polyparasitism is omnipresent but routinely ignored in most epidemiological surveys (Petney & Andrews, 1998; Marti & Koella, 1993). It has been reported many decades ago but only recently gaining interest especially on the coinfection among the major parasitic genera (Cox, 2001; Griffiths *et al*., 2011). The intestinal multiparasitism was documented in greater detail as compared to general multiparasitism (Steinmann *et al*., 2008b). This may be due to many parasitic genera present in the gut and easy to examine in faecal sample. Most of the parasitic coinfection studies were usually focused on the intestinal parasitic infections with plasmodiasis or schistosomiasis but fewer with filariasis. However, several coinfections were unable to be identified, such as mix infection of *Plasmodium* and *Babesia* in malaria endemic areas, due to less detection, misdiagnosis or mistaken as malaria resistance because of similarity in their morphology (Zhou *et al*., 2013).

Polyparasitism has been documented worldwide especially in Africa, Asia and the America regions (Steinmann *et al*., 2008a, 2008b; Ezeamama *et al*. 2005a; Raso *et al*. 2004). In Nigeria, Ugbomoiko *et al*. (2012) had stressed that multiple infections were far more dominant than single parasitic infection in which 23.9% of schoolchildren had multiple intestinal helminths infections including schistosomiasis. Up to 22% of pregnant women from Gabon harboured at least two or more parasites genera (Adegnika *et al*., 2010). It was noted that 29% of the subjects from Tanzania harboured more than one parasite species concomitantly (Mboera *et al*., 2011; Mazigo *et al*., 2010). Prevalence of intestinal protozoans was noted up to 20.2% among patients receiving care in Democratic Republic of Sa˜o Tome´ and Pr´ıncipe (Lobo *et al*., 2014). However, lower prevalence of polyparasitism was reported in Kenya where 9.5% and 1.7% of the

participants had two and three parasitic infections, respectively (Njenga *et al*., 2011). In fact, a large number of African populations were having polyparasitism of at least two parasites genera, simultaneously in the past few decades (Ijagbone & Olagunju, 2006; Nmorsi *et al*., 2009; Fleming *et al*., 2006; Booth *et al*., 1998; Chunge *et al*., 1995; Ashford *et al*., 1992; Buck *et al*., 1978). Polyparasitism was also reported from Latin America such as in Argentina, and the prevalence of intestinal parasites was up to 82.6% (Borda *et al*., 1996), though not specifying the status of multiple infections. In Italy, 48% of the patients were detected with multiple infections with a variety of intestinal parasites species (Belli *et al*., 2014). In China (Poyang Lake region, Jiangxi Province), 27.8% of polyparasitism was found among those above 5-year-old in the communities (Ellis *et al*., 2007). In Laotian children, the prevalence of multiple helminth infections was found up to 40.4% (Sayasone *et al*., 2015). In Orang Asli community of Malaysia, polyparasitism was 62.7% (Al-Delaimy *et al*., 2014), and approximately half of HIV infected (50.4%) patients in Malaysia were being polyparasitised (Asma *et al*., 2011).

Burden of polyparasitism and the potential risk of inter-reactions between coexisting parasites are relatively new research areas that has been lingering. Some studies have highlighted the protective effect of helminth infections associated with decreasing the incidence of malaria attacks (Lemaitre *et al*., 2014; Boel *et al*., 2010; Nacher *et al*., 2000). In contrast, infection with hookworms or *Wuchereria bancrofti* was noted to be associated with higher prevalence of *Plasmodium falciparum* gametocytes in humans (Mboera *et al*., 2011). The documented impact of polyparasitism is stated in Table 2.1, where many unknown and contradictory factors still persist. There is need for more data to clarify the situation.

Table 2.1: Studies on implications of multiple parasitic infections

Authors (year)	Parasitic investigated	Study subjects	Outcome of the study
Nacher *et al*. (2000)	Ascariasis & malaria	Refferal cases at Mahidol University hospital, Bangkok, Thailand	A protective association between *A. lumbricoides* and cerebral malaria; thus parasite coinfections can modulate hosts' immune responses and clinical presentation.
Diallo *et al*. (2004)	*Plasmodium falciparum* & *Schistosoma haematobium*	Children and adults in Saint Louis, Senegal	In children co-infection can increase malaria morbidity but in adults can help to control malaria morbidity
Le Hesran *et al*. (2004)	Ascariasis & malaria	Children in rural Senegal	Associates occurrence of severe malaria and *A. lumbricoides* infection
Ezeamama *et al*. (2005b)	Helminth & malaria	Filipino school children	Increase in risk of anaemia even with low-intensity multiple helminth infections
Brooker *et al*. (2007)	Helminth & malaria	Review on Africa	Co-infection of *P. falciparum* and hookworms has an additive impact on anaemia
Adegnika *et al*. (2010)	Worms & malaria	Pregnant women	*A. lumbricoides* associates with *P. falciparum* infection
Boel *et al*. (2010)	Helminth & malaria	Pregnant Women on the Thai-Burmese Border	Ascariasis decreases risk of malaria & hookworms infection increases risk of malaria & anaemia
Sangweme *et al*. (2010)	Schistosomiasis & malaria	Children 6-17 years old in Zimbabwe	Helminths infection increases susceptibility to malaria
Courtin *et a*l. (2011)	Ascariasis & malaria	General population	A trematode infection increases risk of plasmodial infection or disease
Nacher (2011)	Worms & malaria	Review	Worms can increase or decrease malaria. Thus there should be proper monitoring when de-worming patients to avoid possible increase in malaria/severe malaria incidences
Adegnika & Kremsner (2012)	Helminth & malaria	Review	Ascariasis protects against severe malaria; Hookworms infection increases malaria incidence.
Lyke *et al*., (2012)	Schistosomiasis & malaria	Malian children	Schistosomiasis seems to protects against acquisition of malaria through T-cell regulation
Lemaitre *et al*. (2014)	*Plasmodium falciparum* & *Schistosoma haematobium*	Children in study cohort for malaria, Niakhar, Senegal,	Low intensity urinary schistosomiasis induces protective anti-malarial immune response; high intensity infection promotes the occurrence of malaria attacks.

2.2 Burden, Morbidity and Mortality due to parasitic infections

Parasitic infections have been associated (directly or indirectly) with many chronic diseases such as ability to impair childhood growth, intellectual development and education, causing organ pathology, low birth weight, decreases worker productivity, enhance poverty, as it was disfiguring and stigmatizing (Durrheim *et al*., 2004). The consequences of parasitic diseases burden are enormous as such five major neglected tropical diseases (NTD) (schistosomiasis, hookworm infections, ascariasis, leishmaniasis and trypanosomiasis) caused approximately 500,000 deaths and 57 million disability-adjusted life years (DALYs) lost annually (Hotez *et al*., 2006). The global mortality due to parasitic diseases was noted up to 16.7% in males and 15.6% in females (Hotez, 2008). Moreover, NTD was ranked the fourth most important group of communicable diseases worldwide (Jamison *et al*., 2006), in terms of mortality and years of life lost due to premature disability. Malaria infections have been exposed to approximately 2 billion people with annual estimates of 250 million clinical cases and 900,000 deaths, amongst which 300,000 were children (WHO 2008; Mixson-Hayden *et al*., 2010; Snow *et al*., 2005). In addition, more than 30 million African pregnant women were infected with malaria annually (Nigeria National Population Commission, NNPC 2010). Malaria causes anaemia, low birthweight and significant neonatal mortality (Center for Disease Control, CDC 2011; Shane, 2001; Steketee *et al*., 1996).

Soil-transmitted helminth (STH) infections were the most prevalent and caused debilitating diseases in the world (Bethony *et al*., 2006; Hotez *et al*., 2006; Horton 2003), but it was assumed to have low mortality rate. The mortality due to hookworm infections, ascariasis and trichuriasis were approximately 65000, 60000 and 10000, respectively (Hotez, 2008). Light and moderate infections with STH's can be asymptomatic but heavy infections will lead to life-threatening illnesses such as

intestinal obstruction, acute dysentery and anaemia. Others contribute to malnutrition, protein and iron deficiencies, cognitive impairment, and vitamin A deficiency cumulatively increases income lost (WHO, 2002; Ramos *et al*., 2014; Kosek *et al*., 2003). As for strongyloidiasis, at least 30-100 million people were infected (Keiser & Nutman 2004; Bethony *et al*., 2006; Vadlamudi *et al*., 2006). Heavy infection with *Strongyloides stercoralis* produce enteritis and colitis but systemic hyper-infection is life threatening especially in immunocompromised and immunosuppressed patients (Carvalho & da Fonseca Porto, 2004; Vadlamudi *et al*., 2006; Concha *et al*., 2005).

The mortality due to blood fluke infection (schistosomiasis) was estimated up to 280,000 annually. The infection causes granuloma in the liver (*Schistosoma mansoni* and *S. japonicum*) and bladder or kidneys (*S. haematobium*) (van der Werf *et al*., 2003). Chronic morbidities were documented to be associated with impaired child growth/development, chronic inflammation, anaemia, and other nutritional deficiencies with 3–70 million DALY lost, which is approximately 4–50 folds more than previously estimated DALY lost (King & Dangerfield-Cha, 2008). This estimate exceeds that of malaria or tuberculosis, and was almost equivalent to the DALYs lost to HIV/AIDS (Hotez & Fenwick, 2009; King & Dangerfield-Cha, 2008). Furthermore, there was evidence of female genital schistosomiasis (*S. haematobium*) significantly increasing risk of contracting HIV/AIDS infections (Kjetland *et al*., 2006). A study on assessment of quality adjusted life years (QALY) conducted in China agrees with a previous report from USA, which revealed 9-24% drop in performance due to *S. japonicum* infection (Finkelstein *et al*., 2005). Similarly the liver fluke *Opisthorchis viverrini* infection was documented as the most prevalent food borne trematode in Southeast Asia, resulting in hepatobiliary morbidity (Fürst *et al*., 2012) and chronic infection that may proceed to cholangiocarcinoma, a fatal bile duct cancer (Bouvard *et al*., 2009). About 67.3 million

people were at risk of the liver fluke infection with nearly 10 million people from Thailand and Laos infected (Phongluxa *et al*., 2013; Sithithaworn *et al*., 2012).

As for intestinal protozoan infections, the mortality is ambiguous although about 50 million cases of invasive amoebiasis were documented every year with 100,000 deaths (Farthing, 2003; Clark, 2000; Tannich & Burchard, 2010; Herbinger *et al*., 2011). *Entamoeba histolytica* infection causes dysentery, liver abscess and rarely pulmonary abscess, cerebral and genitourinary amoebiasis (Abd-Alla *et al*., 1998). Despite, *Giardia intestinalis* infection causes diarrhoea, steatorrhea, vitamin A malabsorption and secondary lactose intolerance. *Cryptosporidium parvum* and *Blastocystis* sp. causes severe enteritis and chronic diarrhoea, especially in immunocompromised people (Norhayati *et al*., 2003).

Lymphatic filariasis caused chronic morbidity to nearly 40 million people worldwide (Okorie *et al*., 2013). Auxiliary to these, many other parasitic infections ensures multiple infections consequently aggravating the situations of anaemia (Ezeamama *et al*., 2008; 2005b; Brooker *et al*., 1999), malnutrition (Pullan & Brooker, 2008), organ pathology (Booth *et al*., 2004; Fulford *et al*., 1991), low birth weight (Egwunyenga *et al*., 2001) and other morbidities (Raso *et al*., 2004). Findings on impact of polyparasitism remain obscure due to the multifactorial nature of the infections, specific parasitic species involved, variations in infection intensities and infection dynamics in relation to social and environmental factors. Thus, only a handful of studies have used appropriate statistical methods to assess the biological interaction and report the resultant effect in an additive rather than multiplicative scenario (Mauny *et al*., 2004; Baume *et al*., 2000).

Examination of the independent and interactive effects of schistosomiasis, ascariasis, hookworms and filarial worm infections on several health outcomes such as anaemia,

nutritional status and immune responsiveness are increasingly becoming a favoured study subjects (Ajanga *et al*., 2006; Brito *et al*., 2006; Koukounari *et al*., 2006; Raso *et al*., 2004). Studies of such nature have been advocated especially in people living in the developing world that are vulnerable to a great variety of chronic diseases throughout the course of their lives. Besides individual effects on the disability and quality of life, polyparasitism is likely to have a significant financial impact on affected households, relegated to poverty (Sachs, 2005; Ukwandu & Nmorsi, 2004; Fenwick *et al*., 2005). To make matters worse, many of those who were polyparasitised were among the disadvantageous populations such as pregnant women, children and individuals with HIV.

2.3 Environmental Impact on Parasitic Endemicity

Natural phenomena (climate, temperature and rainfall) and human activities (water projects, road construction, population explosion with the consequent urban expansion, or growth of urban slums and squatter settlements) have led to rapid deterioration of human environment (Nwoke *et al*., 2008; Patz *et al*., 2000) and increases morbidity and mortality due to parasitic diseases (Patz *et al*., 2000). Human induced climate changes through industrialization, which results in depletion of the ozone layer disturbs the ecological balance and encourages the emergence and re-emergence of vector-borne parasitic diseases (Nwoke *et al*., 2008). Infections like malaria, leishmaniasis, cryptosporidiosis, giardiasis, trypanosomiasis, schistosomiasis, filariasis, onchocerciasis, and loiasis are usually affected by environmental changes.

Plasmodium species and their vectors react sharply to the changes in location and climate ecology and habitat, be it deforestation, vegetation, density of human population

and water bodies (Patz *et al*., 2000). Each *Anopheles* species occupies a specific ecological niche and operates at a different level of vector competence. Alteration in their environment may result in the indigenous *Anopheles* failure to adapt and disappear, hence be replaced with a different, opportunist species that moves into the vacated niche. This was demonstrated in the coincidental upsurge of malaria with changes in land-use and human settlements subsequent to deforestation in Africa, Asia and Latin America (Tadei *et al*., 1998; Coluzzi *et al*., 1985; Kaplan *et al*., 1980; Bunnag *et al*., 1979; Coluzzi *et al*., 1979). In addition, changes in temperature have been linked to malaria epidemics in Pakistan and Zimbabwe where incidence and prevalence of malaria have been closely associated with altitude (Freeman & Bradley, 1996; Bouma & van der Kaay, 1994; Taylor & Mutambu, 1986).

Increase in rainfall, leaking of septic tanks and agricultural run-offs lead to heavy contamination of water sources with ruminant/rodents and human faeces which enhances spreading of waterborne parasites especially *Giardia* and *Cryptosporidium* species (Monzingo & Hibler, 1987). Variation in raining seasons, changes in water temperature and water flow as well as changes in vegetation along the water banks all contribute to the abundance of snail vector and enhance parasite population. For instance, in the Nile Delta below Cairo and Sudan, prevalence of bilharziasis parallels with the degree of irrigation intensity, as the snails were pumped along with the water (Jobin, 1999), such was the case in Nigeria with the development of Kianji Dam and tributaries. In Iran, the upgrade in irrigation and construction of canals resulted in an upsurge of bilharziasis prevalence among those working and living within the immediate areas (Patz *et al*., 2000). Similarly in China, after the construction of the Three Gorges High Dam at the Yangtze River, prevalence of human schistosomiasis changed significantly (Xing-jian *et al*., 1999).

2.4 Burden of Parasitic infections in Nigeria

Nigeria claims to be the 'giant of Africa' and indeed is the top in terms of population and parasitic infections. The country is in a "tug of war" where malaria, sleeping sickness, intestinal-helminthiasis, amoebiasis, giardiasis, schistosomiasis, onchocerciasis, lymphatic-filariasis, paragonimiasis and other diseases are on the opposing end. Nigeria is placed in the sub-Saharan region of Africa and was documented to have the highest prevalence in hookworm infections, ascariasis, trichuriasis, schistosomiasis and lymphatic filariasis (Hotez & Kamath, 2009). The prevalence of parasitic infections were reported between 33.3% to 80% among a religious sect that walk bare footed, rural communities, pregnant women and HIV/AIDS patients (Biu & Dauda, 2008; Adeyeba & Essiet, 2001; Okon & Oku, 2001; Ogbuagu *et al*., 2010; Ameh *et al*., 2004; Anosike *et al*., 2004; 2003; Ahmad *et al*., 2001; Gundiri *et al*., 2001). Schistosomiasis (due *S. haematobium* and *S. mansoni*) was reported as endemic (with prevalence between 1.0% and 86%) in various communities in the country (Bassey, 1999; Abdullahi & Sa'idu, 2011; Bassey & Umar, 2004; Bigwan *et al*., 2012; Abdullahi *et al*., 2009; Duwa *et al*., 2009; Daniel *et al*., 2001).

Out of the 774 Local Government Areas (LGAs) in the 36 States and Federal Capital Territory (FCT) of Nigeria, 636 have been mapped and 476 LGAs were found endemic with lymphatic filariasis, though the infection rates varied from 1.5% up to 16.7% (Akogun, 1991; Udonsi, 1988; 1986; Badaki & Akogun, 2001; Dogara *et al*., 2012; Anosike *et al*., 2005; Abel *et al*., 2002). Nigeria is the world most endemic country with onchocerciasis. The disease was reported as hyper and meso-endemic in 31 States and the Federal Capital. Previously, was anticipated to stand at 10 million cases but now stands at 22 million cases of onchocercal dermatitis in the country. This was indicated in records collected from several areas such as Galma Valley area (Crosskey, 1981),

Ibarapa (Wyatt, 1971), Benue State (Dipeolu & Gemade, 1983), Anambra State along the Jarawa Valley and villages around Anambra River (Nwoke *et al*., 2008).

Malaria is the second leading cause of death in Africa, after HIV/AIDS. Malaria is also a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. Almost 97% of Nigerians are at risk of malaria, with reported cases of around 100 million resulting in more than 300,000 deaths per annum. It was estimated to cause up to 11% of maternal mortality in the country (Singh *et al*., 2014a).

2.5 Health Services and Primary Health Care System in Nigeria

In the 1970s, Nigerian government focused on the Basic Health Services System, but due to unclear policy framework and heavy burden especially in terms of infrastructure, professionals and auxiliary health manpower, the system was rendered ineffective. The government then embraced the 1998 joint UNICEF-WHO Alma-Ata Declaration that identified Primary Health Care as essential and accessible to individuals and families in a community. This programme was aimed to theoretically reduce and eradicate certain diseases (including parasitic) through provision of good health care to the people. By means of health education, provision of food, nutritional supplements, essential drugs, immunization against communicable diseases, maternal and child health as well as adequate safe water supply and housing (Udonsi, 2002; Malaria, 2000).

Despite the endorsement of the declaration, Nigerian health care system still face numerous problems such as lacking clear structure for the three tiers (primary, secondary and tertiary levels) of health care systems and ensuring adherence to it at all levels of the government. In the primary health care system, its major aspect is health

education and information that involves the enrolling, training and retraining of the professionals in the medical and paramedical fields. They were involved in giving relevant information to make people understand their everyday health related problems, educate them on practical skills on how to recognize and protect themselves against parasitic diseases (Udonsi, 2002). Health education through school curriculum and community awareness campaign exhibits more profound impact on the population (Udonsi & Ogan, 1993). However, in reality the Nigerian education sector placed the health education as a side issue, as few topics spread across subjects in the curriculum (Udonsi, 2002), which stressed more on personal hygiene rather than focus on specific parasitic diseases. Explanations on diseases were mainly facts, not protective measures, and rarely related to local endemic health problems done entirely at only the primary school level (Udonsi, 2002).

Awareness campaigns are seldom conducted at antenatal clinics in public health centres and once awhile in community centres. Currently, several organisations such as concerned individual professionals, non-governmental organisations and media houses organises and disseminates information about parasitic diseases in the media, all in their own efforts on trying to buttress the related issue. On the other hand, the government emphasized on equipping hospitals with primary health support through referral to higher-ranking health care level when necessary. There is a need for the government to offer workshops to train and retrain health professionals and auxiliary primary health care workers to encourage teamwork and discourage the rather intra-sectoral division and in-fighting currently manifested in the country. Further more, orientation and reorientation of the political class and community leaders is needful for political will to strongly support the primary and secondary health care systems in order to ameliorate the health care services in the country (Asuzu, 2004; Magnussen *et al*., 2004).

To date, the health care system has been improved but still the hospitals (both large and small) management do not provide the adequate type of health care that is needed by the people. The hospital-based health care has been focusing more on curative measures through either secondary or tertiary health care levels rather than emphasizing on the primary health that is community and preventive medicine (Udonsi *et al*., 1980). Furthermore, most of the hospitals and health centres are located in urban and semi urban areas (accounts for less than 30% of total population) and neglecting majority of the rural, which consist of more than 70% of the total population. Indeed, the present health services are such that on the basis of fundamental characteristics, not more than 15% of total Nigerian population (approximately 182 million people) can ever have access to medical facilities under the present organizational structure. Therefore, the Nigerian health care system caters more for the minority elites (Udonsi, 2002) and has actually failed to meet the demand of its people. The 2000 World Health Report was also an attestation to this, in which performance of the health systems of Nigeria was ranked the 187th out of the 191 members worldwide (Asuzu, 2004; Magnussen *et al*., 2004).

2.6 Control programmes of parasitic infections in Nigeria

Control of parasitic infections in Nigeria can be categorized into two groups, direct and indirect approaches. The direct approaches were targeted at controlling or eradication of parasitic infections such as malaria via insecticide residual community spray and fumigation which have been routinely used coupled with antimalarial prophylaxis even though with little favourable results. With the initiation of WHO's Roll Back Malaria programme (RBM), the Nigerian government endorsed and embarked on it by 2001. The programme in collaboration with the Global Fund for

Acquired Immune Deficiency Syndrome (AIDS), tuberculosis (TB) and malaria, the Nigerian government and also other non-governmental organisations operating in the country are struggling to control the pandemic (Gwatkin, 2000; Onwujekwe *et al*., 2004). Among the features of RBM was the initial campaign and public information about the infection (Utzinger *et al*., 2001), which was achieved through media, seminars and lectures especially at hospitals and clinics. This was then followed by interventions with insecticide-treated bed-nets (ITN), indoors house spraying, chemoprophylaxis in childhood or pregnancy and intermittent treatment for pregnant women (Utzinger *et al*., 2001).

Control and eradication programmes have also been carried out against dracunculiasis, filariasis, onchocerciasis, schistosomiasis and African trypanosomiasis, among which share a lot of over lapping foci (co-endemicity). Therefore the programmes gave a huge impact on the population, in which the prevalence and morbidities associated with the diseases were significantly slashed (Coffeng *et al*., 2013; Okorie *et al*., 2013). Many unsuccessful attempts have been made in the efforts to control soil-transmitted helminthic infections, but still these infections are prevalent among the people, especially among children of school age (Ijagbone & Olagunju, 2006).

The indirect approaches are programmes that are not targeted at controlling parasitic infections but their implementation would bring down parasitic infections. These include water resource projects, sanitation and nutrition to improve iron deficiency, vitamin A deficiency and iodine deficiency disorders. Irrefutably, general improvement of potable water supply and sanitation in communities can control and decrease several parasitic infections including water borne protozoan, soil transmitted helminths, schistosomiasis and other trematode infections, malaria and onchocerciasis among

others. Improvement in nutrition helps in lowering morbidities due to parasitic infections and these have been observed though not documented in several communities in the country. From conversation with health workers, improvement in pipe water supply showed reduction of diarrhoea in children and malaria cases. There were less cases of diarrhoea in the communities merely by enlightenment of mothers on nutrition and effects of geophagia.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study design and selected areas

This is a cross-sectional community-based study that includes volunteers of at least one year old and above, in five local government areas of Kano State, Northern Nigeria (Figures 3.1 and 3.2). Kano State was selected based on its population (being the most populated state in Northern Nigeria), have accessible villages, free of conflicts and expect high level of cooperation. The state is located in the sudanian and sahelian savanna region that stretches across the south of the Sahara. The region features savanna vegetation and a hot, semi-arid climate. The state receives an average of about 690 mm (27.2 ins) of rainfall per year, the bulk of which falls between June and September. The capital city of Kano State is Kano city and is typically hot with an average temperature of 33ºC from March to November, but cooler with temperatures ranging between 11°-14 °C from December to February. Kano city is the second largest city in Nigeria; with a population of 9,401,288 (2006 census) and the main inhabitants of the city are the Hausa people, speaking the Hausa language. The Hausa people are predominantly farmers and therefore Kano city is a trade and shipping centre for an agricultural region with wide trade contacts in West and North Africa.

The study areas were selected based on their location in the state, which have fairly equal representation of the state. The state is divided into 2 zones according to the current political zoning of states in Nigeria. In each zone, three local governments were chosen, namely Gwarzo (7.932^0E and 11.915^0N), Shanono (7.983^0E and 12.049^0N) and Minjibir (8.530^0E and 12.226^0N) from the north, while Kura (8.429^0E and 11.774^0N), Doguwa (8.739^0E and 10.743^0N) and Bebeji (8.400^0E and 12.366^0N) were from the south region. However, Doguwa was later excluded due to political crisis. With the help

of primary health care (PHC) personnels and traditional rulers in each local government area, 3 districts were chosen in each local government area. The choice was made considering districts with the most population to ensure reasonable turn out of volunteers.

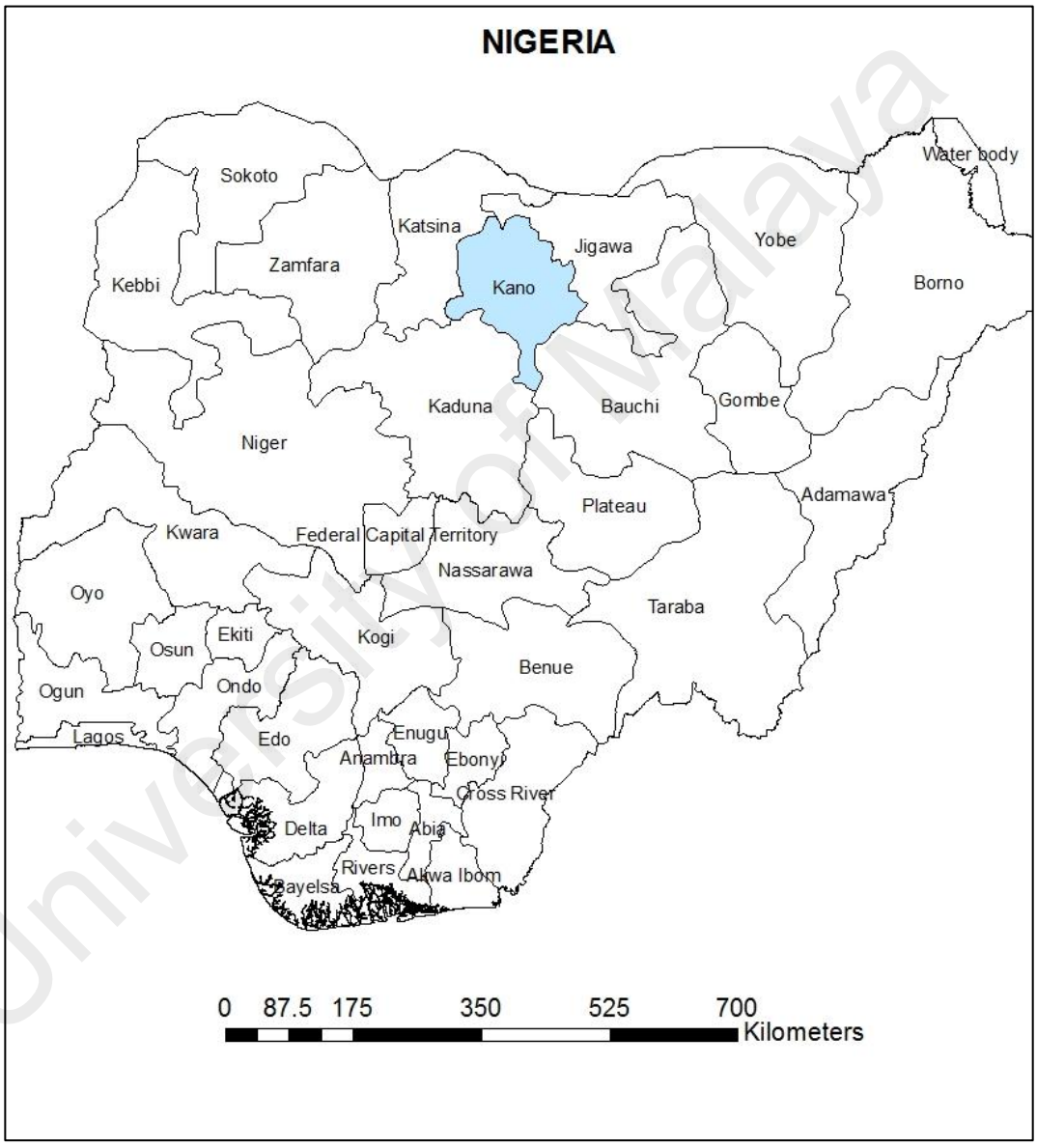

Figure 3.1: Map of Kano State in Nigeria

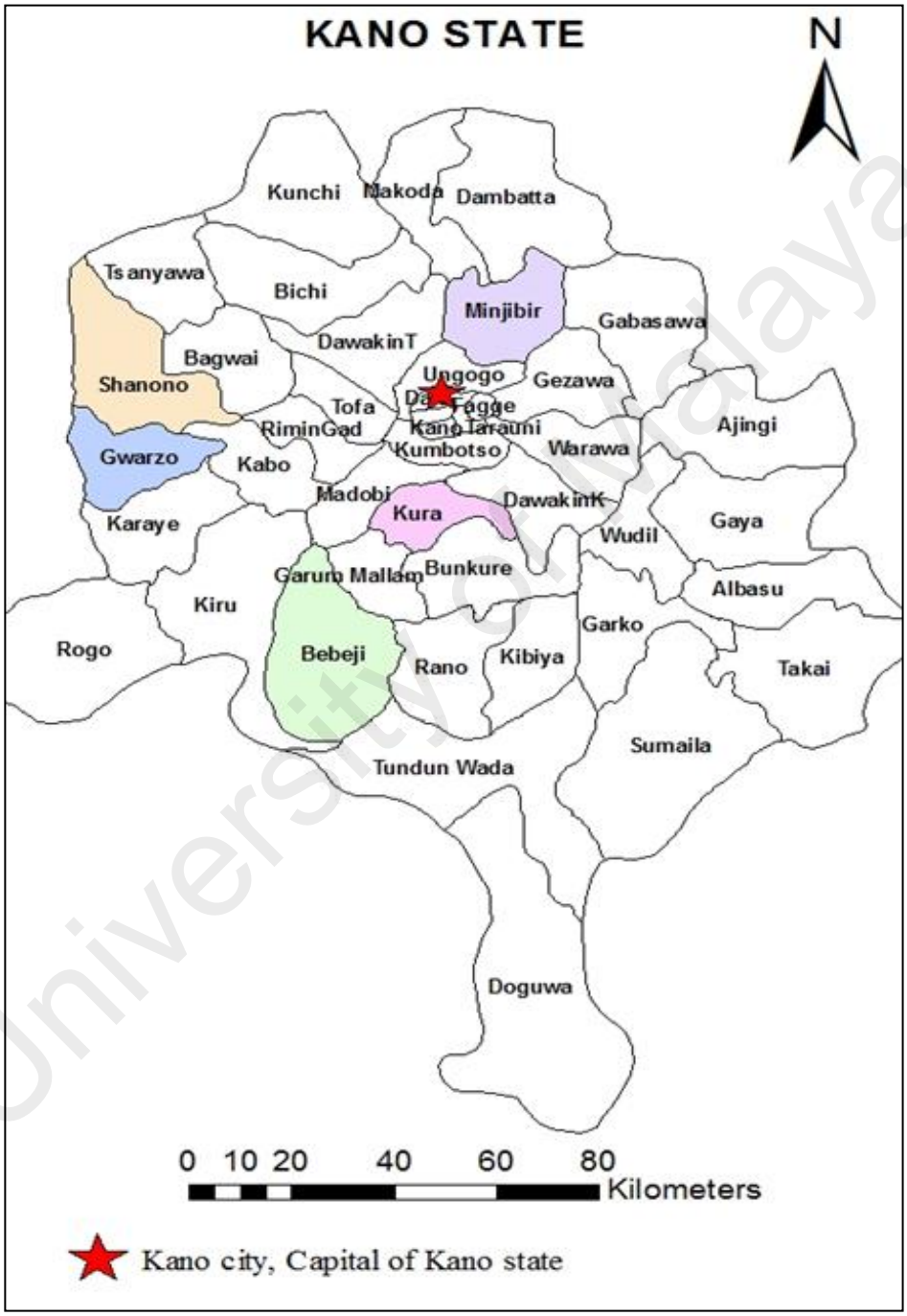

Figure 3.2: Map of study areas (in colours) in Kano State of Nigeria

Basic amenities like provision of pipe-water and electricity were seen throughout the state but there is no constant supply. Water supply was interrupted up to months in some communities. Furthermore, majority of the communities have electricity supply but only for few hours per 24 to 48 hours. The houses in the study areas are made up of either mud or cement with few nomads using hatches (huts) in bushes away from the population, to cater for their herds. All houses in the study areas have functioning toilets but people still defecates outside especially during the day when going about their daily chores as no public conveniences are provided.

3.2 Ethical clearance and study population

Ethical clearance was obtained from University of Malaya, Malaysia (reference number 989.25). Clearance was also approved by the Kano state Ministry of Health through the Kano State Hospitals Management Board, and the respective Local Government Authorities (reference number 08 / 08 / 1434 AH (17 / 06 / 2013). The district heads of each community were briefed on the objectives of the study and were shown the clearance approval from the state authorities. During seeking for consent from volunteers, objectives and importance of the study were clearly explained in the local Hausa language. Participation in this study was on a voluntary basis with written informed consent. However, only volunteers whose family heads were present during the explanation and had meet the exclusion criteria were included in the study. These include age above one-year-old, not sick at the moment (having any fever or diarrhoea), not on any antihelminthic, antidiarrhoeal or antimalarial medication. Lastly, the participant can provide all 3 samples, which were urine, stool and blood. Signed or thumbprint consents were obtained from adult participants and guardians/parents (in case of young children). The selected participants were then given clean sterile bottles

for collection of urine and stool and were requested to return the specimens the next morning.

Appropiate treatment was provided to all infected with parasites through the primary health care personnels in the respective areas. Some under age respodents found infected with *T. vaginalis* were treated and counselled. Individuals with multiple infections (from 5 parasites and above) were also counselled on personal hygiene.

3.3 Sample size

The sample size needed in this study was calculated according to WHO guidelines (Lwanga & Lemeshow, 1991; Naing *et al*., 2006) using the equation formula as below:

$$n = Z^2 P (1- P) / d^2$$

Where
n [sample size]
Z [*Z* value is 1.96 (statistic for a level of 95% confidence level)]
P [expected prevalence or proportion (e.g. if 42%; P = 0.42)]
d [precision (e.g. if 5%, d = 0.05)]

A total of 374 participants were needed for this study. Considering 20% to adjust the anticipated dropout rate ***n*** now becomes 449. This was estimated to give the study at least 80% power at 5% significance.

3.4 Data Collection

3.4.1 Field Work

The fieldwork was conducted within 6 weeks, from 20th June to 4th August 2013. This began with a formal introduction and meeting with district heads and community leaders through the Primary Health Care (PHC) personnels, and subsequently the family heads. Importance of participating in this survey and the implication that can be drawn from it were discussed. The number of participants and samples collected from volunteers is described in the following flow chart:

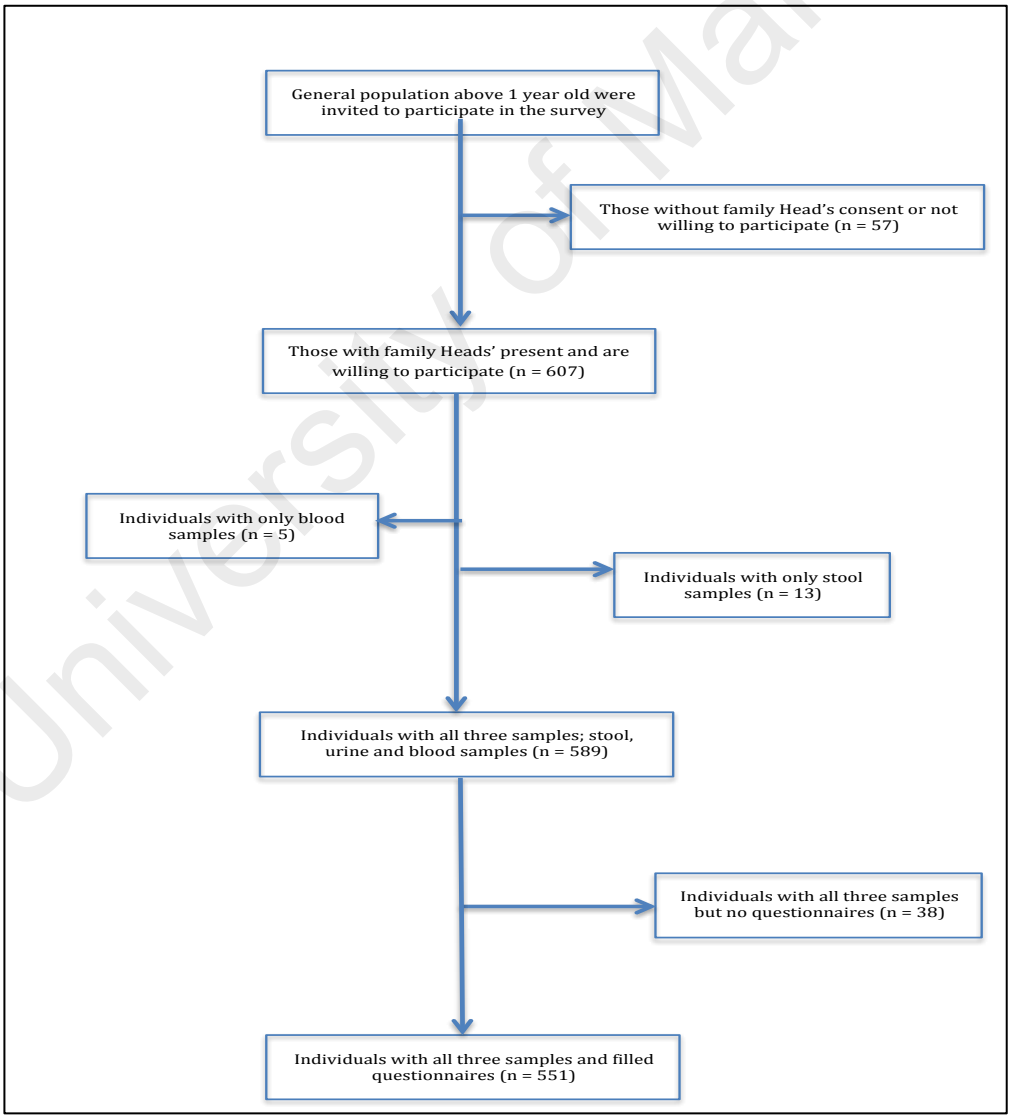

Figure 3.3: Workflow and number of samples collected from volunteers

3.4.2 Questionnaire survey

A validated questionnaire was adapted from Al-Delaimy *et al*. (2014) with some amendments to suite our study. The questionnaire was then used to collect data on the demographic, anthropometric indices, socio-economic, personal hygiene, knowledge and history of infections, contact with animals, living near aquatic environment, source of drinking water and sleeping inside bed nets. In addition, the attitude of volunteers towards parasitic infections was also included. The volunteers were interviewed face-to-face and for children below 8-year-old, the mothers provided the information. During the interviews, direct observation was made on personal hygiene and household cleanliness of the respondents. Observation on the use of bed nets in their homes was also noted. Observations on water activities (bathing / swimming) in the streams and ponds during midday were also carried out. All the houses had toilets but human and animal excreta were observed around water bodies, within farmlands and walkways. Significant numbers of children were seen walking bare footed, or removed their shoes while playing football, hopscotch, etc.

In administering the questionnaire, each participant was given an individual reference number. Then measuring the body weight up to 0.1 decimal point of kilogram using a calibrated SECA scale (SECA 709: Seca, Hamburg, Germany). The precision of the scale was checked regularly to ensure that the scale calibration was accurate and consistent. Height was measured to the nearest cm using meter rule mounted on a wall. Participants were weighed wearing light clothing without shoes, belts, caps or any other material that could interfere with their actual weight and height.

3.4.3 Statistical Analysis

Data was double entered by two different researchers into spreadsheets of IBM Statistical Package for Social Sciences (SPSS), version 20 (IBM Corporation, NY, USA). Then, a third researcher crosschecked the two data sets for accuracy and created a single data set and analysed. Demographic, socioeconomic, environmental and behavioural characteristics were treated as categorical variables and presented as frequencies and percentages. Pearson's Chi Square test and Fisher's Exact test were used to test the associations of infection prevalence with demographic, socioeconomic, environmental and behavioural factors. Odd ratios (OR) at 95% confidence intervals (CI) were computed in order to identify the risk factors significantly associated with the infections.

Knowledge of infection source, symptoms, transmission and prevention are presented as proportions (percentages) and Pearson's Chi Square test was used to test the associations of knowledge of infection with demographic factors (age, gender, and family size), socioeconomic factors (educational and employment status, household monthly income), living near water sources, presence of functioning toilet in the house, housing conditions, using ITNs and insecticide, history of infection, and presence of domestic animals in the households.

Participants were categorized into different age groups for different infection analysis. Age was categorized into two groups (children for those age group ≤ 15 years and adults for those >15 years old) for analysis related to general, single and multiple infections as well regarding *Blastocystis* sp infection. For malaria infection, age was categorised into four groups (≤ 5, >5 – 10, >10 - 18 and >18-year-old). For schistosomiasis, five groups were used in analysis (≤ 10, > 10 – 18, > 18 – 30, > 30 – 50 and > 50-year-old). For risk factors and KAP analysis, age was categorised into two

groups (children for those below 18 years and adults for those 18 years and above). Different categories of age groups were used in analyses to obtain the suitable criteria according to the WHO approved groupings for respective infections. The risk factors that significantly associated with the infection were identified using multivariate logistic regression analysis. In order to retain all possible significant associations variables that showed an association with $P \leq 0.25$ were used in the multiple logistic regression models as suggested by Bendel and Afifi (1977). A P value of < 0.05 was considered statistically significant (Bendel & Afifi, 1977).

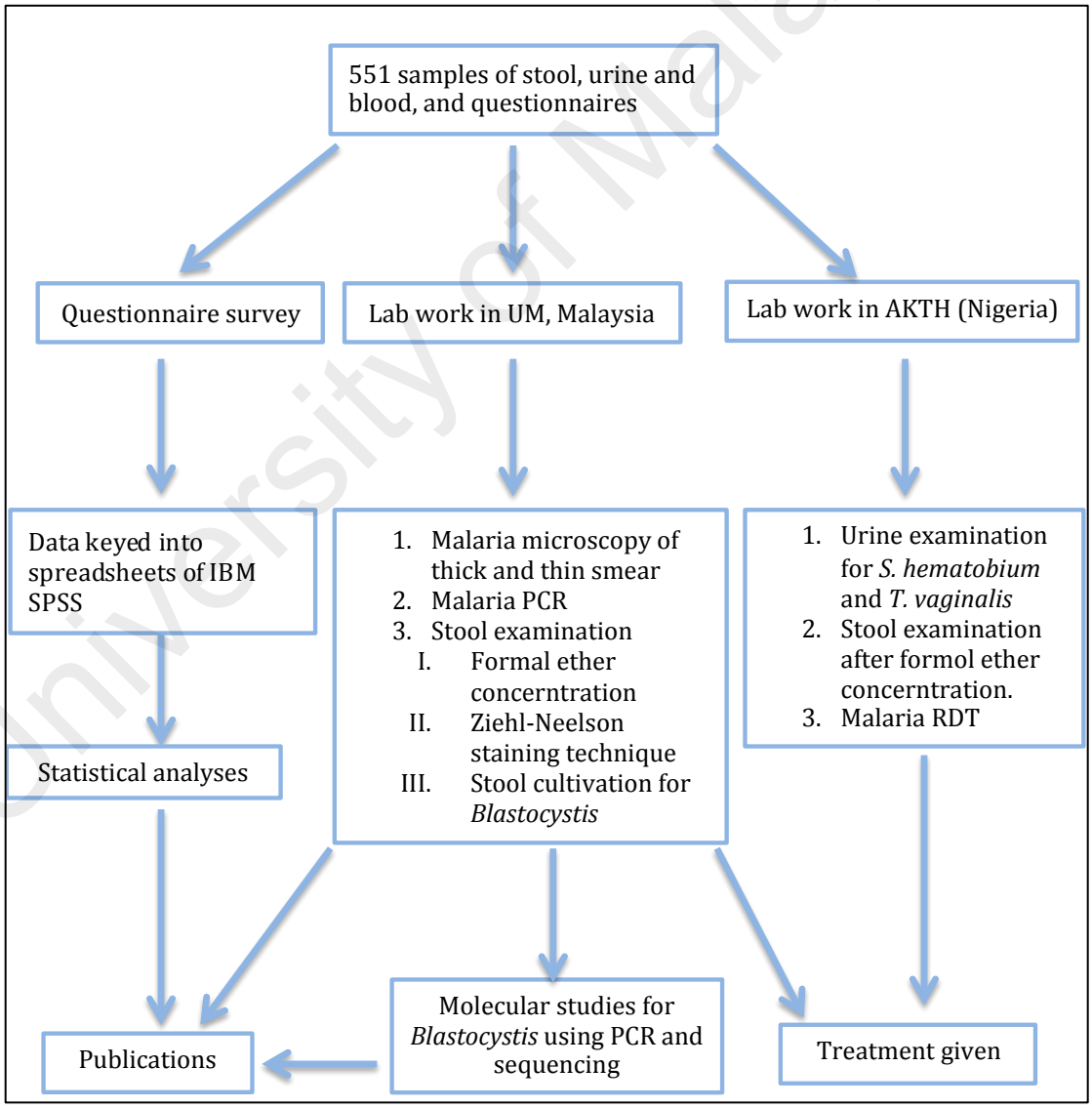

Figure 3.4: Flow chart for methodology

3.5 Collection of samples

The volunteers were given labelled, clean, wide mouthed and screw-capped universal stool containers and universal urine containers (100 mL) and were instructed to bring their early morning stool / urine samples the next day. The collection of morning samples was carried out between 9 am to 2 pm. From each participant, approximately 2−3 mL of venous blood and 2−3 drops of blood samples were respectively collected in an EDTA tube and on 3MM Whatman® filter paper (Whatman International Ltd., Maidstone, England). A medical laboratory technician from the district's health center collected the blood samples. For the urine and stool samples, they were collected and the labelled was checked to appropriately match with the blood sample from each person. All the samples (except blood on filter paper) were kept in a cool box at temperature 10±2°C and transported within 5 hours to Aminu Kano Teaching Hospital for subsequent analysis.

3.6 Processing of Urine, Stool and Blood samples

Processing of samples was carried out on the same day after collection (when possible) or refridgrated at 4^{o}C and was processed the follwing day. Individual urine sample was centrifuged and the sediment was examined microscopically as a wet mount for *Schistosoma* egg and *Trichomonas vaginalis* trophozoite stage at the Aminu Kano Teaching Hospital (as describe in section 3.8). As for stool, each sample was divided into 3 portions. One portion was analysed right away to determine the presence of parasite species by direct smear and formal-ether concentration method. The second portion was preserved in 75% ethanol, and to the third portion normal saline was added (Appendix F, No. 1) to prevent desiccation and was used for cultivation of *Blastocystis*.

As for blood, each sample in the EDTA bottle was used to make 3 sets of thick and thin blood films on glass slides. All the samples (blood film slides, dry blood on filter paper and stool samples in ethanol and normal saline) were packaged and sent by air to the Department of Parasitology, University of Malaya, Malaysia (transportation took 10 days).

3.7 Detection of malaria in blood samples

3.7.1 Detection of *P. falciparum* using rapid diagnostic test (RDT) kit

Rapid diagnostic test (RDT) kit used was "CareStart Malaria HRP2 from Access Bio, Inc" to determine the presence of *P. falciparum* species carried out at the laboratory of Aminu Kano Teaching Hospital. The RDT procedure was carried out according to the manufacturer's description. Briefly, 2 drops of blood from the EDTA bottle was transferred into the sample well of the test cassette using the loop provided in the kit. This was followed by addition of 2 drops of buffer at the buffer port and allowed to stand for 15 minutes before reading. Appearance of 2 purple lines at both the control and test windows indicated positive for *P. falciparum*, a purple line only at the control window indicated negative result. The test was considered invalid and was repeated if only one line appeared at the test window, or no line at either control or test windows.

3.7.2 Detection of malaria species by microscopy

At the laboratory of Parasitology Department, University of Malaya, the thin and thick blood films were stained with Giemsa stain (Appendix F, No.2). The thin films were used to detect the presence of malaria parasite while the thick films were used to

determine the parasiteamia in 200 fields under 1000X magnification, before the slide was considered negative. In the positive slides, number of parasites counted was converted to number of parasites per μL of blood, assuming as standard a WBC count of 8000/μL. Thus parasiteamia was calculated using the following formula:

Parasites / μL of blood = No of asexual parasites counted × 800 WBC/ μL /No of WBC counted

A degree of parasitimia was graded as described by Nwagha *et al*. (2009), which were considered mild (1 – 999 /μL), moderate (1000 – 9999 /μL) and severe (>10 000 /μL). This result was then compared with RDT from section 3.7.1.

3.7.3 Molecular identification of malaria parasites

All malaria positive and randomly selected negative samples were confirmed by PCR. Genomic DNA was extracted from the dry blood samples using Qiagen blood and tissue kit (QIAGEN, DNeasy® Blood & Tissue Kit, Cat. no. 69506, Germany). The blood spot on the filter paper was cut using ethanol flame-sterilized hole-puncher and placed into a sterile 1.5-mL micro centrifuge tube and DNA extraction was done according to the procedure given in the manufacturer's instructions. DNA was eluted using 50 μL AE elution buffer (10 mM Tris-Cl; 0.5 mM EDTA; pH 9.0) and kept at -20^{o}C until used for PCR analysis. *Plasmodium* species were identified by 18s rRNA-based nested PCR using genus and species specific nucleotide primer sets as described by Singh *et al*. (1999).

Brief procedure was first nest reaction mixture of 50 μL contained 5 μL of DNA template, 250 nM of each genus specific primers rPLU1 and rPLU5, 4 mM Magnesium

Chloride ($MgCl_2$) (Thermo Scientific), 5.0 µL of 10× PCR buffer (Thermo Scientific), 200 µM of deoxy nucleoside triphosphate (Thermo Scientific), and 1.25 units of *Taq* DNA polymerase (Thermo Scientific). DNA amplification was carried out in PCR thermal cycler (MultiGene™ II, LabNet, Edison, USA). The amplification conditions were initial denaturation at 94°C for 4 mins, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 4 min. In the second nest, 2 µL of the nest 1 amplification product served as the DNA template in a 20 µL reaction mixture. The concentration of the nest 2 primers and other constituents were identical as nest 1 except that 0.5 unit of Taq polymerase was used. In addition, the nest 2 amplification conditions were identical as in nest 1 except the annealing temperature of 58°C for the specie-specific primers (rFAL 1 and 2, rMar 1 and 2, rVIV 1 and 2 and rOVA1 and 2) and was 62°C for the genus-specific primers (rPLU3 and 4). The PCR products of nest-2 were analysed by gel electrophoresis and stained with ethidium bromide.

The PCR product (10 µL) was mixed with 6× loading dye (2 µL; Thermo Scientific) and was loaded into the well of the electrophoresis gel. Also, 5 µL of 100 bp DNA ladder (Thermo Scientific) and 5 µL of dH_2O were used as a standard marker and negative control, respectively. The gel was set to run at 100 volts for 30 minutes and the DNA bands were visualized under ultraviolet (UV) trans illuminator (3UV™ Trans illuminator, LMS-20E, UVP, USA).

3.8 Detection of *Schistosoma haematobium* ova and *Trichomonas vaginalis* trophozoites in urine samples

Ten mL of the urine sample was centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded and the sediment was transferred to a clean glass slide and examined with 400X magnification to identify *Schistosoma haematobium* ova and *Trichomonas vaginalis* trophozoite. Moreover, egg counts for *S. haematobium* was recorded as eggs per 10 millilitres urine (EP10 mL), and the intensity of the infection was graded with reference to WHO criteria (Montresor et al., 1998) as shown in Table 3.1.

3.9 Detection of helminth eggs and protozoa (oo)cysts in stool samples

The preserved stool samples were subjected to direct faecal smear, formal–ether concentration techniques and Kato-Katz as described by Cheesbrough (2009) for identification of the helminth eggs (i.e nematodes, trematodes and cestodes) and protozoa cysts. A Ziehl-Neelsen stain (Appendix F, No.3) was employed to confirm the morphology of *Cryptosporidium* oocysts. Repeat identification was also carried out by second researcher for formal-ether concentration and Kato Katz techniques. The result of the first diagnosis was blinded to the second microscopists.

3.9.1 Direct faecal smear

A drop of normal saline was placed in the centre of a glass slide. A small amount of faeces was picked using an applicator stick and mixed with normal saline. The mixture

was then covered with a cover slip and examined under light microscope using 100X and then 400X objectives.

3.9.2 Formal ether concentration technique

Approximately 100 mg of faeces was emulsified in 7 mL of 10% formalin. The emulsion was filtered through two layers of gauze into a 15-mL centrifuge tube. Then 3 mL of ether was added and shaken vigorously and the mixture was centrifuged at 3000 rpm for 1 minute. Four layers were formed after the centrifugation, which consisted of the top layer (ether), plug of faecal debris, formalin and bottom layer of sediment containing parasite units. Using an applicator stick, the plug faecal debris was loosened from the sides of the tube and the top three layers were decanted. The remaining sediment was examined as direct smear for the detection of protozoa cyst and eggs / larvae of possible helminths.

3.9.3 Kato-Katz technique

The Kato-Katz procedure was adopted from Cheesbrough (2009). Briefly, a small portion of the stool sample was pressed through a mesh screen to remove large particles and then placed in a hole of a template on a glass slide. The template was removed and the stool sample (approximately 10 mg) was covered with a piece of cello-phane that was soaked in glycerine to clearify the parasites eggs. A set of duplicate Kato-Katz smear was prepared from each faecal sample and examined twice by two different microscopists, first for hookworms within 30 minutes and later for *A. lumbricoides* and *S. mansoni* within one hour. Repeat experiments were carried out against 10% of the

samples by different microscopists for quality control purposes and no large discrepancies were noted. Egg counts were recorded as eggs per gram of faeces (epg) for each positive sample. Intensity of infections was graded as heavy, moderate or light according to the criteria proposed by WHO (Montresor *et al*., 1998) as shown in Table 3.1.

Table 3.1: Gradation of helminth infection intensities proposed by World Health Organisation

Parasite	Number of eggs per gram of faeces or per mL of urine		
	Light intensity	Moderate intensity	Heavy intensity
Ascaris	1 – 4,999	5,000- 49,999	> 50,000
Hookworms	1- 1,999	2,000- 3,999	> 4,000
S.mansoni	1-99	100 - 399	> 400
S.haematobium	< 50	-	> 50

3.9.4 Modified Ziehl-Neelsen staining

The modified Ziehl-Neelsen staining (Appendix F No.3) was performed to confirm the oocyst of *Cryptosporidium*. The procedure was adopted from Getaneh *et al*. (2010). A thin smear was prepared using concentrated specimen (after formal-ether concentration) and was air-dried. The smear was fixed with methanol for 3 minutes, stained with carbol fuchsin for 12 minutes and rinsed with clean running water. The smear was then decolorized with 1% acid alcohol for 15 seconds and counter stained with 0.5% malachite green for 30 seconds. Subsequently, the stained smear was washed, air dried and examined for oocyst of *Cryptosporidium* under 1000X objective using oil emersion. The oocysts (4−6 µm) usually appeared bright pink or dark red with a green background.

3.10 Molecular studies of the most prevalent protozoan (*Blastocystis* species)

The most prevalent intestinal protozoan in this study is *Blastocystis* sp. followed by *Entamoeba* species. Molecular analysis including DNA extraction, PCR, DNA purification and cloning were carried out against *Blastocystis* isolated from positive cultures. Identification of *Entamoeba* species by molecular methods was also carried out but only summary of prevalence will be reported in this study.

3.10.1 Stool Cultivation

The stool samples were cultured in a 15-mL screw-cap tube by inoculating approximately 50 mg of stool specimen (in normal saline) into 5 mL of Jones' medium (Appendix F, No.4) supplemented with 10% horse serum and incubated at 37°C. A new complete medium was replaced every other day up to day 14 by carefully discarding 4 mL of the top medium (not disturbing the cell pellets) followed by adding 4 mL of fresh complete Jones' medium. The presence of *Blastocystis* sp. was observed daily throughout the 14 days of cultivation, by placing 1 drop (50 μL) of cultured sediment onto a glass slide, covered with a cover slip and viewed (100X and 400X objectives) under light microscopy. Positive cultures were defined by identification of any form of *Blastocystis* sp. (vacuolar, granular or amoeboid forms). Cultures were considered negative when there was no growth by the 14^{th} day.

3.10.2 DNA extraction from cultures

Approximately 1−3 x 10^6 cells of each *Blastocystis* isolate were harvested for DNA extraction. The accumulated cells were centrifuged at 2000 rpm for 5 minutes, the

supernatant was discarded and the sediment was washed by transferring to a new sterile tube containing 5 mL sterile phosphate-buffered saline (PBS) (Appendix F, No. 5) then centrifuged and the supernatant discarded. The process was repeated 4 times to minimize bacterial contamination. The cell pellets (approximately 1−3 x 10^6 cells) were then collected by centrifugation and re-suspended in 200 µL of PBS.

DNA extraction was carried out using QIAamp Fast DNA Stool mini kit (QIAGEN, Hilden, Germany) and the procedure was carried out as described by the manufacturer. Briefly, about 200 µL of the cell suspension was kept on ice in a 2-mL micro centrifuge tubes. Then 1 mL of inhibit EX-buffer was added, mixed thoroughly and the suspension was then heated to 70ºC for 5 minutes to lyse the cells. Proteinase K and Buffer AL were then added and the suspension was heated again at 70ºC for 10 minutes. Next, ethanol was added to the lysate and then the DNA was filtered with a QIAamp spin column. The column was washed with buffers AW1 and AW2 and later centrifuged to ensure complete removal of ethanol residual as well as to dry the column. Subsequently the DNA was eluted in 70 µL Buffer ATE, and the eluted DNA was stored at -20ºC until use.

3.10.3 PCR amplification

For each sample, 5 µL of genomic DNA was subjected to PCR analysis, using the primers forward Blast 505–532 (5’ GGA GGT AGT GAC AAT AAATC 3’) (Böhm-Gloning *et al*., 1997) and reverse Blast 998–1017 (5’TGC TTT CGC ACT TGT TCATC 3’) (Santín *et al*., 2011) amplifying an approximately 500 base pairs (bp) fragment of the 1,800 bp small subunit ribosomal RNA gene (SSU-rDNA). The PCR mixture and cycle conditions were adapted from Abdulsalam *et al*. (2013b). In

summary, the PCR volume was 50 µL containing: 5.0 µL of 10× PCR buffer (Thermo Scientific, USA), 3.0 µL of 25 mM Magnesium Chloride ($MgCl_2$) (Thermo Scientific), 2.0 µL of 10 mM Deoxynucleotide triphosphate Mix (Thermo Scientific), 0.5 µL of 5U/µL Taq DNA Polymerase (Thermo Scientific), 2.5 µL of 0.1 g/10 mL Bovine Serum Albumin (BSA) (New England Biolabs, USA) and 0.5 µL each of both Forward and Reverse primers. The DNA amplification was done in PCR thermal cycler (MultiGene^{TM} II, LabNet, Edison, USA), consisting of an initial denaturation at 95°C for 4 minutes, followed by 35 cycles of 95°C for 30 seconds, 54°C for 30 seconds, and 72°C for 30 seconds then finally an extension of 72°C for 5 minutes was included.

3.10.4 Agarose gel electrophoresis

The PCR products were confirmed by electrophoresis on 1.5% agarose gel as in the following procedure. The gel was prepared by dissolving 1.5 g of agarose powder in 100 mL of 1×Tris-acetate EDTA (TAE) buffer (Lonza, USA) (Appendix F, No.6). The mixture was heated until the agarose had completely dissolved. The solution was allowed to cool down to 65ºC and subsequently added with 4 µL of Atlas ClearSight DNA Stain (Bioatlas, Estonia), mixed and poured into a gel-casting tray with a comb (1 mm thick). The gel was allowed to solidify at room temperature and the comb was carefully removed. The tray was then placed into the electrophoresis tank containing 1× TAE buffer. The PCR product (10 µL) was mixed with 6× loading dye (2 µL; Thermo Scientific), and was loaded into the well of the gel. Also, 5 µL of 100 bp DNA ladder (Thermo Scientific) and 5 µL of dH_2O were used as a standard marker and negative control, respectively. The gel was set to run at 100 volts for 30 minutes and the DNA

bands were visualized under ultraviolet (UV) trans illuminator (3UV^{TM} Trans illuminator, LMS-20E, UVP, USA).

3.10.5 Extraction and purification of PCR products

After electrophoresis, the PCR bands were cut and the desired DNA fragment was extracted using QIAquick gel extraction kit (QIAGEN) and purified according to the manufacturer's description. In summary, the amplified band was excised from the gel and weighed. Three volumes of Buffer QG was added to one volume of the gel (100 mg ~ 100 mL) and heated at 50°C for 10 minutes to dissolve the gel slice. To help dissolving gel, the mixture was mixed by vortexing the tube every 2-3 minutes during the incubation period. Equivalent of one gel volume of isopropanol was added and mixed. The mixture was transferred into a QIAquick spin column in a 2-mL collection tube and centrifuged for 1 minute to bind the DNA to the filter. The column was washed with Buffer QG to remove all traces of the gel from the DNA. The column was then washed again with Buffer PE and the column was centrifuged again to ensure complete removal of any residual ethanol from the Buffer PE. Then the column was placed into a sterile 1.5-mL centrifuge-tube and the DNA was eluted with 30 μL of Buffer EB.

3.10.6 Cloning of PCR products

Cloning of the purified DNA of PCR product was carried out using the vector pGEM®-T Vector (Promega, Madison, USA) and was amplified in *Escherichia coli* JM109 competent cells (Promega). The cloning procedure is described below:

3.10.6.1 Ligation

Ligation is the initial step in cloning procedure that involves the insertion of the desired DNA fragment into the vector. First the pGEM®-T Vector DNA fragment, buffer and enzyme were all mixed together. The mixture was incubated overnight at 4°C. The volumes for the mixture are: 5 µL of 2X Rapid Ligation Buffer, 3 µL DNA (in PCR product), 1µL each of pGEM®-T Vector (50 ng) and T4 DNA Ligase (3 Weiss units/µL). By the end of the overnight incubation, it is expected that the vector must have successfully incorporated the DNA fragment into its own to form a recombinant DNA molecule with the help of the enzyme ligase.

3.10.6.2 Transformation

Transformation was carried out to ligate the plasmid into competent bacterial cells (*E. coli* strain, JM 109). In the host cell, the plasmid multiplies, producing numerous identical copies, including the recombinant DNA. After several cell divisions, a colony or clone of identical host cells is produced. Each cell in the clone contains one or more copies of the recombinant DNA molecule (Brown, 2016). The transformation process is performed as below:

Tubes containing the ligation reactions were centrifuged and 5 µL of the sediment was transferred into a sterile 1.5 mL micro centrifuge tube and placed on ice. *Escherichia coli* (JM109) high efficiency competent cells (Promega) were removed from freezer and placed on ice until it thawed (about 5 minutes). The cells were mixed by gently tapping the tube (to ensure proper suspension and avoid destruction of the cells as they are very fragile), 50 µL of the cells were added to the tubes containing the ligation reactions and mixed gently. The tubes were incubated on ice for 30 minutes

then at 42ºC for 45 − 60 seconds in a water bath (heat-shocked) and quickly returned to ice for 2 minutes. Then 950 µL of LB broth (Appendix F, No. 7) was transferred into the tubes containing competent cells with ligation reactions, and was incubated at 37°C for 2 hours at 250 rpm in a shaking incubator. The tubes were then centrifuged at 5000 rpm for 5 minutes and the sediment was resuspended in 200 µL of LB broth. A 100 µL of each transformation culture was plated evenly on each of two LB/ampicillin/IPTG/ X-Gal plates. The plates were allowed to set at room temperature until the transformation mixture was fully absorbed into the agar. Then the plates were inverted and incubated overnight (16–24 hours) at 37°C.

3.10.6.3 Selection of white colonies for recombinant clone

The overnight incubation ensured growth of recombinant colonies that are distinguished based on their colours. The principle is based on the successful cloning of a gene into the pGEM® Vector, interrupts the coding sequence of an enzyme β-galactosidase. Presence or absence of the enzyme β-galactosidase among the recombinant colonies involves a lactose analogue called X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) which when broken down by β-galactosidase produce a deep blue coloured product. When X-gal (plus an inducer of the enzyme such as isopropylthiogalactoside, IPTG) was added to the agar, along with ampicillin, non-recombinant clones that synthesized β-galactosidase, produced blue colonies. Whereas recombinants that are unable to make β-galactosidase, produced white colonies (Brown, 2016). Each of the white (recombinant) colonies were numbered using a permanent marker and subcultured into a new LB-ampicillin plate with the corresponding numbering. The plates were placed upside down in a 37°C incubator for 12-24 hours.

3.10.6.4 Identification of recombinant colony by PCR

To confirm the presence and orientation of the DNA cloned, PCR was employed. The white colonies were individually picked with a sterile pipette tip and suspended in 50 μL of the PCR master mix (as in section 3.10.3 with the same primers), and then subjected to PCR amplification (as described in section 3.10.3). The PCR products were electrophoresed in an agarose gel for the appropriate band size (approximately 500 bp).

3.10.7 DNA sequencing of recombinant clones

Three to four clones containing inserts of the expected size were randomly selected for each sample and sequenced on both strands using M13 forward (5'GTA AAA CGA CGG CCA GT'3) and M13 reverse primers (5'GCG GAT AAC AAT TTC ACA CAG G'3). Sequencing was carried out using the ABI Big Dye® Terminator Cycle Sequencing Ready Reaction Kit v3.1, using the ABI PRISM® 3730xl DNA Analyser (Applied Biosystems, USA).

3.10.8 Phylogenetic analyses of *Blastocystis* subtypes

Upon receiving the vector sequence chromatograms, sequence of the inserted DNA fragment was isolated by Gene Runner software (version 3.05). These sequences were BLAST in the Genbank database for confirmation. *Blastocystis* subtypes were identified by determining the exact match or closest similarity against all known *Blastocystis* subtypes according to the last classification by Stensvold *et al*. (2007b). Sequences downloaded from Genbank as well as those obtained from this study were aligned using ClustalW of Bio Edit software (version 7.0.9.0) (Hall, 1999).

Phylogenetic tree was constructed using the neighbor-joining methods. The neighbor-joining (NJ) method was carried out using the software MEGA version 4 (Tamura *et al*., 2007) and molecular distances were estimated by the Kimura two-parameter model (Kimura, 1980). Branch reliability was assessed using bootstrap analysis (1000 replicates). *Proteromonas lacerate* (U37108), an organism phylogenetically closely related to *Blastocystis* sp. was used as the out-group.

CHAPTER 4: RESULTS

4.1 General characteristics of the respondents

Three types (faecal, urine and blood) of samples from each of the 551 participants of at least 1 year old and above were successfully collected. Of these, 340 (61.7%) were males and 211 (38.3%) were females comprising of 171 (31.0%) children below the age of 15 years and 380 (69.0%) adolescents and adults. They were 127 (23.0%) from Kura, 119 (21.6%) from Bebeji, 97 (17.6%) from Gwarzo, 99 (18.0%) from Shanono and 109 (19.8%) from Minjibir, respectively. Overall, 191 (34.7%) respondents had attained at least 6 years of formal-education while only 270 (49.0%) were employed. Accordingly, those with an overall family monthly income of NGN3200 (NGN=Nigerian Naira 3200 = USD200) and above were 320 (58.1%).

Characteristically, the Hausas live in extended family mode with many members in a large house, thus 267 (48.5%) of the respondents had 1–10 members in the family, 183 (33.2%) had 11−20 family members and 101 (18.3%) had > 20 family members. Typically, in all the rural communities houses are made up of mostly mud (78%) or concreate (27%). This followed by mud floors, 57.2%, 40.3% were concrete and 2.5% were tiled. Majority (86.0%) of the respondents had electricity supply but not constant. All houses except 5 (located in bush encamped settlements) had functioning toilets, but mostly (86.4%) were of the traditional pit latrines. About two thirds of the houses had piped water supplies (64.6%). A little more than one-third 228 (41.4%) of the participants live with domestic animals around and claimed they had contact with the animals or their dung. Table 4.1 provides detailed demographic, socioeconomic and general characteristics of the respondents.

Table 4.1: General characteristics of the respondents

VARIABLE	N (551)	%
Gender		
Male	340	61.7
Female	211	38.3
Age groups (yrs)		
Adults>15	380	69.0
Children≤15	171	31.0
Address		
Kura	127	23.0
Bebeji	119	21.6
Gwarzo	97	17.6
Shanono	99	18.0
Minjibir	109	19.8
Educational status		
At least 6years of primary	191	34.7
Less than 6 years of primary	360	65.3
Employment status		
Employed	270	49.0
Unemployed	281	51.0
Monthly income per house		
> NGN 3200	320	58.1
≤ NGN 3200	231	41.9
Family size		
1-10 members	267	48.5
11-20 members	183	33.2
>20 members	101	18.3
Type of housing		
Mud	430	78.0
Concrete	121	22.0
Type of floor		
Mud	315	57.2
Concrete	222	40.3
Tiled	14	2.5
Have electricity	474	86.0
Type of Toilet facility		
Pour flush toilet	70	12.7
Pit latrine	476	86.4
No Toilet	5	0.9
Use treated water for		
Drinking	356	64.6
Domestic purposes	348	63.2
Have domestic animals	228	41.4

NGN (Nigerian naira); N (number examined)

Table 4.2: Frequency of risk factors of parasites infection among the respondents

Risk factor	n	%
Having treated water supply	203	36.8
Unsafe toilet facility	481	87.3
Have domestic animals	228	41.4
Have contact with domestic animals	242	43.9
Eat with hands	461	83.7
Wash hands before eating	524	95.1
Wash hands after toilet	492	89.3
Wash vegetables before consumption	317	57.5
Wash fruits before consumption	403	73.1
Lives near stagnant water body	380	69.0
Have contact with stagnant water	139	25.2
Cutting nails frequently	366	66.4
Not wearing shoes outside house	142	25.8
Not having bed nets	113	20.5
Do not use bed nets	202	36.7
Do not use insecticide	348	63.2

n (respondents number with respective criteria)

From the respondents' responses, many possible risk factors that probably predisposed the population to parasitic infections are presented in Table 4.2. These included not having treated water supply (36.8%) and using unsafe toilet facility (87.3%). The habit of eating with hands, a culture of the Hausa community was practiced by majority (83.7%) of the respondents. However, they claim to wash their

hands before eating (95.1%) and after going to toilet (89.3%). Most respondents considered washing their fruits and vegetables before eating (73.1% and 57.5%, respectively). Even though quite a number of the respondents (69.0%) lives near a water body, yet majority claimed not having contact with the water body. Also the tendency of not wearing shoes outside house was less (25.8%). Though only 20.5% of the respondents have no bed net, yet 36.7% do not use the nets and many do not use insecticide (63.2%).

4.2 Prevalence of parasite species infection as detected by microscopy

A total of 15 parasites (genus / species) were recovered from the three types of samples (blood, faeces and urine) using light microscopy and are shown in Table 4.3. Amongst which includes a hemoprotozoan in the blood, intestinal protozoan and helminths in faeces, blood flukes in faeces and urine, and a vaginal protozoan in urine samples. Overall 463 (84%) participants were infected with the above-mentioned parasite species (Figure 4.1 A). Complete range of parasitic genus/species recovered and their percentage is presented in Figure 4.1 B. The most prevalent was *Plasmodium* (60.6%), followed by *Blastocystis* (29.2%), *Entamoeba* (16.3%) [Including *E. histolytica* 14.3% (13), *E. dispar* 33.3% (30) and *E. moshkhovskii* 52.4% (47) revealed from molecular study that is not report in detail in this study], hookworms (15.4%), *Schistosoma mansoni* (9.4%), *Giardia intestinalis* (9.4%), *Schistosoma hematobium* (8.9%), *Ascaris lumbricoides* (8.0%), *Cryptosporidium* (7.4%), *Trichomonas vaginalis* (5.6%), *Strongyloides stercoralis* (3.4%), *Fasciola hepatica* (2.5%), *Balantidium coli* (2.2%), *Entrobius vermicularis* (2.0%) and the least was *Hymenolepis nana* (0.9%).

Table 4.3: Parasite species recovered from a single collection of faecal, urine and blood among 551 volunteers of Hausa communities

Parasite	Designated	Infected Sample (%)		
		Blood (n=551)	Faecal (n=551)	Urine (n=551)
1. *Plasmodium* sp.	*a*	334 (60.6)	-	-
2. *Blastocystis* sp.	*b*	-	161 (29.2)	-
3. *Entamoeba species*	*c*	-	90 (16.3)	-
4. Hookworm species	*d*	-	85 (15.4)	-
5. *Schistosoma mansoni*	*e*	-	52 (9.4)	-
6. *Giardia intestinalis*	*f*	-	52 (9.4)	-
7. *Schistosoma haematobium*	*g*	-	-	49 (8.9)
8. *Ascaris lumbricoides*	*h*	-	44 (8.0)	-
9. *Cryptosporidium species*	*i*	-	41 (7.4)	-
10. *Trichomonas vaginalis*	*j*	-	-	31 (5.6)
11. *Strongyloides stercoralis*	*k*	-	19 (3.4)	-
12. *Fasciola hepatica*	*l*	-	14 (2.5)	-
13. *Balantidium coli*	*m*	-	12 (2.2)	-
14. *Enterobius vermicularis*	*n*	-	11(2.0)	-
15. *Hymenolepis nana*	*o*	-	5 (0.9)	-

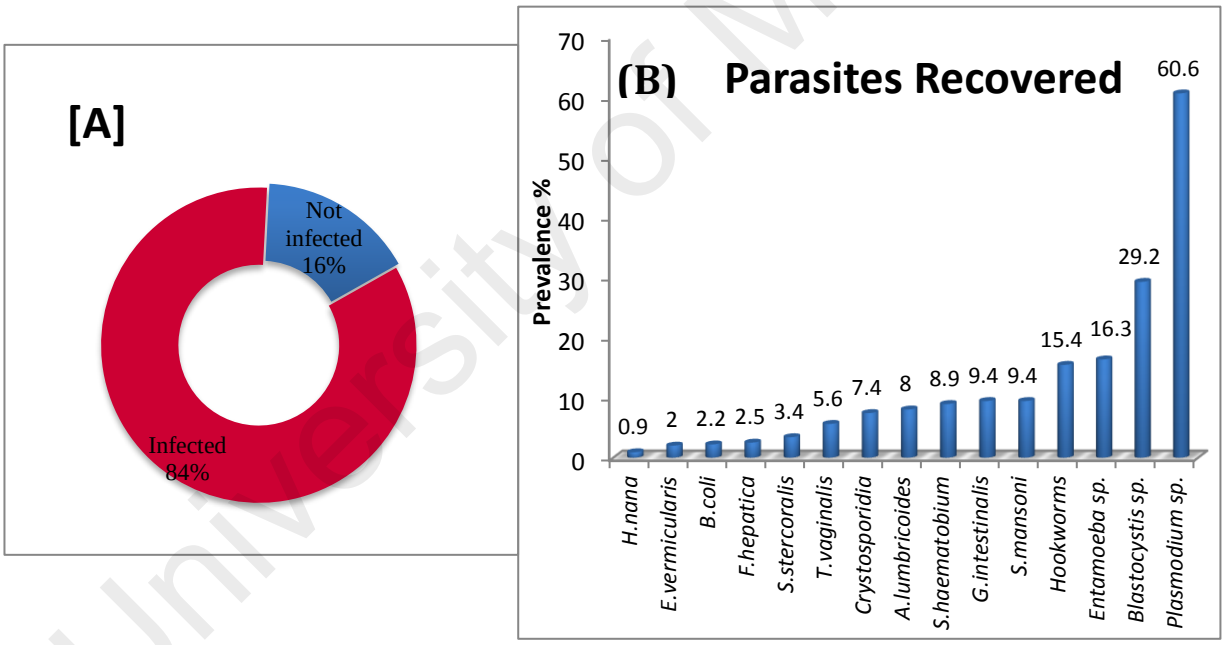

Figure 4.1 (A and B): Prevalence of parasitic infections in five communities of Kano State.

Table 4.4 presents overall prevalence of infection by location with gender and age groups. Prevalence of parasitic infections varied with location, which was most prevalent in Kura (19.1%), followed by Minjibir (17.2%), Bebeji (16.9%), Gwarzo (15.8%) and least in Shanono (15.1%). Among the infected respondents from Kura

majority were males (14.3%) and adults (15%). Among the parasite species recovered were malaria (13.4%) as the most prevalent, *Blastocystis* sp. (6.4%), hookworms (4.4%), *S. mansoni* (3.1%), *Entamoeba histolytica / dispar / moshkhovskii* (2.7%) and *G. intestinalis* (2.4%). Infection with *H. nana* was not seen amongst Kurans. In contrast, in Bebeji parasitic infections were higher among females (8.9%) and adults (14.3%). Amid respondents from Bebeji, malaria (12.3%) was most prevalent followed by *Blastocystis* sp. (5.6%), *Entamoeba histolytica / dispar / moshkhovskii* (2.9%) and hookworms (2.5%). Lowest prevalence was recorded in infection with the trematodes, *F. hepatica* and *H. nana* (0.2% each).

Respondents with parasitic infections in Gwarzo, were mostly males (14.7%) and younger than 15 years of age (10.3%). Regarding parasite species, *Plasmodium* and *Blastocystis* sp. (11.3% and 6.4%, respectively) were most prevalent, followed by *S. haematobium* (4.4%) least were *Cryptosporidium* and *B. coli* (0.2% each). Infection with *H. nana* was not found in Gwarzo. However, in Shanono more females (8.2%) than males had parasitic infections, and more in adults (12.5%) above 15 years old. Malaria (10.7%) was most prevalent and infection with hookworms parallels *Blastocystis* sp. (3.8%) among respondents from Shanono. Least prevalence was recorded in infection with *A. lumbricoides* and *F. hepatica* (0.4% each) while *E. vermicularis* was not seen. Considering Minjibir, parasitic infections were more prevalent amongst males (10%) and adults (10.5%). Same as in all of these rural communities, malaria and *Blastocystis* sp. (12.9% and 7.1%, respectively) were the highest recorded infections. Subsequent infections were with *Entamoeba histolytica / dispar / moshkhovskii* (4.2%), *Giardia intestinalis* (2.5%) and *Cryptosporidium* sp. (2.0%). Infections with *E. vermicularis* and *H. nana* were not recorded in Minjibir.

Table 4.4: Distribution of parasitic infections among the population in Kano State by gender and age (N = 551)

Site	Number of infected subject (%) by gender and age groups			Number of infected subject and recovered parasite species from a single collection of faecal, urine and blood samples														
	(M=male, F=female)			*a*	*b*	*c*	*D*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*o*
Kura	M 79 (14.3)	≤15yrM 18(3.4)	≤15years 23 (4.2)	12	4	4	3	4	2	1		2	2	1	2	2	2	
n=127		>15yrM 61(11.1)		45	20	5	19	10	5	1	7	6	5	2	3	2	1	
	F 26 (4.7)	≤15yrF 5(0.9)	>15years 82(15.0)	3	1					1		3	1					
		>15yrF 21(3.8)		14	10	6	2	3	6	1	3			1	2		1	
Total (%)	105 (19.1)			74	35	15	24	17	13	4	10	11	8	4	7	4	4	0
				(13.4)	(6.4)	(2.7)	(4.4)	(3.1)	(2.4)	(0.7)	(1.8)	(2.0)	(1.5)	(0.7)	(1.3)	(0.7)	(0.7)	
Bebeji	M 44 (8.0)	>15yrM 10(1.8)	≤15years 14 (2.5)	5	4		2		2	3	1				1	1		
n=119		≤15yrM 34(6.2)		25	16	6	7	2	2	5	5	3	1	1		1		
	F 49 (8.9)	>15yrF 4(0.7)	>15years 79(14.3)	3	2	1	2	1	2		1						1	
		≤15yrF 45(8.2)		35	9	9	5	3	2		4	5	2	2			4	1
Total (%)	93 (16.9)			68	31	16	14	6	8	8	11	8	3	3	1	2	5	1
				(12.3)	(5.6)	(2.9)	(2.5)	(1.1)	(1.5)	(1.5)	(2.0)	(1.5)	(0.5)	(0.5)	(0.2)	(0.4)	(0.9)	(0.2)
Gwarzo	M 81 (14.7)	≤15yrM 56(10.2)	≤15years 57(10.3)	40	24	9	10	3	5	15	7		6	2		1		
n=97		>15yrM 25(4.5)		18	10	6	6	4	3	8	6	1	1		2		2	
	F 6 (1.1)	≤15yrF 1(0.2)	>15years 30 (5.4)	1														
		>15yrF 5(0.9)		3	1	1	1		1	1	1		1					
Total (%)	87 (15.8)			62	35	16	17	7	9	24	14	1	8	2	2	1	2	0
				(11.3)	(6.4)	(2.9)	(3.1)	(1.3)	(1.6)	(4.4)	(2.5)	(0.2)	(1.5)	(0.4)	(0.4)	(0.2)	(0.4)	
Shanono	M 38 (6.9)	≤15yrM 10(1.8)	≤15years 14 (2.5)	4	4	3	4	1	1	2	1			1				2
n=99		>15yrM 28(5.1)		21	10	8	7	4	4	1		2	2	4	1			1
	F 45 (8.2)	≤15yrF 4(0.7)	>15years 69(12.5)	3	1	1		2	1			1	1					
		>15yrF 41(7.4)		31	6	8	10	9	2	2	1	7	2	2	1	4		1
Total (%)	83 (15.1)			59	21	20	21	16	8	5	2	10	5	7	2	4	0	4
				(10.7)	(3.8)	(3.6)	(3.8)	(2.9)	(1.5)	(0.9)	(0.4)	(1.8)	(0.9)	(1.3)	(0.4)	(0.7)		(0.7)
Minjibir	M 55 (10.0)	≤15yrM 33(6.0)	≤15years 37 (6.7)	23	15	10	3	1	6	4	2	4	1	1				
n= 109		>15yrM 22(4.0)		15	7	2		1	1	2	1	1	1	1	1			
	F 40 (7.3)	≤15yrF 4(0.7)	>15years 58(10.5)	2	1	1			1		1	2	1					
		>15yrF 36(4.5)		31	16	10	6	4	6	2	3	4	4	1	1	1		
Total (%)	95			71	39	23	9	6	14	8	7	11	7	3	2	1	0	0
	(17.2)			(12.9)	(7.1)	(4.2)	(1.6)	(1.1)	(2.5)	(1.5)	(1.3)	(2.0)	(1.3)	(0.5)	(0.4)	(0.2)		
Grand total	463			334	161	90	85	52	52	49	44	41	31	21	14	12	11	5
(%)	(84.0)			(60.6)	(29.2)	(16.3)	(15.4)	(9.4)	(9.4)	(8.9)	(8.0)	(7.4)	(5.6)	(3.8)	(2.5)	(2.2)	(2.0)	(0.9)

a (*Plasmodium* spp.), *b* (*Blastocystis* sp.), *c* (*Entamoeba* complex), *d* (Hookworm species), *e* (*Schistosoma mansoni*), *f* (*Giardia intestinalis*), *g* (*Schistosoma haematobium*), *h* (*Ascaris lumbricoides*), *i* (*Cryptosporidium* species), *j* (*Trichomonas vaginalis*), *k* (*Strongyloides stercoralis*), *l* (*Fasciola hepatica*), *m* (*Balantidium coli*), *n* (*Enterobius vermicularis*), *o* (*Hymenolepis nana*), % (percentage), n (sample size),

4.3 Prevalence of single and multiple parasitic infections

Overall, 51.2% (282/551) of the participants had polyparasitism and 32.8% (181/551) had monoparasitism. That said, among the 463 infected participants 282 (60.9%) individuals had polyparasitism while 181 (39.1%) had only one parasite specie infection (figure 4.2A). Regarding gender, both single and multiple parasitic infections were almost doubled in male than in female respondents. Similarly, prevalence of single and multiple parasitic infections in adults were doubled that in children. In Male respondents, single infection was slightly more than multiple infections (64.6% vs 63.7%). However in females multiple infections was seen slightly more than single infection (36.3% vs 35.4%). Although, prevalence of single infections was marginally higher than multiple infections (69.6% vs 68.3%) among the adult respondents, in children the reverse was the case (Figure 4.2 B).

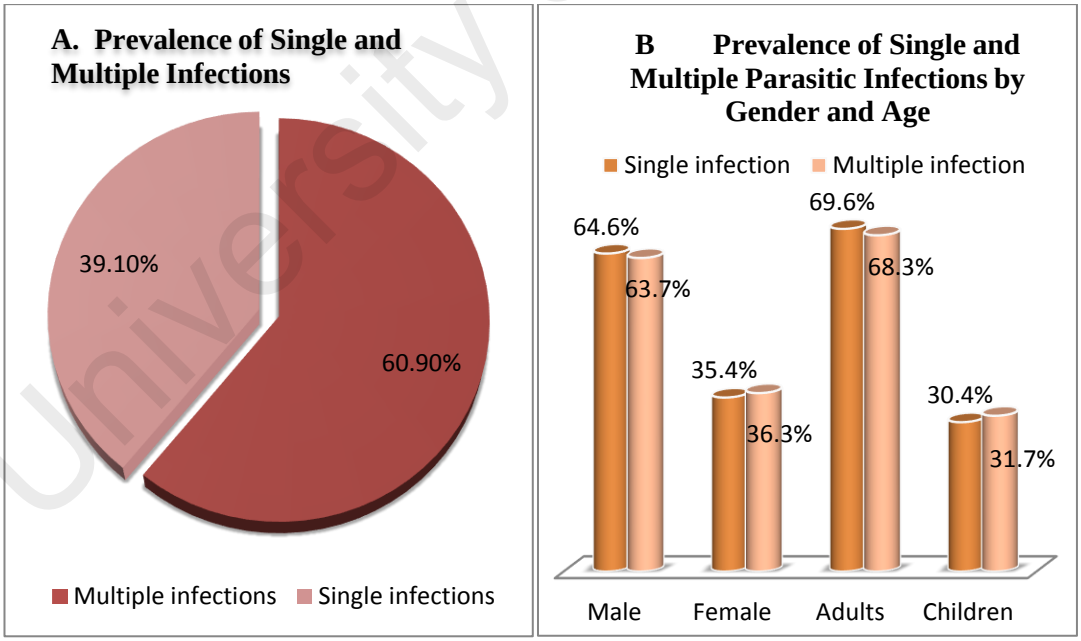

Figure 4.2 (A and B): Prevalence of single and multiple parasitic infections in the study

4.3.1 Distribution of single parasitic infection among the study population by gender, age and location

Table 4.5 presents details of single species parasitic infection in this study. Generally, 181 (39.1%) respondents were infected with only one parasite species and *Plasmodium* (23%) had the highest prevalence. Up to 3.3% of respondents were infected with only *Blastocystis* sp. followed by 1.5% with *S. haematobium* and 1.3% had only hookworms. Prevalence of *A. lumbricoides* and *Cryptosporidium species* was 1.1% each, for *Entamoeba histolytica / dispar / moshkhovskii* and *Giardia intestinalis* was 0.5% each, while *T. vaginalis* and *S. stercoralis* was 0.4% each. *S. mansoni* and *F. hepatica* showed the least prevalence of 0.2% each. Other parasite species recovered in the study were not found in the single specie infections among the study population. Regarding the study locations, prevalence of single specie infection was highest in Kura (8.5%), then Bebeji (6.9%) and Minjibir (6.7%). Lower prevalence was recorded among respondents from Gwarzo (5.4%) and Shanono (5.3%). Prevalence of infection was higher among male than female respondents in Kura (6.5%), Gwarzo (5.1%) and Minjibir (4.7%) whereas in Bebeji and Shanono prevalence was higher in females. Regarding age, prevalence was predominantly higher among adults in all the rural areas except in Gwarzo where prevalence was considerably higher in children than in adults (4.2% vs 1.3%).

Regarding single infection with specific parasite species, in all the studied areas, plasmodiasis showed the highest prevalence. Among residents of Kura and Bebeji infection with *Blastocystis* sp. was second followed by infections with hookworms and *Cryptosporidium species* but among residents of Gwarzo, *S. haematobium* infection was second then followed by infection with *Blastocystis* sp, hookworms and *T. vaginalis*. In Shanono, infections with hookworms and *Cryptosporidium species* were the second most prevalent single infection while *Blastocystis* sp. was completely absent. In

Minjibir community, infection with *Blastocystis* sp. was second followed by *S. haematobium.*

Table 4.5: Prevalence of single parasitic infection among the study population by gender, age and location (N = 181)

Site	Number of infected subject (%) by gender and age groups			Number of subject infected with a single parasite species recovered from a single collection of faecal, urine and blood samples											
	(M=male, F=female)			*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*
Kura	M 36 (6.5)	≤15yrsM 8 (1.5)	≤15yrs 10 (1.8)	7			2							1	
n=127		>15yrsM 28 (5.1)		23					1	1		1			1
	F 11 (2.0)	≤15yrs F 2 (0.4)	>15yrs 37 (6.7)	1								1			
		>15yrs F 9 (1.6)		6	3										
Total %)	47 (8.5)			37 (6.7)	3 (0.5)		2 (0.4)		1 (0.2)	1 (0.2)		2 (0.4)		1 (0.2)	1 (0.2)
Bebeji	M 14 (2.5)	≤15yr M 4 (0.7)	≤15yrs 4 (0.7)	2	1				1						
n=119		>15yrs M 10 (1.8)		6	2		1					1			
	F 24 (4.4)	≤15yrs F 0	>15yrs 34 (6.2)												
		>15yrs F 24 (4.4)		20		1						1	1	1	
Total (%)	38 (6.9)			28 (5.1)	3 (0.5)	1 (0.2)	1 (0.2)		1 (0.2)			2 (0.4)	1 (0.2)	1 (0.2)	
Gwarzo	M 28 (5.1)	≤15yrs M 22 (4.0)	≤15yrs 23 (4.2)	13	3	1				3			2		
n=97		>15yrs M 6 (1.1)		2	1		1			2					
	F 2 (0.4)	≤15yrs F 1 (0.2)	>15yrs 7 (1.3)	1											
		>15yrs F 1 (0.2)					1								
Total (%)	30 (5.4)			16 (2.9)	4 (0.7)	1 (0.2)	2 (0.4)			5 (0.9)			2 (0.4)		
Shanono	M 13 (2.4)	≤15yrs M 3 (0.6)	≤15yrs 4 (0.7)	2			1								
n=99		>15yrs M 10 (1.8)		7		1	1						1		
	F 16 (2.9)	≤15yrs F 1 (0.2)	>15yrs 25 (4.5)	1											
		>15yrs F 15 (2.7)		12				1				1	1		
Total (%)	29 (5.3)			22 (4.0)		1 (0.2)	2 (0.4)	1 (0.2)				1 (0.2)	2 (0.4)		
Minjibir	M 26 (4.7)	≤15yrs M 12 (2.2)	≤15yrs 14 (2.5)	8						1		1			
n=109		>15yrs M 14 (2.5)		8	4				1	1					
	11 (2.0)	≤15yrs F 2 (0.4)	>15yrs 23 (4.2)	1							1				
		>15yrs F 9 (1.6)		7	2										
Total (%)	37 (6.7)			24 (4.4)	8 (1.5)				1 (0.2)	2 (0.4)	1 (0.2)	1 (0.2)			
GT (%)	181 (32.8)			127 (23.0)	18(3.3)	3(0.5)	7(1.3)	1(0.2)	3(0.5)	8(1.5)	6(1.1)	6(1.1)	2(0.4)	2(0.4)	1(0.2)

a (*Plasmodium* spp.), *b* (*Blastocystis* sp.), *c* (*Entamoeba* complex), *d* (Hookworm species), *e* (*Schistosoma mansoni*), *f* (*Giardia intestinalis*), *g* (*Schistosoma haematobium*), *h* (*Ascaris lumbricoides*), *i* (*Cryptosporidium* species), *j* (*Trichomonas vaginalis*), *k* (*Strongyloides stercoralis*), *l* (*Fasciola hepatica*), % (percentage), n (sample size), GT (grand total)

4.3.2 Distribution of multiple parasitic infections among the study population by gender, age and location

Distribution and prevalence of multiple parasitic infections among the respondents are presented in Table 4.6. Overall, 282 (51.2%) respondents had two or more simultaneous parasitic infections. In all the localities, multiple parasitic infections were seen most in Minjibir (10.5%), followed by Kura and Gwarzo (10.3% each), Bebeji (10%) and Shanono (9.8%) had the lowest prevalence. Disparities observed when stratifying by gender of the respondents included higher frequencies in males from Kura, Bebeji and Gwarzo. However, in Minjibir similar prevalence was recorded among male and female respondents (5.3%) and in Shanono, prevalence was higher among females (5.3% vs 4.5%). Relatedly, prevalence of multiple infections was considerably higher among adults than children in all these rural areas except in Gwarzo where 6.2% of children had multiple parasitic infections compared to 4.2% of adults.

Several variations were observed regarding the explicit number of multiple infections. In Figure 4.3, double parasitic infections were recorded in all communities and highest in Minjibir (5.6%) and Bebeji (5.3%) but low in Shanono (4.2%). Triple infections were high among respondents from Gwarzo (3.8%), Shanono (3.6%) and Bebeji (3.3%), but low in Minjibir (2.2%). Nonetheless, concurrent infections with four parasite species were most prevalent amid respondents from Kura (2.0%) and Gwarzo (1.3%), least was among respondents from Bebeji (1.1%). Infection with five parasite species simultaneously was mostly in Minjibir (0.7%) followed by Kura (0.5%), Bebeji (0.5%) and Shanono (0.5%) while only one respondent had five concurrent infections from Gwarzo. Infection with six parasite species concurrently was observed in four respondents from Gwarzo and one individual each from Shanono and Minjibir. None was observed in Kura and Bebeji. Besides, infection with seven parasite species was

seen only among three respondents from Kura and one case with eight species also in Kura.

Concerning coinfections with specific parasite species, Table 4.6 revealed that dual infections in Kura were mostly malaria / *Entamoeba* complex and malaria / hookworms indicating malaria drives the coinfection. In Bebeji and Gwarzo, malaria / *Entamoeba* complex and *Entamoeba* complex / *S. haematobium* predominated although there was an increase of *Blastocystis* sp. / *Entamoeba* complex. In Shanono, double infections were led by *Entamoeba* complex / *S. mansoni* and *Entamoeba* complex / *Cryptosporidium* species. Whereas, in Minjibir, complexes of malaria / *Entamoeba*, then malaria / *Blastocystis* sp. and *Blastocystis* sp. / *Entamoeba* were leading. Triple coinfections were dictated by *Entamoeba* complex with an unspecific pattern. In Kura, triple coinfections mostly involved *Entamoeba* complex / hookworms / *A. lumbricoides*. In Bebeji, it was the *Entamoeba* complex / malaria / *Blastocystis* sp. coinfection and in Gwarzo it was *Entamoeba* complex / malaria / *A. lumbricoides* alongside *Entamoeba* complex / malaria / *S. haematobium.*

Infection with four parasite species presented dominance of *Entamoeba* complex / malaria / hookworm / *A. lumbricoides* and *Entamoeba* complex / malaria / *Blastocystis* sp. / *G. intestinalis* in Kura. Moreover in Gwarzo, coinfection with quadruple parasitic species involved *Entamoeba* complex / malaria / hookworm / *S. haematobium* in male respondents. Then in Minjibir, coinfection with quadruple parasitic species was propelled by malaria mostly in combination of malaria / *Blastocystis* sp. / *Entamoeba* complex. However in Bebeji and Shanono, there were no specific combinations although *Entamoeba* complex predominated. Coinfection with five parasitic species involved *Entamoeba* complex / *Blastocystis* sp. in all the sites. Additionally, malaria and hookworms infections further complicated the coinfection in 2 out of the three cases

in Kura, Shanono and the only case in Gwarzo. Regarding concurrent infection with six parasite species, malaria and *S. haematobium* were the most frequent in Gwarzo. As in Minjibir the only respondent was harbouring malaria / *Blastocystis* sp. / *Entamoeba* complex / *S. mansoni* / *S. haematobium* / *A. lumbricoides* simultaneously. Multiple infections with seven species were observed only in Kura and in all three instances malaria / *Blastocystis* sp. / hookworms / *S. mansoni* subjugated. Coinfection involving eight parasitic species (malaria, *Entamoeba* complex, hookworms, *S. mansoni*, *S. haematobium*, *F. hepatica*, *B. coli* and *E. vermicularis*) was seen in one young male respondent from Kura.

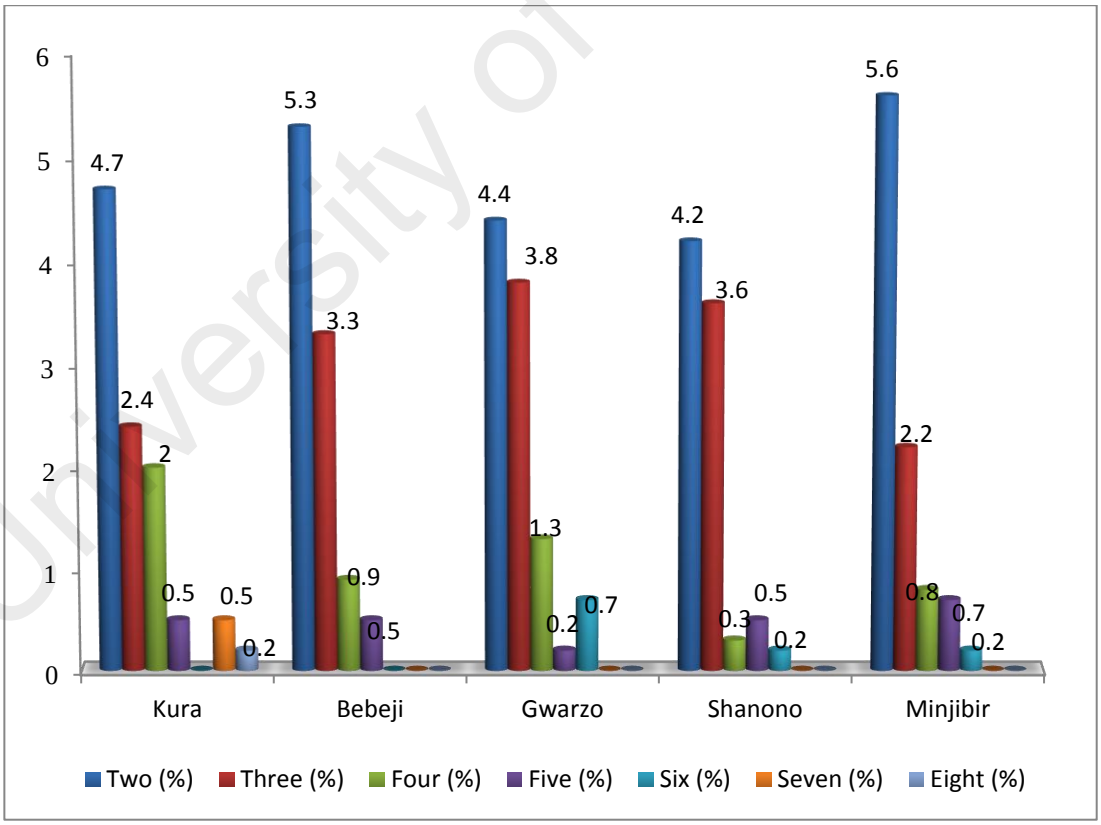

Figure 4.3: Prevalence of multiple parasitic infections by the study communities

Table 4.6: Multiple parasite species recovered from infected participants by gender, age and location (N = 282)

Site	Number of infected subject (%) by gender (M=male, F=female) and age groups			Number of subject infected with multiple parasite species recovered from a single collection of faecal, urine and blood samples						
				Two	*Three*	*Four*	*Five*	*Six*	*Seven*	*eight*
Kura **n**=127	M 42 (7.6)	≤15yrsM 9 (1.6)	≤15yrs 12 (2.2)	*aj, ej, bi, ce,* (4)	*cfn, abl,* (2)	*cbfi,* (1)	*abcem,* (1)			*Acdeglmn (*1)
		>15yrs M 33 (6.0)		*bf, ad, ad, ae, ae, de, ch, cj, cj, ac, cd, ad, ac, ac, hi, ac* (16)	*aek, bfl, aci, cde, bdj, cdh, cid,* (7)	*acdh, acdh, acfj, acin, cikl, acde* (6)	*abcde, abcde* (2)		*abcdehj, abcdemn*(2)	
	F 15 (2.7)	≤15yrs F 3 (0.5)	>15yrs 45 (8.2)	*ai, ci* (2)	*cgj,* (1)					
		>15yrs F 12 (2.2)		*ef, ab, ac, ec* (4)	*cdh, acf, bck,* (3)	*abcf, abfh, cghl, abcf,* (4)			*abdefln* (1)	
Total (%)	57 (10.3)			26 (4.7)	13 (2.4)	11 (2.0)	3 (0.5)	0	3 (0.5)	1 (0.2)
Bebeji **n**=119	M 30 (5.4)	≤15yrsM 6 (1.1)	≤15yrs 10 (1.8)	*ag, lm, ad, cg,* (4)	*cdg,* (1)	*Acfh* (1)				
		>15yrs M 24 (4.4)		*ab, ac, cg, dg, ch, ce, ac, ac, ci, cg, ck,* (11)	*abd, bcd, acd, agh, abd, acg, abc, ach, ach, acf* (10)	*abcd, cfhi, cejm (3)*				
	F 25 (4.5)	≤15yrs F 4 (0.7)	>15yrs 45 (8.2)	*ah, cf,* (2)		*acdf,* (1)	*Bcden* (1)			
		>15yrs F 21 (3.8)		*eh, np, bc, ao, bh, ac, cd, ci, ac, ci, ac, fi, cn* (13)	*abc, abh, bcd, abc, acj, cin,* (6)	*aceh,* (1)	*Abcef* (1)			
Total (%)	55 (10.0)			30 (5.4)	17 (3.1)	6 (1.1)	2 (0.4)	0	0	0
Gwarzo **n**=97	M 53 (9.6)	≤15yrsM 34 (6.2)	≤15yrs 34 (6.2)	*ac, cg, ac, bj, df, cd, bc, cf, cg, ac, ac, ac, ac,* (13)	*agh, acg, abc, aef, ach, cdj, cgj, acg, acg, bcj, bdk, ach, ach, acf, ehl* (15)	*bghk, acdg, aceg, acdg,* (4)	*abcdm,* (1)	*abdfgh,* (1)		
		>15yrs M 19 (3.4)		*cg, bc, ac, cg, cg, bc, cl, cd* (8)	*ace, gij, cdh, abc, ace* (5*)*	*abcd, achn, cdeh (3)*		*abdfgh,abcef h acfghn* (3)		
	F 4 (0.7)	≤15yrs F 0	>15yrs 23 (4.2)							
		>15yrs F 4 (0.7)		*bf, cg, cj* (3)	*ach,* (1)					
Total (%)	57 (10.3)			24 (4.4)	21 (3.8)	7 (1.3)	1 (0.2)	4 (0.7)	0	0
Shanono **n**=99	M 25 (4.5)	≤15yrsM 7 (1.3)	≤15yrs 10 (1.8)	*gk, ab, ce,* (3)	*ahp, adf,* (2)	*abdo, bcdg,* (2)				
		>15yrs M 18 (3.3)		*ab, df, ac, ci, ce, ac, bc* (7)	*abk, acf, ack, cdo, cde, abc* (6)	*efil, bcdj, acdg,* (3)	*abcdk,* (1)	*abcefk* (1)		
	F 29 (5.3)	≤15yrs F 3 (0.5)	>15yrs 44 (8.0)		*afj, bce, cei,* (3)					
		>15yrs F 26 (4.7)		*dg, ab, eh, bi, cj, ci, cd, ce, ci, ce, ci, bd, bc,* (13)	*acg, cde, ade, cdm, bcm, bcd, dim, ace, cdk,* (9)	*ackl, cefi,* (2)	*abcfo, bcdem,* (2)			
Total (%)	54 (9.8)			23 (4.2)	20 (3.6)	7 (1.3)	3 (0.5)	1 (0.2)	0	0
Minjibir **n**=109	M 29 (5.3)	≤15yrsM 21 (3.8)	≤15yrs 23 (4.2)	*fi, ab, ab, cg, ad, ci, ck, bc, ci, ac, ac* (11)	*abf, dhi, abc, bcf,* (4)	*abcf, abch, abcg, acfg, acdf, abce*(6)				
		>15yrs M 8 (1.5)		*ac, cg, ce, cj, bc,* (5)	*abi, acl, chk,* (3)					
	F 29 (5.3)	≤15yrs F 2 (0.4)	>15yrs 35 (6.4)		*cij,* (1)	*abfh,* (1)				
		>15yrs F 27 (4.9)		*ab, em, ac, ck, ab, bc, cj, cd, cf, cj, ac, ac, ac, ci,bc,* (15)	*ace, bcd, cfj, acd,* (4)	*abcf, acdf, acdj,* (3)	*bghil, abcfi, abcdf, bcehi* (3)	*abcegh* (1)		
Total (%)	58 (10.5)			31 (5.6)	12 (2.2)	10 (1.8)	3 (0.5)	1 (0.2)	0	0
GT (%)	282 (51.2)			134 (24.3)	83 (15.1)	41 (7.4)	12 (2.2)	6 (1.1)	3 (0.5)	1 (0.2)

a (*Plasmodium* spp.), *b* (*Blastocystis* sp.), *c* (*Entamoeba* complex), *d* (Hookworm species), *e* (*Schistosoma mansoni*), *f* (*Giardia intestinalis*), *g* (*Schistosoma haematobium*), *h* (*Ascaris lumbricoides*), *i* (*Cryptosporidium* species), *j* (*Trichomonas vaginalis*), *k* (*Strongyloides stercoralis*), *l* (*Fasciola hepatica*), *m* (*Balantidium coli*), *n* (*Enterobius vermicularis*), *o* (*Hymenolepis nana*), % (percentage), **n** (sample size), % (percentage), n (sample size)

4.3.3 Variables of parasitic infections stratified by gender

Variables of parasitic infections stratified by gender are presented in Table 4.7. Overall, 52.3% had parasite species in faeces, 60.6% in blood and 14% in urine samples. Among which in males, 33.2% were in faeces, 37.7% in blood and 10.7 in urine. In females, 19.1% were in faeces, 22.9% in blood and 3.3% in urine. In all, significantly more male (53.9%) than female (30.1%) respondents had parasitic infections (P = 0.05; OR = 1.4; 95%CI = 1.0, 2.0). Similarly, prevalence of single parasitic infection among males was almost doubled that of females (P = 0.02; OR = 1.6; 95%CI = 1.1, 2.4). Specifically, in males 4.2% were in faeces, 14.2% in blood and 2.2% in urine and in females 2.2% were in faeces, 8.9% in blood and 0.4% in urine. Despite the higher population of male (32.7%) respondents with multiple parasitic infections compared to females (18.5%), the difference was not statistically significant (P > 0.05; OR = 0.9; 95%CI = 0.7, 1.4).

Prevalence of intestinal helminths was 21.9% (13.9% in males and 8% in females), intestinal protozoan was 45% (28.7% in males and 16.3% in females), blood and liver flukes was 19.2% (13.9% in males and 5.3% in females), malaria was 60.6% (37.7% in males and 22.9% in females) and genital infection with *Trichomonas vaginalis* was 5.6% (3.4% in males and 2.2% in females). In the above infections dissimilarity of prevalence between males and females were not statistically significant except in infection with blood and liver flukes (P = 0.01; OR = 1.8; 95%CI = 1.2, 2.9). In sorting out some coinfections among the respondents, 32.5% had simultaneous infections with malaria and intestinal parasites of which 20.1% in males and 12.3% in females though the difference was not significant (P > 0.05). Concurrent infections of malaria and schistosomiasis was 11.6%, of which 8.2% in males and 3.4% in females. Coinfections of schistosomiasis and intestinal parasites were 13.6% (8.9% males and 4.7% females).

In both coinfections increased prevalence amid males more than females was not statistically significant ($P > 0.05$).

Table 4.7: Variables of parasitic infections stratified by gender

Characteristics	Infected samples (%)				Infected Male respondents (%)				Infected Female respondents (%)				*P*	OR (95%CI)
	Faecal (n=551)	Blood (n=551)	Urine (n=551)	Total	Faecal (n=551)	Blood (n=551)	Urine (n=551)	Total	Faecal (n=551)	Blood (n=551)	Urine (n=551)	Total		
Infected (n=551)	288(52.3)	334(60.6)	77(14)	463(84)	183(33.2)	208(37.7)	59(10.7)	297(53.9)	105(19.1)	126(22.9)	18(3.3)	166(30.1)	0.05★	1.4(1.0,2.0)
Single infection (refer to Table 4.5)	35(6.4)	127(23)	14(2.5)	181(32.8)	23(4.2)	78(14.2)	12(2.2)	117(21.2)	12(2.2)	49(8.9)	2(0.4)	64(11.6)	0.02★	1.6(1.1,2.4)
Polyparasitised (refer to Table 4.6)	253(45.9)	207(37.6)	63(11.4)	282(51.2)	160(29)	130(23.6)	47(8.5)	180(32.7)	93(16.9)	77(13.9)	16(2.9)	102(18.5)	0.85	0.9(0.7,1.4)
Intestinal helminths (*d, e, h, s, o* and *p*)	121(21.9)	-	-	121(22)	77(13.9)	-	-	77(13.9)	44(8)	-	-	44(8)	0.31	1.2(0.8,1.9)
Intestinal protozoa (*b, c, f, i* and *n*)	248(45)	-	-	248(45)	158(28.7)	-	-	158(28.7)	90(16.3)	-	-	90(16.3)	0.43	1.1(0.8,1.7)
Blood + liver flukes (*S.haematobium* + *S.mansoni* +*F.hepatica*)	66(12)	-	49(8.9)	106(19.2)	40(7.3)	-	42(7.6)	77(13.9)	26(4.7)	-	7(1.3)	29(5.3)	0.01★	1.8(1.2,2.9)
Malaria (*Plasmodium* sp.)	-	334(60.6)	-	334(60.6)	-	208(37.7)	-	208(37.7)	-	126(22.9)	-	126(22.9)	0.79	1.1(0.7,1.8)
Genital Infection (*T.vaginalis*)	-	-	31(5.6)	31(5.6)	-	-	19(3.4)	19(3.4)	-	-	12(2.2)	12(2.2)	0.96	1.0(0.5,2.1)
Co-infections														
Intestinal parasitic + malaria	179(32.5)	179(32.5)	-	179(32.5)	111(20.1)	111(20.1)	-	111(20.1)	68(12.3)	68(12.3)	-	68(12.3)	0.92	1.0(0.7,1.5)
Schistosomiasis + malaria	37(6.7)	64(11.6)	30(5.4)	64(11.6)	22(4)	45(8.2)	25(4.5)	45(8.2)	15(2.7)	19(3.4)	5(0.9)	19(3.4)	0.13	1.5(0.9,2.7)
Intestinal parasitic + schistosomiasis	75(13.6)	-	26(4.7)	75(13.6)	49(8.9)	-	21(3.8)	49(8.9)	26(4.7)	-	5(0.9)	26(4.7)	0.49	1.2(0.7,2.0)

b (*Blastocystis* sp.), *c* (*Entamoeba* complex), *d* (Hookworm species), *e* (*Schistosoma mansoni*), *f* (*Giardia intestinalis*), *h* (*Ascaris lumbricoides*), *i* (*Cryptosporidium* species), *n* (*Enterobius vermicularis*), *o* (*Hymenolepis nana*), % (percentage), n (sample size), ★Indicates significance, P (Pearson Chi-Square), OR (odd ration/ risk), CI (confidence interval)

4.4 Quantitative analysis of helminth and malaria infections

Results obtained from counting intestinal helminth eggs from the Kato Katz slides were categorized into different intensity groups following WHO's reference values for schistosoma and STH's and are presented in Table 4.8. Majority of *Schistosoma* infection was of light intensity although a considerable number of respondents had heavy urogenital schistosomiasis. Ascariasis and hookworm infections were both mainly of light intensity. Malaria was caused by two species, 97.3% *P. falciparum* and 2.6% *P. malariae*, both were mostly of mild intensity. More than 35% and nearly 8% of *P. falciparum* were of moderate and severe infections, respectively. Other helminth infections were of low intensity except for *H.nana*. *H. nana* was found in five participants and all were heavily infected with eggs counted as much as 75 EPG as minimum and 345 EPG maximum.

Table 4.8: Intensity of helminth and malaria infections

Parasite	Intensity (%)			Total	Mean EPG
Blood flukes	Light	Moderate	Heavy		
S.mansoni	39 (75)	9 (17.3)	4 (7.7)	52	64.8
S.heamatobium	34 (69.4)	-	15(30.6)	49	98
STH's					Arithmetic mean EPG
Hookworm	48 (75)	16 (25)	-	64	605
Ascaris	41 (93.2)	3 (6.8)	-	44	22
Malaria	Mild	Moderate	Severe		
P. falciparum	180 (53.9)	119 (35.6)	26 (7.8)	325 (97.3)	
P. malariae	9 (2.6)	-	-	9 (2.6)	

4.5 Potential risk factors associated with multiple parasitic infections

Univariate and multivariate analyses of potential risk factors associated with multiple parasitic infections are presented in Table 4.9. In this finding, parallel risks of multiple parasitic infections were established by analysing the characteristics of respondents' behaviours. For instance, despite varying frequency of polyparasitism with respect to age and gender, risk of multiple infections was similar ($P > 0.05$; OR = 1.08 and 1.36 respectively). Regarding educational status, all respondents had corresponding risk of infection though infection was slightly more in those not educated and slightly less among respondents with only primary education compared to respondents with higher education ($P > 0.05$; OR = 1.04; 0.95, respectively). Notwithstanding, there was increased risk of multiple infections among respondents having a polyparasitised family member ($P = 0.017$; OR 1.52; 95% CI = 1.08, 2.13). Likewise among respondents that seldom wear proper foot wear outside home ($P = 0.043$; OR = 1.50; 95%CI = 1.01, 2.18). In both instances, risk of infection increased by more than 50%.

Table 4.9: Univariate and multivariate analyses of potential risk factors associated with polyparasitism

Variables	Polyparasitism			
	No examine	No infected (%)	OR (95% CI)	*P*
Age				
< 18 years	198	110 (55.6)	1.32 (0.93, 1.87)	0.124
≥ 18 years	353	172 (48.7)	1	
Gender				
Male	340	180 (52.9)	1.20 (0.85, 1.69)	0.294
Female	211	102 (48.3)	1	
Educational levels				
Non educated	88	46 (52.3)	1.06 (0.69, 1.78)	0.862
Primary education	272	139 (51.1)	1.01 (0.85, 1.57)	0.732
Secondary/tertiary education	191	97 (50.8)	1	
Occupational status				
Not working	281	149 (53.0)	1.16 (0.83, 1.63)	0.377
Working	270	133 (49.3)	1	
Household monthly income				
< NGN 32,000 (low)	231	123 (53.2)	1.15 (0.82, 1.61)	0.410
≥ NGN 32,000	320	159 (49.7)	1	
Family size				
> 10 members (large)	284	148 (52.1)	1.08 (0.77, 1.51)	0.651
≤ 10 members	267	134 (50.2)	1	
Type of house				
Mud	430	210 (48.8)	0.85 (0.57, 1.27)	0.431
Concrete	121	64 (52.9)	1	
Type of floor				
Mud	315	165 (52.4)	1.12 (0.80, 1.57)	0.515
Concrete/tile	236	117 (49.6)	1	
Type of toilet in house				
Pit latrine	481	251 (52.2)	1.37 (0.83, 2.28)	0.217
Pour flush toilet	70	31 (44.3)	1	
Source of drinking water				
Unsafe source (stream, rain & well)	195	98 (50.3)	0.94 (0.67, 1.34)	0.748
Safe source (pipe)	356	184 (51.7)	1	
Source of household water				
Unsafe source (stream, rain & well)	203	105 (51.7)	1.04 (0.73, 1.46)	0.845
Safe source (pipe)	348	177 (50.9)	1	
Washing hands before eating				
No	27	11 (40.7)	0.68 (0.31, 1.50)	0.338
Yes	524	263 (50.2)	1	
Washing fruits before eating				
No	148	81 (54.7)	1.22 (0.83, 1.77)	0.312
Yes	403	202 (49.9)	1	
Washing vegetables before eating				
No	234	119 (50.9)	0.98 (0.70, 1.34)	0.896
Yes	317	163 (51.4)	1	
Eating soil habit (Geophagy)				
Yes	227	112 (49.3)	0.99 (0.86, 1.14)	0.879
No	324	162 (50.0)	1	
Water proximity				
Near (≤ 250 meters)	380	187 (49.2)	0.94 (0.65, 1.34)	0.717
Far (> 250 meters)	171	87 (50.9)	1	
Presence of domestic animals				
Yes	228	119 (52.2)	1.07 (0.76, 1.51)	0.689
No	323	163 (50.5)	1	
Presence of infected family member				
Yes	320	179 (55.9)	1.58 (1.12, 2.22)	0.007*†
No	231	103 (44.6)	1	
Cutting nails periodically				
No	185	95 (51.4)	1.01 (0.71, 1.44)	0.954
Yes	366	187 (51.1)	1	
Wearing shoes when going outside				
No	142	83 (58.5)	1.50 (1.01, 2.18)	0.043*†
Yes	409	199 (48.7)	1	

NGN (*Nigerian Naira*) US$1 = NGN 165. OR (Odds ratio), CI (Confidence interval),
*Significant association ($P < 0.05$),† Confirmed as significant predictors by logistic regression analysis

4.6 Malaria

4.6.1 Prevalence of malaria among the population

Our findings showed that the proportion of asymptomatic carriers among the respondents was high, with 60.6% (Figure 4.1B) of the population infected with malaria, yet majority reported not having malaria episode for a long time (more than 3 months). Distribution of malaria among the population is presented in Table 4.10. The prevalence was recorded slightly higher among male respondents than female respondents (61.2% vs 59.7%, respectively). Regarding age, prevalence peaked among those > 10 – 18-year-old (66.2%), followed by > 18-year-old (61.7%) and less among children ≤ 5-year-old (37.5%). Possession of bed nets did not reduce malaria since prevalence of malaria was similar among those with bed net and those without (60.5% vs 61.1%). However use of bed net significantly reduced malaria (45.8% vs 59.4%), while use of insecticide marginally reduced malaria (59.1% vs 61.5%). The habit of storing water-in-house increases malaria prevalence (63.1%).

Table 4.10: Prevalence of malaria among the respondents (N = 551)

Characteristic	No. Examined	No. Infected (%)
Gender		
Male	340	208 (61.2)
Female	211	126 (59.7)
Age group		
≤ 5 years old	32	12 (37.5)
> 5 – 10 years old	24	10 (41.7)
> 10 -18 years old	148	98 (66.2)
> 18 years old	347	214 (61.7)
Possess Bed net		
Yes	438	265 (60.5)
No	113	69 (61.1)
Use Bed net		
Yes	236	108 (45.8)
No	202	120 (59.4)
Use insecticide		
Yes	203	120 (59.1)
No	348	214 (61.5)
Store water in-house		
Yes	222	140 (63.1)
No	329	194 (60.0)

4.6.2 Potential risk factors of malaria

Table 4.11 shows result of univariate and multivariate analyses carried out to determine the potential of having malaria among the respondents. Even though prevalence of malaria was slightly different between corresponding groups but still most possessed similar risk of being infected. For instance concerning age groups, children ≤ 5 years old were twice at risk of acquiring malaria infection as compared to other age groups (P = 0.008; OR = 2.68; 95%CI = 1.27, 5.66). Considering education, respondents having primary education had the highest malaria prevalence (62.1%) compared to those not educated (54.5%). Comparably, prevalence of malaria was more between working respondents though had similar risk of infection with their counterparts (OR = 1.18; 95%CI = 0.84, 1.66).

As for income, prevalence of malaria was less among respondents with higher income. An odd of malaria among respondents with low income was almost double (*P* = 0.008; OR = 1.61%; 95%CI = 1.13, 2.29). Lack of proper usage of bed net also increases risk of malaria in this population (*P* 0.004; OR = 1.66; 95%CI = 1.17, 2.34). Nonetheless, not using insecticide (61.5%), storing water at home (63.1%), having animals (57.9%) in the house and previous infection with malaria (59%) all shared comparable risk with respective corresponding groups (P > 0.05 in all groups).

Table 4.11: Univariate and multivariate analyses of potential risk factors associated with malaria among the Nigerian participants (N = 551)

Variables	No. Examined	No. Infected (%)	OR (95% CI)	*P*
Age groups				
≤ 5 years	32	12 (37.5)	2.68 (1.27, 5.66)	0.008
> 5 – 10 years	24	10 (41.7)	2.25 (0.97, 5.22)	0.053
> 10 – 18 years	148	98 (66.2)	0.82 (0.55, 1.23)	0.338
> 18 years	347	214 (61.7)	1	
Gender				
Male	340	208 (61.2)	1.06 (0.75, 1.51)	0.733
Female	211	126 (59.7)	1	
Educational status				
Not educated	88	48 (54.5)	0.76 (0.46, 1.27)	0.289
Primary education	272	169 (62.1)	1.04 (0.71, 1.52)	0.849
Secondary/tertiary education	191	117 (61.3)	1	
Occupation				
Working	270	169 (62.6)	1.18 (0.84, 1.66)	0.352
Not working	281	165 (58.7)	1	
Family income				
< NGN32, 000	231	155 (67.1)	1.61 (1.13, 2.29)	0.008*†
≥ NGN32, 000	320	179 (55.9)	1	
Family size				
>10 members	284	173 (60.9)	1.01 (0.85, 1.21)	0.882
≤10 members	267	161 (60.3)	1	
Type of houses				
Mud	430	260 (60.5)	0.97 (0.64, 1.47)	0.891
Concrete	121	74 (61.2)	1	
Type of toilet				
Pit (ground dug)	481	297 (61.7)	1.44 (0.87, 2.38)	0.155
Pour flush system	70	37 (52.9)	1	
Having bed net				
No	113	69 (61.1)	1.01 (0.92, 1.10)	0.914
Yes	438	265 (60.5)	1	
Using bed net				
No	202	120 (59.4)	1.66 (1.17, 2.34)	0.004*†
Yes	236	51 (21.6)	1	
Using insecticide				
No	348	214 (61.5)	1.07 (0.85, 1.33)	0.581
Yes	203	120 (59.1)	1	
Have domestic animals				
Yes	228	132 (57.9)	0.82 (0.58, 1.16)	0.272
No	323	202 (62.5)	1	
Storing water in house				
Yes	222	140 (63.1)	1.02 (0.68, 1.42)	0.742
No	329	194 (60.0)	1	
Living near water sources (stream, dam, lake, pond, etc.)				
Yes (< 250 meter)	380	228 (60.0)	0.92 (0.64, 1.33)	0.659
No	171	106 (62.0)	1	
Recent history of infection				
Yes	183	108 (59.0)	0.97 (0.86, 1.09)	0.588
No	368	226 (61.4)	1	

* Significant association ($P < 0.05$); † Confirmed as significant predictors by logistic regression analysis;
OR (odd ratio / risk);CI (confidence interval), % (percentage); NGN (Nigerian naira)

4.6.3 Respondents' knowledge, attitude and practices (KAP) against malaria

Generally the respondents were well informed about malaria, 483 (95.6%) knew about malaria (excluding children below the age of 10 years, thus n = 505). Knowledge of cause, prevention and symptoms of malaria as well as perception of its seriousness amongst others is presented in Table 4.12. Expectedly, knowledge about malaria was mostly through the media (307: 63.6%) or personal and relative's experiences (255: 52.8%). Few (101: 20.9%) do not know the cause of malaria but majority ascribed it to mosquito (374: 77.4%). Subsequently, 336 (69.6%) of the respondents indicated avoiding mosquito through using bed nets or insecticide, but about 49 (10%) replied not knowing how to prevent malaria. About three-quarters of them mentioned fever (376: 77.8%), a little less than half mentioned body weakness (233: 48.2%) and barely a quarter mentioned vomiting (104: 21.5%) and abdominal pain (104: 21.5%) as symptoms of malaria.

In spite of the bulk of the subjects' regards malaria as a serious infection, 18 (3.3%) regarded it as other wise and 29 (5.2%) were indecisive. The distribution of bed nets was thorough in the study area (79.5%) but high percentage (36.7%) of them did not use the net. Only 1/3 of them used insecticide (36.8%). As for treatment seeking behaviour, considerable number of the respondents (407: 73.9%) claimed that they went to hospitals or clinics when having an episode of any malaria symptoms. A negligible amount (17: 3.1%) of them patronised the traditional practitioners. Approximately 106 (19.2%) of the respondents self-declared that they bought antimalarial (or other drugs), either from vendors on the street or went to unrecognized chemists to buy antimalarial drugs without prior laboratory diagnosis or doctor's consultation. A low percentage (21: 3.8%) of the respondents do not treat malaria infection.

Table 4.12: Knowledge, attitude and practices of respondents against malaria in Kano State, Nigeria (N = 505, excluding ≤10yr)

Variables (n=505)	n	%	Malaria Attitude (n=505)	n	%
Malaria knowledge	483	95.6	Harmful	504	91.5
Source of information (n=483)			Not harmful	18	3.3
Media	307	63.6	Do not know	29	5.2
Awareness campaign	56	11.6	Practices		
Family & friends	255	52.8	Having bed net	438	79.5
Causes			Not having bed net	113	20.5
Mosquito	374	77.4	Using bed net	236	42.8
Hygiene	47	9.7	Not using bed net	202	36.7
Stagnant water	22	4.6	Using insecticide	203	36.8
Do not know	101	20.9	Not using insecticide	348	63.2
Prevention			Treatment seeking behaviour		
Using nets/insecticide	336	69.6	Hospitals/clinics	407	73.9
Improved hygiene	103	21.3	Self-medication	106	19.2
Anti-malaria prophylaxis	34	7.0	Traditional/herbal medicine	17	3.1
Do not know	49	10.1	Others (no treatment)	21	3.8
Symptoms					
Fever	376	77.8			
Weakness	233	48.2			
Vomiting	104	21.5			
Abdominal pain	104	21.5			
I do not know	62	12.8			

4.6.4 KAP against malaria and it's association with gender and age

Most of the respondents were familiar with malaria. However, a few unfamiliar with malaria were mostly among the female 16 (7.6%) respondents. The odd value of unfamiliarity was doubled among the females (*P* = 0.08; OR = 2.1; 95%CI 1.0, 0.4) though the difference was not significant. On the other hand, the difference of unfamiliar characteristic between adults (3.7%) and children (8.8%) was significant (*P* = 0.02; OR = 0.4; 95%CI = 0.2, 0.8). Knowledge of malaria amongst respondents in the five communities varied, in which most of those unfamiliar with malaria were from Shanono (11.5%; P ≤ 0.001). Likewise, the attitude of respondents towards malaria was positive even though few of them considered malaria as not harmful. The negative attitude was similar in both gender (8.5% both; *P* = 1.00; OR = 1.0), but notably more children regarded malaria as not harmful (14.0% vs 6.1%; *P* = 0.003; OR = 2.5).

Regarding the localities, though attitude towards malaria differed but not significant (*P* = 0.37). Malaria was considered as harmful by most of the respondents from Kura (94.5%) followed by Shanono (93.9%), Minjibir (90.8%), Gwarzo (89.2%) and Bebeji (88.2%) as shown in Table 4.13.

Table 4.13: Association of malaria knowledge (N = 483) and attitude (N = 505) with gender and age in five rural communities of Kano State, Nigeria

Variables	Knowledge [heard (H), Not heard (NH)] n (%)		*P*	OR(95%CI)	Attitude n (%)		*P*	OR(95%CI)
	H malaria	NH malaria			Harmful	Not harmful		
Gender								
Male	327 (96.2)	13 (3.8)	0.076	2.1(1.0,4.4)	311 (91.5)	29 (8.5)	1.000	1.0(0.5,1.8)
Female	195 (92.4)	16 (7.6)			193 (91.5)	18 (8.5)		
Age groups								
Adults>15yrs	366 (96.3)	14 (3.7)	0.021★	0.4 (0.2,0.8)	357 (93.9)	23 (6.1)	0.003★	2.5(1.4,4.6)
Children	156 (91.2)	15 (8.8)			147 (86.0)	24 (14.0)		
Location								
Kura	107 (98.2)	2 (1.8)	0.008★	NA	120(94.5)	7 (5.5)	0.366	NA
Bebeji	106 (96.4)	4 (3.6)			105 (88.2)	14 (11.8)		
Gwarzo	91 (95.8)	4 (4.2)			87 (89.2)	10 (10.3)		
Shanono	77 (88.5)	10 (11.5)			93 (93.9)	6 (6.1)		
Minjibir	102 (98.1)	2 (1.9)			99 (90.8)	10 (9.2)		

* Significant association (*P* < 0.05); *P* (Pearson Chi Square); OR (odd ratio / risk); CI (confidence interval); NA (not analysed)

4.6.5 Respondents' knowledge, attitude and other characteristics against malaria infection

Table 4.14 shows the association of respondents' characteristics (education, occupation, family income and family size) with knowledge and attitude towards malaria. Among the different groups, knowledge of malaria slightly differed however, it was significantly different with educational status. Up to 6.7% of the not educated respondents were ignorant of malaria compared to 2.6% of the educated (*P* = 0.04). The employed (95.9%) and unemployed (93.9%) respondents were all familiar with malaria. Both families with low income (304, 95.0%) and high income (218, 94.4%) were

conversant with malaria. Similarly, respondents with small family members (251, 94%) and large family members (271, 95.4%) knew about malaria. On the other hand, respondent's attitude was found significantly different by occupational status and family monthly income ($P < 0.05$). Odds of acknowledging the detrimental nature of malaria were more than doubled amid the corresponding groups (OR = 2.7 and 2.0 respectively). Nonetheless, respondents' attitude was not significantly different with educational status and family size ($P > 0.05$).

Table 4.14: Association of malaria knowledge (N = 483) and attitude (N = 505) with other characteristics of the respondents in five rural communities of Kano State, Nigeria

Variables	Knowledge [heard (H), Not heard (NH)] n (%)				Attitude n (%)			
	H of malaria	NH of malaria	*P*	OR (95%CI)	Consider harmful	Not harmful	*P*	OR (95%CI)
Educational status								
Educated (> 6 years formal primary education)	186 (97.4)	5 (2.6)	0.046*	0.4(0.1,1.0)	179 (93.7)	12 (6.3)	0.201	1.6 (0.8,3.2)
Not educated (≤ 6 years formal primary school)	336 (93.3)	24 (6.7)			325 (90.3)	35 (9.7)		
Occupational status								
Employed	259 (95.9)	11 (4.1)	0.255	0.6(0.3,1.3)	257 (95.2)	13 (4.8)	0.002*	2.7(1.4,5.3)
Unemployed	263 (93.9)	18 (6.4)			247 (87.9)	34 (12.1)		
Family income								
≤ NGN 32000	304 (95.0)	16 (5.0)	0.847	1.1(0.5,2.4)	286 (89.4)	34 (10.6)	0.044*	2.0(1.0,3.9)
>NGN 32000	218 (94.4)	13 (5.6)			218 (94.4)	13 (5.6)		
Family size								
≤ 10 members	251 (94.0)	16 (6.0)	0.568	1.3(0.6,2.8)	244 (91.4)	23 (8.6)	1.000	1.0(0.5,1.8)
> 10 members	271 (95.4)	13 (4.6)			260 (91.5)	24 (8.5)		

* Significant association ($P < 0.05$); *P* (Pearson Chi Square); OR (odd ratio / risk); CI (confidence interval); NGN Nigerian Naira (NGN165 = USD1)

4.6.6 Association of malaria practices with other characteristics of the respondents

Table 4.15 presents the respondents practices and behaviours against malaria. Majority of the respondents had bed nets and claimed to use it. Respondents with > 6 years education (145: 75.9%) and those with ≤ 6 years formal primary education (264: 73.3%) claimed to have and use bed net at their homes. Similar claim was also made by the employed (74.1%) and unemployed (74.4%), as well as among respondents with small family size (74.5%) and large families (73.9%). The use of insecticide was rather less but similar irrespective of occupational status (172: 63.7% vs 187: 66.5%), family monthly income (201: 62.8% vs 158: 68.4%) and family size (180: 67.4% vs 179: 63.0%). Use of insecticide was significantly different among educated (155, 81.2%) and not educated (204, 56.7%) participants ($P < 0.001$). Similarly, the habit of going to hospital as the first choice when infected with malaria was significantly different among educated and not educated participants ($P = 0.032$), where odd of preferring hospital was almost doubled (OR = 1.6; 95%CI = 1.1, 2.4). Participants' preference of hospital treatment was similar irrespective of their characteristics of employed or unemployed, families with low income or high income and small families or large families.

Table 4.15: Association of malaria practices with other characteristics of the respondent (N = 505)

Variables	Bed net n (%)		*P*	OR (95%CI)	Insecticide n (%)		*P*	OR (95%CI)	Treatment n (%)		*P*	OR (95%CI)
Educational status	Uses	Not use			Uses	Not use			Hospital	Others		
Educated (> 6 years formal primary education)	145(75.9)	46 (24.1)	0.540	0.9(0.6,1.3)	155 (81.2)	36 (18.8)	0.00★	0.3 (0.2,0.5)	152 (79.6)	39 (20.4)	0.032★	1.6 (1.1,2.4)
Not educated (≤ 6 years formal primary school)	264(73.3)	96 (26.7)			204 (56.7)	156 (43.3)			255 (70.8)	105 (29.2)		
Occupational status												
Employed	200(74.1)	70 (25.9)	1.000	1.0 (0.7,1.5)	172(63.7)	98 (36.3)	0.531	1.1 (0.8,1.6)	194 (71.9)	76 (28.1)	0.332	0.8 (0.6,1.2)
Unemployed	209(74.4)	72 (25.6)			187 (66.5)	94 (33.5)			213 (75.8)	68 (24.2)		
Family income												
≤ NGN 32000	244(76.2)	76 (23.8)	0.236	1.3 (0.9,1.9)	201 (62.8)	119 (37.2)	0.205	0.8 (0.5,1.1)	232 (72.5)	88 (27.5)	0.432	1.2 (0.8,1.7)
> NGN 32000	165(71.4)	66 (28.6)			158 (68.4)	73 (31.6)			175 (75.8)	56 (24.2)		
Family size												
≤ 10 members	199(74.5)	68 (25.5)	0.922	1.0 (0.7,1.4)	180 (67.4)	87 (32.6)	0.285	0.8 (0.6,1.2)	191 (71.5)	76 (28.5)	0.245	0.8 (0.5,1.2)
> 10 members	210(73.9)	74 (26.1)			179 (63.0)	105 (37.0)			216 (76.1)	68 (23.9)		

★Indicates significance; (*P* < 0.05); *P* (Pearson Chi Square); OR (odd ratio / risk); CI (confidence interval); NGN Nigerian Naira (NGN165 = USD1)

4.7 Schistosomiasis among the study population

4.7.1 Prevalence and distribution of schistosomiasis

Overall, 17.8% of the participants were detected positive for *Schistosoma* infection (Table 4.16) due to *S. mansoni* (8.9%), *S. haematobium* (8.3%) and both species (0.5%). The infection was significantly higher among males than females (20.6% vs 13.3%). Moreover, the highest prevalence was reported among participants aged 11-18 years (27.8%) while those aged 31-50 years had the lowest prevalence (10.6%). With regards to communities, Gwarzo showed the highest (30.9%) prevalence and the lowest was in Bebeji (11.8%).

Table 4.16: Prevalence of *Schistosoma* species among respondents (N = 551) in Kano State, Nigeria

Characteristics	No. Examined	No infected (%)
Schistosomiasis	551	98 (17.8)
S. mansoni	551	49 (8.9)
S. haematobium	551	46 (8.3)
Mixed infection	551	3 (0.5)
Gender		
Male	340	70 (20.6)
Female	211	28 (13.3)
Age groups (years)		
< 10	46	5 (10.9)
>10 - 18	158	44 (27.8)
>18 – 30	146	24 (16.4)
>30 -50	142	15 (10.6)
> 50	59	10 (16.9)
Location		
Kura	127	20 (15.7)
Bebeji	119	14 (11.8)
Gwarzo	97	30 (30.9)
Shanono	99	21 (21.2)
Minjibir	109	13 (11.9)

4.7.2 Potential risk factors associated with schistosomiasis

Results of univariate and multivariate analyses showing association of schistosomiasis with demographic, socioeconomic, environmental and behavioural factors is presented in Table 4.17. Besides the significant association of schistosomiasis with age and gender, the results showed that the prevalence of schistosomiasis was significantly higher among those who were not working (21.7%) when compared with working participants (13.7%). Moreover, presence of an infected family member significantly associated with the high infection rate among the participants ($P < 0.001$). Likewise, prevalence of schistosomiasis was significantly higher among those who had history of infection compared to their counterparts ($P < 0.001$).

Three factors were retained by multiple logistic regression model analysis as the significant risk factors of schistosomiasis among the examined participants. The results confirmed that participants aged < 18 years were at higher odds for schistosomiasis when compared with their counterparts' ≥ 18 years (adult) by 2 times (OR = 1.76; 95% CI = 1.13, 2.73). Moreover, the presence of infected family member showed increased infection risk in participant by almost 3 times (OR = 3.36; 95% CI = 1.85, 6.10). Similarly, participants who had history of schistosomiasis had more than 2 times odds of infection when compared with their counterparts (OR = 2.62; 95% CI = 1.68, 4.09).

Table 4.17: Univariate analyses of factors associated with schistosomiasis among participants (N = 551) in Kano State, Nigeria

Variables	Schistosomiasis			
	Total N	% Infected	OR (95% CI)	*P*
Age				
< 18 years	198	23.2	1.76 (1.13, 2.73)	0.012*, †
≥ 18 years	353	14.7	1	
Gender				
Male	340	20.6	1.70 (1.05, 2.73)	0.029*
Female	211	13.3	1	
Educational levels				
Non educated	88	17.0	0.83 (0.43, 1.60)	0.573
Primary education	272	16.5	0.80 (0.50, 1.29)	0.355
Secondary/tertiary education	191	19.9	1	
Occupational status				
Not working	281	21.7	1.75 (1.12, 2.74)	0.014*
Working	270	13.7	1	
Household monthly income				
< NGN 32,000 (low)	231	18.2	1.05 (0.67, 1.63)	0.836
≥ NGN 32,000	320	17.5	1	
Family size				
> 10 members (large)	284	20.4	1.46 (0.94, 2.27)	0.095
≤ 10 members	267	15.0	1	
Type of toilet in house				
Pit latrine	481	17.3	0.77 (0.41, 1.42)	0.394
Pour flush toilet	70	21.4	1	
Source of drinking water				
Unsafe source (stream, rain, well)	195	15.9	0.82 (0.51, 1.30)	0.391
Safe source (pipe)	356	18.8	1	
Source of household water				
Unsafe source (stream, rain, well)	203	16.7	0.89 (0.57, 1.41)	0.627
Safe source (pipe)	348	18.4	1	
Water proximity				
Near (≤ 250 meters)	380	16.6	0.77 (0.49, 1.22)	0.269
Far (> 250 meters)	171	20.5	1	
Water contact				
Yes	257	18.7	1.04 (0.66, 1.63)	0.877
No	248	18.1	1	
Presence of domestic animals				
Yes	228	18.0	1.02 (0.66, 1.59)	0.919
No	323	17.6	1	
Presence of infected family member				
Yes	55	38.2	3.36 (1.85, 6.10)	< 0.001*,†
No	496	15.5	1	
Wearing shoes when go outside				
No	142	19.0	1.12 (0.68, 1.83)	0.657
Yes	409	17.4	1	
History of schistosomiasis				
Yes	214	26.6	2.62 (1.68, 4.09)	< 0.001*,†
No	337	12.2	1	

NGN, *Nigerian Naira*; (US$1 = NGN 165). OR (Odds ratio); CI (Confidence interval); % (percentage),
* Significant association ($P < 0.05$).
† Confirmed as significant predictors by logistic regression analysis

4.7.3 Knowledge towards schistosomiasis

Table 4.18 presents data on respondents' knowledge about schistosomiasis. Most participants have heard about schistosomiasis (376, 74.5%) through family or neighbours (268: 71.3%). However, understanding the infection source, transmission and preventive measures were comparably less. Many participants (168, 44.7%) knew that the disease was due to worms while others (188, 50.0%) mentioned water body as a source of infection. Moreover, some of the participants (222, 59.0%) mentioned that haematuria is a sign but very few mentioned blood in stool (56, 14.9%) and other symptoms. Likewise, about one third (145, 38.6%) of them did not know any of the symptoms and 252 (67.0%) had no idea how it is transmitted with an overwhelming 282 (75.0%) ignorant on how to prevent the infection.

Table 4.18: Knowledge towards schistosomiasis among the respondents in Kano State, Nigeria

Variables (n = 505)	N	%
Have heard of schistosomiasis	376	74.5
Source of information (n = 376)		
Mass media	81	21.5
Awareness campaign	46	12.2
Family/Neighbours	268	71.3
Causes		
Worms	168	44.7
Polluted water	188	50.0
Salty or sour food	71	18.9
Poor personal hygiene	20	5.3
Do not know	72	19.1
Signs and symptoms		
Haematuria	222	59.0
Blood in stool	56	14.9
Burning urination	15	4.0
Abdominal pain	13	3.5
Do not know	145	38.6
Transmission		
Contaminated water	105	27.9
Sharing toilet	23	6.1
Poor personal hygiene	9	2.4
Do not know	252	67.0
Prevention		
Medication	90	23.9
Avoid contaminated water	23	6.1
Personal hygiene	13	3.5
Do not know	282	75.0

4.7.4 Knowledge towards schistosomiasis associated with gender and age

Table 4.19 shows the association of respondents' knowledge about schistosomiasis with their age and gender. With regards to age groups, more respondents aged ≥ 18 years have heard about schistosomiasis but < 18 years old had better knowledge about the cause of schistosomiasis and were significantly more in terms of mentioning worms (54.7%) and polluted water (58.5%) as infection cause. Similarly, percentage of those aged < 18 years old that mentioned medication as preventive measure was significantly higher than those aged ≥ 18 years (35.8% vs 23.7%). It was found that male respondents had significantly higher knowledge about schistosomiasis than the females (81.3% vs 63.6%; OR = 2.49; 95% CI = 1.65, 3.74). With regards to knowledge about the cause, significantly higher percentages of males mentioned worms (51.6% vs 30.6%) and polluted water (57.5% vs 34.7%) compared to females. Likewise, percentages of males who mentioned haematuria as a sign of schistosomiasis (63.9% vs 49.2%; OR = 1.83; 95% CI = 1.18, 2.83), contaminated water as a mode of transmission (31.7% vs 20.2%; OR = 1.84; 95% CI = 1.10, 3.08), and medication as a preventive measure (34.5% vs 12.1%; OR = 3.83; 95% CI = 2.11, 6.97) were significantly higher when compared to female respondents.

Table 4.19: Knowledge toward schistosomiasis associated with age and gender of participant (N = 376)

Variables	Age (years), n (%)				Gender, n (%)			
	≥ 18	< 18	OR	95% CI	Female	Male	OR	95% CI
Heard about schistosomiasis	270 (76.5)	106 (69.7)	0.71	0.46, 1.08	124(63.6)	252(81.3)	2.49*	1.65, 3.74
Causes								
1.Worms	110 (40.7)	58 (54.7)	1.76*	1.12, 2.77	38(30.6)	130 (51.6)	2.41*	1.53, 3.80
2.Polluted water	126 (46.7)	62 (58.5)	1.61*	1.02, 2.54	43(34.7)	145 (57.5)	2.55*	1.63, 4.00
3.Salty or sour food	53 (19.6)	18 (17.0)	0.84	0.47, 1.51	19(15.3)	52 (20.6)	1.43	0.81, 2.56
4.Poor personal hygiene	20 (7.4)	0 (0.0)	NA	NA	7 (5.6)	13 (5.2)	0.91	0.35, 2.34
5.Do not know	67 (24.8)	5 (4.7)	0.15	0.07, 0.38	38 (30.6)	34 (13.5)	0.35*	0.21, 0.30
Signs and symptoms								
1.Haematuria	157 (58.1)	65 (61.3)	1.14	0.72, 1.81	61 (49.2)	161 (63.9)	1.83*	1.18, 2.83
2.Blood in stool	41 (15.2)	15 (14.3)	0.92	0.49, 1.75	14 (11.3)	42 (16.7)	1.57	0.82, 3.00
3.Burning urination	14 (5.2)	1 (0.9)	0.21	0.04, 1.60	2 (1.6)	13 (5.2)	3.32	0.74, 14.93
4.Abdominal pain	12 (4.4)	1 (0.9)	0.17	0.03, 1.34	2 (1.6)	11 (4.4)	2.78	0.61, 12.76
5.Do not know	108 (40.0)	37 (34.9)	0.80	0.51, 1.28	62 (50.0)	83 (32.9)	0.49	0.32, 0.76
Transmission								
1.Contaminated water	77 (28.5)	28 (26.4)	0.90	0.54, 1.49	25 (20.2)	80 (31.7)	1.84*	1.10, 3.08
2.Sharing toilet	19 (7.0)	4 (3.8)	0.52	0.17, 1.56	5 (4.0)	18 (7.1)	1.83	0.66, 5.05
3.Poor personal hygiene	8 (3.0)	1 (0.9)	0.1	0.05, 2.52	1 (0.8)	8 (3.2)	4.03	0.50, 32.51
4.Do not know	178 (65.9)	74 (69.8)	1.20	0.74, 1.94	90 (72.6)	162 (64.3)	0.68	0.43, 1.09
Prevention								
1.Medication	64 (23.7)	38 (35.8)	1.80*	1.11, 2.93	15 (12.1)	87 (34.5)	3.83*	2.11, 6.97
2.Avoid water contact	20 (7.4)	3 (2.8)	0.37	0.11, 1,25	4 (3.2)	19 (7.5)	2.45	0.81, 7.35
3.Good personal hygiene	12 (4.4)	1 (0.09)	0.21	0.04, 1.59	3 (2.4)	10 (4.0)	1.67	0.45, 6.17
4.Do not know	213 (78.9)	69 (65.1)	0.50	0.31,0.82	107(86.3)	175 (69.4)	0.36	0.21, 0.64

* Significant association; OR (Odds ratio); CI (Confidence interval); NA (Not applicable)

4.7.5 Knowledge towards schistosomiasis associated with other characteristics

Table 4.20 shows the association of respondents' knowledge about schistosomiasis with educational level and employment status. Significantly more of the educated respondents mentioned several characteristics such as haematuria (62.4% vs 40.4%; OR = 2.45; 95% CI = 1.38, 4.36), considered contaminated water as a principal means of schistosomiasis transmission (30.1% vs 15.8%; OR = 2.30; 95% CI = 1.08, 4.87) and deworming as preventive measure (29.2% vs 15.8%; OR = 2.20; 95% CI = 1.04, 4.65) as compared to those without formal education. Furthermore significantly more employed respondents knew avoiding the contaminated water could prevent the infection (8.7% vs 3.3%; OR = 2.79; 95% CI = 1.07, 7.23). Pertaining respodents that were ignorant of cause (23.6%; OR = 1.84; 95% CI = 1.08, 3.13) and prevention (80%; OR = 1.75; 95% CI = 1.09, 2.80) of schistosomiasis majority were among the employed.

Table 4.20: Knowledge towards schistosomiasis associated with educational and employment status of respondents (N = 376) in Kano State, Nigeria

Variables	Educational level: ≥ 6yrs of primary education				Occupation: not working (NW), working (W)			
	No n (%)	Yes n (%)	OR	95% CI	NW n (%)	W n (%)	OR	95% CI
Heard about schistosomiasis	57 (80.3)	319 (73.5)	0.68	0.37, 1.27	181 (73.3)	195 (75.6)	1.13	0.76,1.68
Causes								
Worms	20 (35.1)	148 (46.4)	1.60	0.89, 2.88	86 (47.5)	82 (42.1)	0.80	0.53, 1.21
Polluted water	24 (42.1)	164 (51.4)	1.46	0.82, 2.57	95 (52.5)	93 (47.7)	0.83	0.55, 1.24
Salty or sour food	7 (12.3)	64 (20.1)	1.79	0.78, 4.14	32 (17.7)	39 (20.0)	1.16	0.70, 1.96
Poor personal hygiene	4 (7.0)	16 (5.0)	0.70	0.23, 2.17	9 (5.0)	11 (5.6)	1.14	0.46, 2.83
Do not know	16 (28.1)	56 (17.6)	0.55	0.29, 1.04	26 (14.4)	46 (23.6)	1.84*	1.08, 3.13
Signs and symptoms								
Haematuria	23 (40.4)	199 (62.4)	2.45*	1.38, 4.36	108 (59.7)	11 (58.5)	0.95	0.63, 1.44
Blood in stool	8 (14.0)	48 (15.0)	1.09	0.48, 2.43	24 (13.3)	32 (16.4)	1.28	0.72, 2.28
Burning urination	1 (1.8)	14 (4.4)	2.57	0.33,19.94	7 (3.9)	8 (4.1)	1.06	0.38, 3.00
Abdominal pain	1 (1.8)	12 (3.8)	2.19	0.28,17.17	7 (3.9)	6 (3.1)	0.79	0.26, 2.39
Do not know	33 (57.9)	112 (35.1)	0.39*	0.22, 0.70	68 (37.6)	77 (39.5)	1.08	0.72, 1.64
Transmission								
Contaminated water	9 (15.8)	96 (30.1)	2.30*	1.08, 4.87	46 (25.4)	59 (30.3)	1.27	0.81, 2.00
Sharing toilet	5 (8.8)	18 (5.6)	0.62	0.22, 1.75	12 (6.6)	11 (5.6)	0.84	0.36, 1.96
Poor personal hygiene	1 (1.8)	8 (2.5)	1.4	0.18,11.74	4 (2.2)	5 (2.6)	1.16	0.31, 4.41
Do not know	42 (73.7)	210 (65.8)	0.69	0.36, 1.30	127 (70.2)	125(64.1)	0.76	0.49, 1.17
Prevention								
Medication	9 (15.8)	93 (29.2)	2.20*	1.04, 4.65	53 (29.3)	49 (25.1)	0.81	0.51, 1.28
Avoid contact with contaminated water	4 (7.0)	19 (6.0)	0.84	0.28, 2.56	6 (3.3)	17 (8.7)	2.79*	1.07, 7.23
Good personal hygiene	3 (5.3)	10 (3.1)	0.58	0.16, 2.19	3 (1.7)	10 (5.1)	3.21	0.87,11.85
Do not know	48 (84.2)	234 (73.4)	0.52	0.24, 1.10	126 (69.6)	156 (80.0)	1.75*	1.09, 2.80

* Significant association; OR (Odds ratio); CI (Confidence interval)

4.7.6 Attitude and practices of respondents towards schistosomiasis

Generally, the detrimental nature of schistosomiasis was recognized by 338 (67.0%) of the respondents who agreed that schistosomiasis is a serious disease (Table 4.21). With regards to practices, 257 (50.9%) respondents admitted to have contact with a water body due to domestic purposes (175, 68.1%) and swimming (66, 25.7%) as the most common reasons. About two-thirds of the participants (348, 68.9%) lived near water body (< 250 meters) while 176 (34.9%) used safe drinking water and 185 (36.6%) used safe water for domestic purposes. Inclination towards self-medication among the respondents was substantial (241, 47.7%) followed by seeking treatment from hospitals (175, 34.7%). Yet 5.0% indicated that they do nothing.

Table 4.21: Attitude and practices of respondents towards schistosomiasis (N = 505) in Kano State, Nigeria

Variables Attitude	N	%
Is schistosomiasis a serious disease?		
Yes	338	67.0
No	34	6.7
Do not know	133	26.3
Practices		
Using safe drinking water	176	34.9
Using safe water for domestic purposes	185	36.6
Living near water body (< 250 meter)	348	68.9
Had contact with a water body	257	50.9
Reasons for contact:		
Swimming	66	25.7
Fishing	13	5.1
Domestic purposes	175	68.1
Waste disposal	3	1.2
Seeking treatment behaviour:		
Hospital / clinic	175	34.7
Traditional medicine	64	12.7
Self-medication	241	47.7
Not treated (do nothing)	25	5.0

4.7.7 Association between attitude and practices about schistosomiasis with age and gender

Table 4.22 shows the comparison between attitude and practices toward schistosomiasis with age and gender of the respondents. The respondents under age group < 18-years-old considered the disease as less serious (59.9% vs 70.0%, OR = 0.64; 95% CI = 0.43, 0.95), had significantly higher contact with a water body (mainly due to swimming) (48.0% vs 14.4%, OR = 5.47; 95% CI = 3.54, 8.45) and fewer considered going to hospital for the treatment of abdominal pain and/or haematuria (27.6% vs 37.7%, OR = 0.63; 95% CI = 0.42, 0.96). However, self-medication for schistosomiasis was significantly higher among those aged < 18 years old as compared to their older counterparts (88: 57.9% vs 153: 43.3%; OR = 1.80; 95% CI = 1.22, 2.64).

Regarding gender, more male respondents agreed to the seriousness of the disease (72.3% vs 58.5%, OR = 1.85; 95% CI = 1.27, 2.70), had contact with a water body (for swimming though not for domestic purposes) (31.3% vs 13.8%, OR = 2.83; 95% CI = 1.77, 4.54) and declared going to the hospital when suffering abdominal pain and/or haematuria (38.4 vs 28.7%, OR = 1.55; 95% CI = 1.05, 2.27) as compared to female counterparts. The percentage of those who did nothing for such signs and symptoms was significantly lower among male as compared to female respondents (2.9% vs 8.2%, OR = 0.34; 95% CI = 0.15, 0.77).

Table 4.22: Association of attitude and practices about schistosomiasis with age and gender (N = 505) in Kano State, Nigeria

Variables	Age (years)				Gender			
	≥ 18	< 18	OR	95% CI	Female	Male	OR	95% CI
Attitude								
Schistosomiasis is a serious disease	247 (70.0)	91 (59.9)	0.64	0.43, 0.95*	114 (58.5)	224 (72.3)	1.85	1.27, 2.70*
Practices								
Using unsafe drinking water	84 (31.1)	42 (39.6)	0.69	0.43, 1.10	36 (29.0)	90 (35.7)	1.36	0.85, 2.16
Using unsafe water for domestic purposes	91 (33.7)	40 (37.7)	0.84	0.53, 1.34	39 (31.5)	92 (36.5)	1.25	0.79, 1.98
Living near water body	190 (70.4)	69 (65.1)	1.27	0.79, 2.05	83 (66.9)	176 (69.8)	1.14	0.72, 1.81
Had contact with a water body	51 (14.4)	73 (48.0)	5.47	3.54, 8.45*	27 (13.8)	97 (31.3)	2.83	1.77, 4.54*
Reasons for contact								
- Swimming	13 (3.7)	53 (34.9)	14.00	7.33, 26.72*	4 (2.1)	62 (20.0)	11.94	4.27, 33.39*
- Fishing	11 (3.1)	2 (1.3)	0.42	0.11, 1.89	1 (0.5)	12 (3.9)	7.81	1.01, 42.56*
- Domestic purposes	92 (34.1)	30 (28.3)	0.69	0.46, 1.04	44 (35.5)	78 (31.0)	0.82	0.52, 1.28
Seeking treatment behaviour								
- Hospital/clinic	133 (37.7)	42 (27.6)	0.63	0.42, 0.96*	56 (28.7)	119 (38.4)	1.55	1.05, 2.27*
- Traditional medicine	47 (13.3)	17 (11.2)	0.82	0.45, 1.48	21 (10.8)	43 (13.9)	1.33	0.77, 2.33
- Self-medication	153 (43.3)	88 (57.9)	1.80	1.22, 2.64*	102 (52.3)	139 (44.8)	0.74	0.52, 1.06
- Do nothing	20 (5.7)	5 (3.3)	0.57	0.21, 1.54	16 (8.2)	9 (2.9)	0.34	0.15, 0.77*

* Significant association; OR (Odds ratio); CI (Confidence interval)

4.7.8 Association between attitude and practices about schistosomiasis with other characteristics

The educated and employed respondents were more aware of schistosomiasis seriousness (OR = 2.22; 95% CI = 1.34, 3.70 and OR = 1.74; 95% CI = 1.19, 2.53 respectively) and indicated that they had less contact with a water body (13.6% vs 36.0%; OR = 0.28; 95%CI = 0.18, 0.43) but higher percentage had a swimming history in these waters (OR = 5.97; 95% CI = 1.43, 24.95 and OR = 0.12; 95%CI = 0.07, 0.25 respectively). More of the not educated respodents had contact with polluted waters for domestic purposes (OR = 0.56; 95% CI = 0.32, 0.99). Nonetheless, the educated and employed respodents declared going to hospital more for treating urinary and intestinal signs and symptoms of schistosomiasis (OR = 1.31; 95%CI = 0.76, 2.26 and OR = 1.26; 95%CI = 0.87, 1.82 respectively) as compared to their non-educated and unemployed counterparts. Thus majority of respodents that do not treat schistosomiasis are not educated (OR = 0.40; 95% CI = 0.16, 0.99), Table 4.23.

Table 4.23: Association of attitude and practices about schistosomiasis with educational and employment status of respondents (N = 505) in Kano State, Nigeria

Variables	Educational level: ≥ 6yrs of primary education				Occupation: not working (NW), working (W)			
	No	Yes	OR	95% CI	NW	W	OR	95% CI
Attitude								
Schistosomiasis is a serious disease	36 (50.7)	302 (69.6)	2.22	1.34, 3.70*	150 (60.7)	188 (72.9)	1.74	1.19, 2.53*
Practices								
Using safe drinking water	18 (31.6)	108 (33.9)	1.11	0.61, 2.03	69 (38.1)	57 (29.2)	0.67	0.43, 1.03
Using safe water for domestic purposes	23 (40.4)	108 (33.9)	0.76	0.43, 1.35	71 (39.2)	60 (30.8)	0.69	0.45, 1.05
Living near water body	39 (68.4)	220 (69.0)	1.03	0.56, 1.88	118 (65.2)	141 (72.3)	1.39	0.90, 2.16
Had contact with a water body	16 (22.5)	108 (24.9)	1.14	0.63, 2.07	89 (36.0)	35 (13.6)	0.28	0.18, 0.43*
Reasons for contact								
- Swimming	2 (2.8)	64 (14.7)	5.97	1.43, 24.95*	57 (23.1)	9 (3.5)	0.12	0.07, 0.25*
- Fishing	4 (5.6)	9 (2.1)	0.36	0.11, 1.18	7 (2.8)	6 (2.3)	0.82	0.27, 2.46
- Domestic purposes	25 (43.9)	97 (30.4)	0.56	0.32, 0.99*	60 (33.1)	62 (31.8)	0.94	0.61, 1.45
Seeking treatment behaviour								
- Hospital/clinic	21 (29.6)	154 (35.5)	1.31	0.76, 2.26	79 (32.0)	96 (37.2)	1.26	0.87, 1.82
- Traditional medicine	9 (12.7)	55 (12.7)	1.00	0.47, 2.13	33 (13.4)	31 (12.0)	0.89	0.52, 1.50
- Self-medication	34 (47.9)	207 (47.7)	0.99	0.60, 1.64	122 (49.4)	119 (46.1)	0.88	0.62, 1.24
- Do nothing	7 (9.9)	18 (4.1)	0.40	0.16, 0.99*	13 (5.3)	12 (4.7)	0.88	0.39, 1.96

* Significant association; OR (Odds ratio); CI (Confidence interval)

4.8 Prevalence and distribution of *Blastocystis* infection

4.8.1: Prevalence of *Blastocystis* infection based on microscopy

Overall prevalence of *Blastocystis* sp. in this study was 29.2% (161/551) based on microscopy of direct feacal smear. The percentage of infection against several characteristics is presented in Table 4.24. The infection was seen more in males (33.5%) than in females (22.3%), and higher in children than adults (32.7% vs 27.6%, respectively). Distinctive prevalence was recorded between the localities as well. Infection was highest among participants from Gwarzo (36.1%) and Minjibir (35.8%), followed by residents of Kura (27.6%) and Bebeji (26.1%). Lowest prevalence was observed among residents from Shanono (21.2%).

Table 4.24: Prevalence of *Blastocystis* infection based on microscopy of direct feacal smear (N = 551) in Kano State, Nigeria

Characteristic	No. Examined	No. Infected (%)
Gender		
Male	340	114 (33.5)
Female	211	47 (22.3)
Age group		
≤15 years old (children)	171	56 (32.7)
>15 years old (adults)	380	105 (27.6)
Address		
Kura	127	35 (27.6)
Bebeji	119	31 (26.1)
Gwarzo	97	35 (36.1)
Shanono	99	21 (21.2)
Minjibir	109	39 (35.8)

Further univariate analysis to determine the association of *Blastocystis* infection with demographic and some socioeconomic factors is shown in Table 4.25. The logistic analysis indicated that infection was not associated with age of the respondents despite the varying prevalence (OR = 1.28; *P* = 0.222). However, significantly higher prevalence was observed among males as compared to females (OR = 1.76; 95% CI =

1.19, 2.61; *P* = 0.005) and among not educated respondents (30.9% vs 20.5%; OR= 1.74; *P* = 0.049). Correspondingly, substantial association was established between *Blastocystis* infection and presence of infected family member (92.3% vs 24.4%; OR = 37.15; *P* = 0.001) as well as coinfection with other intestinal protozoan (35.1% vs 4.3%; OR = 4.48; *P* = 0.001).

Table 4.25: Univariate analyses of factors associated with *Blastocystis* infection among the participants (N = 551) in Kano State, Nigeria

Variables	*Blastocystis* infection			
	Total	Infected (%)	OR (95% CI)	*P*
Age				
< 15 years	171	56 (32.7)	1.28 (0.86, 1.89)	0.222
≥ 15 years	380	105 (27.6)	1	
Gender				
Male	340	114 (33.5)	1.76 (1.19, 2.61)	0.005*,†
Female	211	47 (22.3)	1	
Educational levels				
Less than secondary education	463	143 (30.9)	1.74 (0.99, 3.03)	0.049*
Secondary/tertiary education	88	18 (20.5)	1	
Occupational status				
Not working	281	83 (29.5)	0.97 (0.67, 1.40)	0.867
Working	270	78 (28.9)	1	
Household monthly income				
< NGN 32,000 (low)	320	87 (27.2)	1.26 (0.87, 1.83)	0.217
≥ NGN 32,000	231	74 (32.0)	1	
Family size				
> 10 members (large)	284	82 (28.9)	1.04 (0.72, 1.50)	0.854
≤ 10 members	267	188 (70.4)	1	
Type of toilet in house				
Pit latrine	481	139 (28.9)	1.13 (0.66, 1.94)	0.664
Pour flush toilet	70	22 (31.4)	1	
Source of drinking water				
Unsafe source (stream, rain, well)	195	59 (30.3)	1.08 (0.74, 1.58)	0.692
Safe source (pipe)	356	102 (28.7)	1	
Source of household water				
Unsafe source (stream, rain, well)	203	57 (28.1)	0.91 (0.63, 1.34)	0.653
Safe source (pipe)	348	104 (29.9)	1	
Have unhygienic water storage				
Yes	222	66 (29.7)	0.96 (0.66, 1.39)	0.829
No	329	95 (28.9)	1	
Water proximity				
Near (≤ 250 meters)	380	106 (27.9)	1.23 (0.83, 1.81)	0.308
Far (> 250 meters)	171	55 (32.2)	1	
Water contact				
Yes	139	44 (31.7)	1.17 (0.77, 1.77)	0.465
No	412	117 (28.4)	1	
Presence of domestic animals				
Yes	228	64 (28.1)	1.10 (0.76, 1.60)	0.618
No	323	97 (30.0)	1	
Having an infected family member				
Yes	39	36 (92.3)	37.15 (11.25, 122.73)	< 0.001*,†
No	512	125 (24.4)	1	
History of other intestinal protozoan infection				
Yes	248	87 (35.1)	4.48 (3.73, 5.39)	< 0.001*,†
No	303	13 (4.3)	1	

NGN, *Nigerian Naira*; (US$1 = NGN 165). OR (Odds ratio); CI (Confidence interval)
* Significant association (*P* < 0.05).
† Confirmed as significant predictors by logistic regression analysis

4.8.2: Distribution of *Blastocystis* subtypes

Out of 161-microscopy feacal smear positive samples, only 54 (33.5%, 54/161) were able to be cultured in complete Jones medium. These samples were from Bebeji (54.8%, 17/54), Shanono (33.3%, 7/54), Kura (31.4%, 11/54), Gwarzo (25.7%, 9/54) and Minjibir (25.6%, 10/54). On the other hand, these samples were 39 (34.2%) male, 15 (31.9%) female, 33 (31.4%) adults and 21 (37.5%) young respondents. The cultured samples were grown and each individual isolate was harvested at the exponential phase for molecular identification. Four subtypes were identified as ST1 (23), ST2 (4), ST3 (17) and ST4 (7). There were three samples with mixed subtype infections of ST4 & ST2, ST1 & ST3 and ST2 & ST1 as shown in Table 4.26. Subtypes ST1 and ST3 were obviously more among male and adult respondents. In addition, mix infections were also observed in males only. Among all the subtypes, ST1 was seen most prominent in all studied communities.

Table 4.26: Prevalence of *Blastocystis* Subtypes among the Respondents in Kano State, Nigeria

	Blastocystis subtypes							
Variables	ST1	ST2	ST3	ST4	ST4 & ST2	ST1 & ST3	ST2 & ST1	Total (%)
Over all (n=161)	23	4	17	7	1	1	1	54 (32.3)
Gender								
Males (n=114)	17	2	12	5	1	1	1	39 (34.2)
Females (n=47)	6	2	5	2	0	0	0	15 (31.9)
Age groups								
Adults >15yrs (n =105)	15	2	11	4	0	0	1	33 (31.4)
Children ≤ 15yrs (n = 56)	8	2	6	3	1	1	0	21 (37.5)
Location								
Kura (n= 35)	6	1	4	0	0	0	0	11 (31.4)
Bebeji (n=31)	6	2	7	2	0	0	0	17 (54.8)
Gwarzo (n=35)	3	0	1	3	1	1	0	9 (25.7)
Shanono (n=21)	5	0	0	1	0	0	1	7 (33.3)
Minjibir (n=39)	3	1	5	1	0	0	0	10 (25.6)

Data on *Blastocystis* isolates clones and related subtypes are summarized in Table 4.27. Two to four clones were randomly selected (namely a, b, c, d) from each isolate and subjected to DNA sequencing. Each sequence was then exploited in ClustalW programme to establish multiple alignments and found that nucleotide sequences in the four clones (a, b, c, d) from each of the 46 isolates and two clones (a and b; c and d) from each of the eight isolates were identical. A high homology was exhibited between the test sequences and the closest match reference sequences from Genbank.

Table 4.27: *Blastocystis* isolates and percentage homology with their closest match reference sequence from Genbank

Blastocystis Isolates [clones]	ST	Reference isolate (Accession no.)	Sequence homology %
KN60[a,b,c,d], **KN200**[a,b,c,d], **KN206**[a,b,c,d], KN231[a,b,c,d], KN266[a,b,c,d], KN286[a,b,c,d], KN303[c,d], **KN435**[a,b], KN530[a,b,c,d], KN805[a,b,c,d], KN1022[a,b,c,d], KN1045[c,d], KN1049[a,b,c,d], **KN1097**[a,b], **KN1102**[a,b,c,d], KN57[a,b,c,d]	1	GU992416	99.7
KN320[a,b,c,d], **KN334**[a,b,c,d], KN354[a,b,c,d], KN359[a,b,c,d], KN509[a,b,c,d], KN518[a,b,c,d]			97.0
KN215[a,b,c,d], **KN338**[a,b,c,d],	2	GU992412	99.0
KN415[a,b], **KN1036**[a,b,c,d], **KN1097**[c,d], **KN80**[a,b,c,d],			99.0
KN26[a,b,c,d], **KN70**[a,b,c,d], **KN1012**[a,b,c,d], KN1047[a,b,c,d], KN1187[a,b,c,d], KN88[a,b,c,d]			100
KN234[a,b,c,d], **KN249**[a,b,c,d], KN265[a,b,c,d], KN288[a,b,c,d], KN315[a,b,c,d], **KN329**[a,b,c,d], KN341[a,b,c,d], **KN435**[c,d], KN521[a,b,c,d]	3	AB107963	99.7
KN1109[a,b,c,d], **KN1173**[a,b,c,d], KN87[a,b,c,d]			99.5
KN415[c,d], **KN522**[a,b,c,d], KN533[a,b,c,d], KN1087[a,b,c,d]			100
KN241[a,b,c,d], **KN433**[a,b,c,d]	4	AY590114	99.7
KN313[a,b,c,d], **KN374**[a,b,c,d]			

Isolates from Kano (KN); clones (a, b, c, d); Subtype (ST); isolate clones with bold blue were used in phylogenetic tree construction

The *Blastocystis* subtypes of the tested isolate clones were analysed based on SSUrDNA gene sequence retrieved from Genbank as a reference in a phylogenetic tree (Figure 4.4) and *Proteromonas lacertae* (U37108) used as an out-group (due to their close relation phylogenetically). Applying the rooted neighbor-joining tree, 9 clades

were recognised (ST1- ST9) in a moderate to strongly supported bootstrap values. Phylogenetic analysis of isolates from this study (clustered under four subtypes ST1, ST2, ST3 and ST4) supported by good bootstrap formed four independent monophyletic groups. The tree showed isolates of all three subtypes (ST1, ST2 and ST3) clustered in one group each. However, isolates belonging to ST4 had two distinct clades with well-supported bootstrap (64% and 99%).

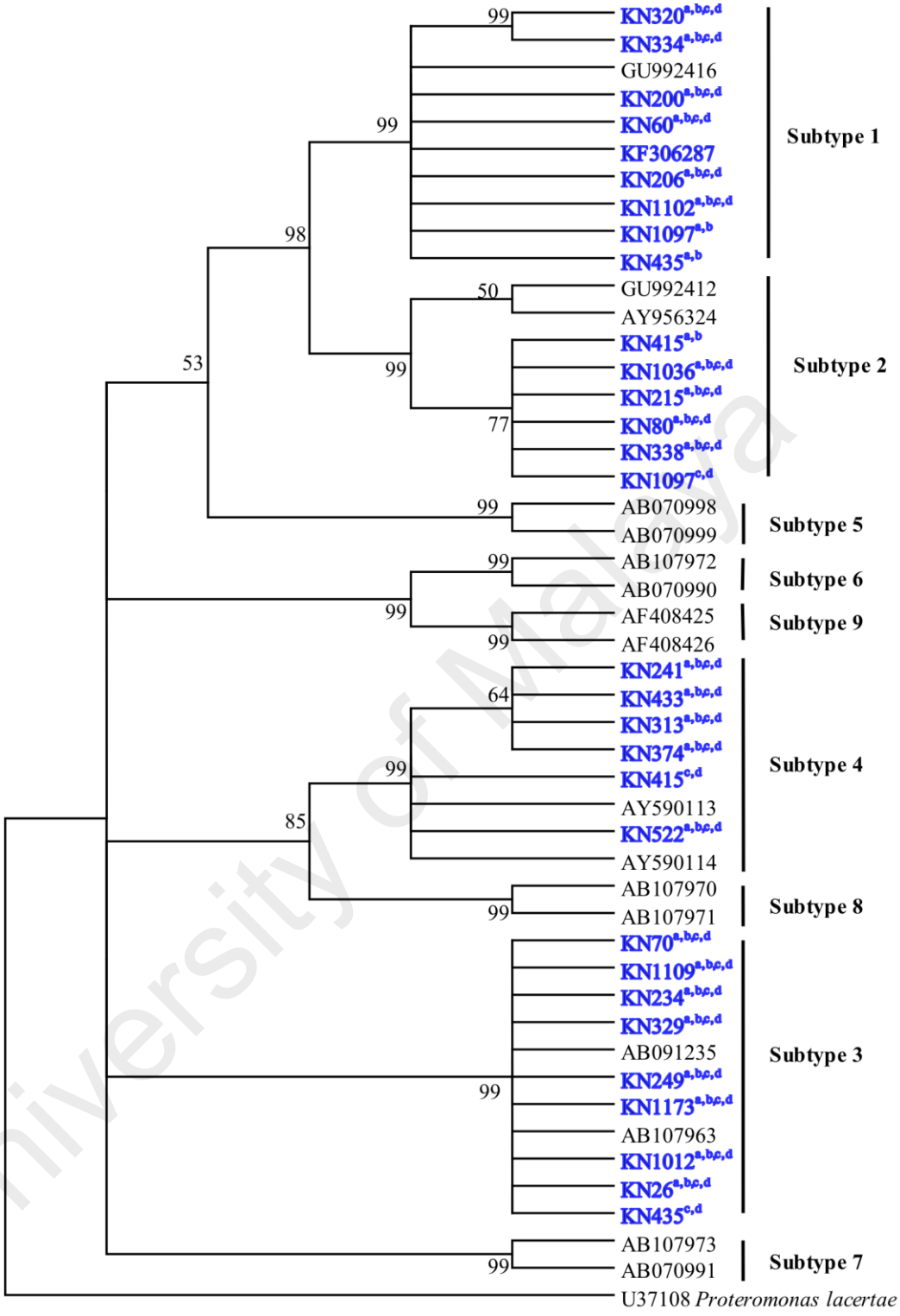

Figure 4.4: Phylogenetic tree of the SSU rDNA gene sequences of *Blastocystis* isolates as inferred using the neighbour-joining method. Reference isolates and accession numbers are available in Genbank. Sequences generated in this study (designated as 'KN—' followed by clones 'a, b, c, d'). *Proteromonas lacertae* (U37108) served as the out-group. Bootstrap values (%) are indicated at the internal nodes (1,000 replicates). Bootstrap values of less than 50% are not shown.

CHAPTER 5: DISCUSSION

5.1 Parasite species by morphological detection

Population of Hausa communities were confirmed as having at least 15 different parasites species / genus that were recovered in a single collection of stool, urine and blood samples based on the morphology of eggs, larvae, (oo)cysts and trophozoites as diagnostic stages. Regarding intestinal helminths, the obtained result might be under estimation of true extent of the infections, because egg-output in faecal sample varies from day-to-day. Hence collection of multiple stool samples would have increased the diagnostic sensitivity (Coulibaly *et al*., 2012; Utzinger *et al*., 2011; Booth *et al*., 2003). However, we consider this data meaningful being a base line study of multiple parasitic infections in these communities, taking into account that those missed infections were most likely of light intensity.

The prevalence of parasitic infections in this population was 84%, which included malaria (60%), soil transmitted helminths (STH) (22%), intestinal protozoa (45%) and blood flukes (19.2%). Previously, Gundiri *et al*. (2001) recovered 8 parasite species among school aged Fulani children in Guduso, Gire Local Government Area of Adamawa State. Anosike and colleagues (2004) observed 14 different parasite species among the nomadic Fulani of southeastern Nigeria. Houmsou *et al*. (2010) reported 10 parasite species among primary school children in Makurdi, Benue State. Abah and Arene (2015) recorded 6 species among primary school children in Rivers State. Despite single collection of sample as in this study, the differences in recovery of number of species could be associated to the diagnostic technique employed and the type of samples examined (Ogbuagu *et al*., 2010).

For malaria, *Plasmodium falciparum* was dominant and showed the most prevalence among the participants in Kano State. The recent malaria risk maps estimated that malaria prevalence in Nigeria varied from < 20% in certain areas to > 70% in others (Onyiri, 2015). Gunn *et al*. (2015) reported 99% of pregnant women in Enugu State had malaria, although Nwagha *et al*. (2009) recorded much less (58.4%) in prevalence. This variation can be attributed to the different climatic conditions, less rainfall and surface water that serve as breeding sites for mosquito. Hence, people are subjected to hundreds of infectious mosquito bites each year, making it hard to avoid contracting malaria, thus malaria is holoendemic in the population.

Many parasites were found in the faecal samples in which *Blastocystis* sp. (29.2%) showed the highest prevalence followed by *Entamoeba histolytica / dispar / moshkhovskii* (16.3%), hookworms (15.4%), *Schistosoma mansoni* (9.4%), *Giardia intestinalis* (9.4%), *Ascaris lumbricoides* (8.0%), *Cryptosporidium species* (7.4%), *Strongyloides stercoralis* (3.4%), *Fasciola hepatica* (2.5%), *Balantidium coli* (2.2%), *Enterobius vermicularis* (2.0%) and *Hymenolepis nana* (0.9%). While *Schistosoma haematobium* (8.9%) and *Trichomonas vaginalis* (5.6%) were detected in urine samples. Previous parasitological surveys in Nigeria hardly reported *Blastocystis* sp., probably due to inexperience or less prominence in terms of disease seriousness. To the best of our knowledge, only two previous works were reported on *Blastocystis* infection, among which in HIV patients (2.83% HIV positive and 0.94% HIV negative) (Ochigbo *et al*., 2011) and in school children from Ibadan, Oyo State (only one child out of 1,273 children) (Adekunle, 2002). As for *E. histolytica* (the detail result is not reported in this study), it was previously observed as the most prevalent protozoa in stool samples with respectively 40% (Muhammad *et al*., 2015) and 43.9% (Ogbuagu *et al*., 2010).

In this study, hookworms were found to be the most dominant among the STH although less prevalent (15.4%) as compared to previous reports which were higher (50%, 37.3% and 26.5% by Biu *et al*. (2013), Obiukwu *et al.* (2008) and Adekunle (2002) in different Nigerian communities, respectively). Muhammad *et al*. (2014) also documented hookworms dominating *A. lumbricoides* where the prevalence of hookworms was 17% and *A. lumbricoides* was 11.4% among patients attending Maiduguri hospital. On the other hand, our finding showed that hookworms were more prevalent than previous reports by Ibrahim and Zubairu, (2010) and Biu *et al*. (2012), which documented 8.7% and 8.8%, respectively. As for *A. lumbricoides* infection, it was found less prevalent than in many previous reports. It was documented as the most dominant (46%) helminth followed by hookworms (20.5%) among school children in Osun State (Ijagbone & Olagunju, 2006). Abah and Arene, (2015) also found higher prevalence of *A. lumbricoides* (51.78%) than hookworms (25.00%) and *T. trichiura* (15.18%) more than any other intestinal helminths. *A. lumbricoides* and hookworms were highlighted to cause significant effect on Nigerian children's development due to nutritional deficiencies (Adekunle, 2002).

Hookworm infections were seen as occupational disease of the farming communities, due to use of human faeces as fertilizers and walking without proper foot-ware especially in the tropical farms (Mordi & Ngwodo, 2007; Ekpo *et al*., 2008a). Moreover, open defaecation practiced in near-by bushes and walking bare foot within the contaminated residential areas promotes transmission of hookworms. In rural northern Nigerian communities, food hawking and poor sanitation are outstanding factors promoting infection and re-infection of STHs especially hookworms (Ekpo *et al*., 2008a). These hawkers usually roam around the streets selling their products without proper footwear. In general, poor environmental hygiene and socioeconomic conditions promotes survival and transmission of STHs in Nigeria (Adeyeba &

Akinlabi, 2002; Ugbomoiko *et al*., 2012; Ekpo *et al*., 2008a).

Blood flukes, *S. mansoni* (9.4%) and *S. haematobium* (8.9%) were also found in our study but with less prevalence as compared to previous reports that ranged from 11% to 19% in Nigerian communities (Ivoke *et al*., 2014; Gutman *et al*., 2008; Nale *et al*., 2003; Gazzinelli *et al*., 1998). Higher prevalence rate for *S. haematobium* (44.2% and 42.7%) and *S. mansoni* (50.3%) were reported earlier in the same state of Kano (Bassey & Umar, 2004; Abdullahi *et al*., 2009; Duwa *et al*., 2009, respectively). Similarly, high prevalence of schistosomiasis (above 50%) has been reported from other states in Nigeria (Amuta & Houmsou, 2014; Ekpo *et al*., 2008b). The lower prevalence reported in this study could be attributed to the integrated and cost-effective control measures implemented by the Federal Ministry of Health (Hotez *et al*., 2012).

One of the important STH, *T. trichiura* was not observed in this study; perhaps the infection was sparse in our study area or was missed due to low intensity. Previously, the prevalence of *T. trichiura* was reported to range from 0.2% to 7% (Mordi & Ngwodo, 2007; Ijagbone & olagunju, 2006; Nmorsi *et al*., 2009; Abubakar *et al*., 2008; Tinuade *et al*., 2006). Few studies that evaluated implication of socioeconomic status of urban and rural populations with STH infections insinuated that hookworms were found more often in rural areas, whereas *A. lumbricoides* and *T. trichiura* were more prevalent in urban and sub-urban slums (Pullan & Brooker, 2012; Rosewell *et al*., 2010). Relatedly, Humphries *et al*. (2013) reported not detecting *A. lumbricoides* and *T. trichiura* in neighbouring country of Ghana. Hookworms were more widespread than other STHs infections while ascariasis or trichuriasis were not detected among the study subjects in Tanzania (Mazigo *et al*., 2010). The difference in prevalence from different rural communities has been attributed to ecological factors as well as use of different diagnostic techniques by various researchers. Further disparing prevalence were linked

to people's level of education, standard of personal/environmental hygiene and perhaps social habits (Ogbuagu *et al*., 2010).

Related to study sites, this study showed that Kura community had the highest prevalence of parasitic infections followed by Minjibir, Bebeji, Gwarzo and Shanono. In Kura, the infections were dominant in adult males with *Plasmodium*, *Blastocystis* and hookworm species. Majority of male residents (24.4%) of Kura were farmers thus the higher parasites prevalence were highly associated with their occupation. In Minjibir the highest prevalence was in adults yet the peak was in male children particularly with *Plasmodium* species. This may be due to the tradition of allowing male children (boys) to go out to play in the early evening hours in the dirty playgrounds and streets, whereas girls were mostly restricted at home or spend lesser hours at the playgrounds. In addition, the playgrounds were seen full of rubbish piles and the street were characterized with open drainage that serves as breeding site for malaria vector as well as source of transmission for many other parasitic species. In Gwarzo community, urinary *Schistosomiasis* was highest (15/49: 30.6%) among males, mainly young children and teenagers who practice peer group-swimming competitions.

Respondents from all of these five communities were predominantly farmers and living in close contact with domestic animals. Previous reports inferred human transmission of some helminths and protozoa via direct contact with infected persons (anthroponotic transmission) or animals (zoonotic transmission), apart from contaminated food and water (foodborne / waterborne transmission) (Xiao, 2010; Lobo *et al*., 2014). Lobo and colleagues (2014) suggested that infection with *C. parvum* in children in Sa˜o Tome´ Island was likely due to zoonotic origin after genotypic analysis.

5.2 Single and multiple parasites recovery

In this study, single infection (32.8%, 181/551) was found less than multiple infections (51.2%). Twelve different parasitic genus or species were found as a single infection in a host. They included *P. falciparum* (23%), *Blastocystis* sp. (3.3%), *Entamoeba* complex (0.5%), hookworms (1.3%), *S. mansoni* (0.2%), *Giardia intestinalis* (0.5%), *S. haematobium* (1.5%), *A. lumbricoides* (1.1%), *Cryptosporidium specie* (1.1%), *T. vaginalis* (0.4%), *S. stercoralis* (0.4%) and *F. hepatica* (0.2%). Highest prevalence of single infection was found in Kura community (8.5%) followed by Bebeji (6.9%), Minjibir (6.7%), Gwarzo (5.4%) and Shanono (5.3%). The infection was also seen high in those above 15-years-old in all communities except Gwarzo. This may be due to participants from Gwarzo are predominantly less than 18-years-old and the most infected group.

As for multiple infections, almost 2/3 of the infected population were polyparasitised (60.9%). This result is in line with Amuta *et al*. (2009), who reported 58% of primary school children in Makurdi Benue state had parasitic infections and 27% of them were single while 31% had multiple infections. In Osun State, southwestern Nigeria, Ugbomoiko and colleagues (2012) found children of school aged harbouring multiple parasite species concurrently more than single (46.5% vs 31.9%). In contrast, higher prevalence of single (63%) than multiple (18%) infections was observed among the Almajiris in Borno State Northeastern Nigeria (Damen *et al*., 2011). Others documented 70% single and 2% multiple infections among school children (Ijagbone & Olagunju, 2006), 60% single and 14.85% multiple infections among prison inmates in Zaria, Kaduna State (Amuga *et al*., 2006).

Contemporaneous multiple parasitic infections recurred in most of developing and underdeveloped communities (Al-Agha & Teodorescu, 1999; Tchuem Tchuente *et al*.,

2003; Keiser *et al*., 2002; Thiong'o *et al*., 2001; Ashford *et al*., 1993; Rietveld *et al*., 1987). To date, there are not many published epidemiological data to demonstrate the extent of the problem in Nigeria. Consequently, polyparasitism is prevalent in Nigeria, although the underlying parasite species may vary from one community to the other. Many previous studies were carried out in the country, but did not mention high prevalence of polyparasitism (Adekunle, 2002; Ochigbo *et al*., 2011; Houmsou *et al*., 2010; Chukwuma *et al*., 2009; Dada-Adegbola *et al*., 2005). Our finding is in accordance with reports from other parts of Africa whom have been vulnerable to similar chronic parasitic infections especially in the rural communities including Kenya, Cote d'Ivoire, Sudan, Gabon, Zimbabwe and Senegal (Bisanzio *et al*., 2014; Hürlimann *et al*. 2014; Abou-Zeid *et al*., 2012; Adegnika *et al*., 2010; Sangweme *et al*., 2010; Le Hesran *et al*., 2004). Multiple parasitic infections have also been observed in other parts of the world including Malaysia, Laos, Philippines and Bangladesh (Nasr *et al*., 2013; Sayasone *et al*., 2011; Ezeamama *et al*., 2008; Persson *et al*., 2000).

In the present study, multiple parasites of up to 8 different species or genus were recovered concurrently in a single host. Similar multiple infections were reported elsewhere from rural communities in Cote d'Ivoire of 9 and 10 species simultaneously (Hürlimann *et al*., 2014; Raso *et al*., 2004). Mazigo and colleagues (2010) reported triple co-infections of the four parasites recovered among Tanzanians. Handzel *et al*. (2003) found 2.9% of children in Kenya infected with three parasites (*A. lumbricoides*, hookworms and *T. trichiura*) among (291) all infected. Likewise in Philippines, majority of the study subjects were infected with multiple of 3 or 4 parasites (Ezeamama *et al*., 2008). Al- Delaimy *et al*. (2014) found the Orang Asli in Malaysia harbouring as many as five different parasite species concurrently. Another study reported less number in which a maximum of 2 species simultaneously in a single host among immigrants and travellers to Spain (Norman *et al*., 2010).

In this study, majority of multiple infections were of double infections (24.3%, 134/551), which were due to *P. falciparum*/*Entamoeba* complex detected in all communities except Shanono. Coinfections with *P. falciparum* and either *Entamoeba* complex or hookworms were prominent in Kura community. Coinfections of *Entamoeba* complex with either *P. falciparum* or *S. haematobium* were prominent in Bebeji and Gwarzo communities. Coinfections with *S. mansoni* were detected more in Shanono adult females. Further coinfections of *Blastocystis* sp. and either *P. falciparum* or *Entamoeba* complex were also high among the communities. Almost all the houses in the communities have in-house water storage and stagnant domestic open sewers that supports breeding of mosquitoes the malaria vectors (Mazigo *et al*., 2010), which spread the *Plasmodium* infection in these communities. The in-house water storage couple with other environmental factors enhances the transmission of water borne as well as STH parasites.

The seasonal factors of rainy and dry season irrigation were prominent in Kura area. The rainy season could increase in vector breeding sites that were associated with the higher prevalence of multiple infections (precisely malaria and schistosomiasis) in Kura community. Contact with polluted water body during the dry season could be another apparent factor that relates to the transmission of parasitic infections. In Shanono, *S. haematobium* coinfections were replaced with a higher prevalence of *S. mansoni* especially among adult females. During our sampling field trip, some women in this community were seen using untreated water from the near-by ponds for their domestic activities. This activity would have increased during the dry season when most of their personal dug wells dried up. Previous authors commented that prevalence of parasitic infections in Nigerian communities was the consequences of unhygienic characteristic especially the practice of defecating indiscriminately or arbitrary dumping of refuse (Obiamiwe & Nmorsi, 1991; Ekundayo *et al*., 2007). The non-functional drainage

systems and lack of constant supply of treated water (Damen *et al*., 2011; Ogbuagu *et al*., 2010) are other important factors. Unfortunately, to date the situation has not changed much in both rural and urban Nigerian communities, apparently the blanket high prevalence of *Plasmodium* in the communities.

Coinfections of malaria and intestinal parasites have been reported in many studies throughout Africa such as from Zimbabwe, Cameroon, Tanzania, Côte d'Ivore, and Kenya (Midzi *et al*., 2008; Nkuo-Akenji *et al*., 2006; Raso *et al*., 2004; Handzel *et al*., 2003; Olsen *et al*., 1998). This indicated that African population were infected mainly with multiple parasitic coinfections (Steinmann *et al*., 2008a; Brooker *et al*., 2006; Fleming *et al*., 2006; Raso *et al*., 2004). Therefore, distress concern on clinical implication of multiple parasitic infections was highlighted (Mwangi *et al*., 2007). Studies have clearly documented the correlation between polyparasitism and cognitive functions, growth retardation and malnutrition, exacerbation of iron deficiency anaemia as well as increased organ pathology (Jardim-Batelho *et al*., 2008; Brooker *et al*., 2006; Shapiro *et al*., 2005; Sakti *et al*., 1999). Pregnant women who were co-infected with malaria and helminths produced neonates with much lower mean birth-weights as compared to infected mothers with malaria alone (Egwunyenga *et al*., 2001).

Majority of the infections detected in this study were of light intensity, which can further go undetected and results in chronic infections as well as increased morbidity. There was a report that bladder cancer was the sixth most frequent cancer presented in Kano state and subsequent histologic analyses of the squamous cell carcinoma showed association with chronic *S. haematobium* infection (Mohammed *et al*., 2008). Moreover, anaemia and malnutrition, which are typical features of chronic parasitic infections, continue to be important public health problems in Nigeria, especially protein-energy malnutrition, micronutrient malnutrition and iron deficiency anaemia (Adelekan, 2001).

On the other hand, low intensity malaria was found to be associated with a stable transmission of infection. Individuals with low parasitemia serve as reservoirs of infection with themselves having acquired immunity, which were observed in halo endemic African countries (Owusu-Agyei *et al*., 2002).

5.3 Possible risk factors of parasites' infections

The risk factors for parasitic infections in Nigerian communities are always related to human behavioural and environmental factors (Hürlimann *et al*., 2014; Ugbomoiko *et al*., 2012; Cunin *et al*., 2003) of a particular region. In this study, many respondents were found living in a large family (a peculiar custom in Hausa land) where an infected member can easily transmit infection and eventually increase the infection risk to other members. We also found that multiple infections associated with the presence of an infected family member. This coupled with cultural influences including the norm of group eating with hands from the same plate (a custom believed to strengthen affection and good relations among families, friends and neighbours) could increase risk and promote transmission of parasitic infection especially with poor hygiene. Having large family members in an economically stressed family usually promotes unhygienic practices. Example in a typical family of ten, using one toilet facility will be more challenging to keep the toilet clean. In the same way storing water especially for drinking purpose, different hands and mouths subsequently reused cups before being washed. From our observation, the cups on the water storage containers were dirty and seldom washed. The dirty buckets used to fetch water from hand-dug wells were also potential contaminants of the well-water source. All these characteristics highly supported the fact of parasitic transmission (protozoa and STH) through ingestion of contaminated food and water (Hürlimann *et al*., 2014).

Another noteworthy factor that caused an upsurge in risk of multiple infections in this study was the habit of not wearing proper foot wears outside home. Walking bare footed aids transmission of STHs especially hookworms that showed high prevalence in this study. In addition, young peers were seen playing on polluted grounds bare footed as well as swimming in polluted waters regularly. The respondents also walk/work bare footed in the farms that was contaminated with untreated human and animal faeces used as manures in their cultivation. There were also scores of children and young adults working as scavengers with torn-shoes, roaming around refuse dumps searching for reusable cans. Furthermore, it has been reported that living in a mud house, having mud flooring and pit toilet also influences incidence of multiparasitism. The facilities could have served as a hiding and breeding places for mosquitoes. These are characteristics of low socioeconomic status prevailing in rural Africa at large (Bassey *et al*., 2007). The correlation between helminths coinfections and socio-economic status is ambiguous (Brooker *et al*., 2007).

The high prevalence of malaria, schistosomiasis and STH in Kenya were incriminated for coinfections around the coastal region (Bisanzio *et al*., 2014). Similar facts have been found in our study area of widespread prevalence of malaria, schistosomiasis, STH's and possibly many more that have not been carried out due to several limitations. Incidences of multiple infections between communities were documented to vary across East Africa. Bisanzio *et al*. (2014) established that demographic and socioeconomic conditions influenced co-infections to a greater extent than environmental factors at village scale.

Many investigators consistently emphasized on sanitation being crucial to spread of multiple parasitic infections (Ugbomoiko *et al*., 2012; 2010 and 2009). The practice of geophagia, widely acquiesced by mothers of children (Chukwuma *et al*., 2009; Nock *et*

al., 2003; Seppo *et al*., 2002) and the propensity of considering either to wash or not to wash vegetables and fruits increased tremendously the risk of multiple parasitic infections. Ogbuagu *et al.* (2010) ratified the significant increase in parasites load amongst respondents who indulged in eating raw vegetables, particularly when cultivated with untreated sewage water or untreated manures (Garba *et al.*, 2010; Ozumba *et al*., 2005).

5.4 Prevalence, infection risk as well as knowledge, attitude and practices of respondents against malaria

This study reported an alarmingly high prevalence of malaria (60.6%) dominated by *P. falciparum* in Kano State, Northern Nigeria. This is in agreement with the recent malaria risk maps estimated that malaria prevalence varied from < 20% up to > 70% across Nigeria (Onyiri, 2015) depending on the different climatic conditions. Less rainfall and surface water serve as breeding sites for mosquito. The prevalence of *P. falciparum* in healthy school children from Kebbi State was reported up to 59.6% (Singh *et al*., 2014a) and 58.0% among children in three hospitals and a Nursery school in Awka Metropolis, Anambra State (Mbanugo & Ejims, 2000). It was 64.0% among children (aged between 2–15 years) that attended Gwarinpa General Hospital in Abuja (Nmadu *et al*., 2015), respectively 62.4% and 57.1% in pregnant women from Ogun and Delta States, respectively (Madukaku *et al*., 2012; Idowu *et al*., 2008a). Prevalence of 80.1% was reported among pregnant women from Imo State (Ohalete *et al*., 2011), 78.9% in Ogun State (Oladeinde *et al*., 2012), 76.9% in Gboko, Benue State (Houmsou *et al*., 2010), 72% in Osogbo, Osun State (Adefioye *et al*., 2007) and 68.3% in Makurdi, Benue State (Amuta *et al*., 2014).

Moreover, an extraordinary population-based prevalence rate (99.2%) was reported among 2069 pregnant women in Enugu State, southeastern Nigeria (Gunn *et al*., 2015). A prevalence of 81.5% was reported among 708 participants attending the Department of Health Services, University of Agriculture in Abeokuta, Ogun State, located in the forest zone of southwestern Nigeria (Okonko *et al*., 2009) and 80% among 200 fresh (first year) university students in Anambra State, South-Eastern Nigeria (Ibekwe *et al*., 2009).

In contrast, lower prevalence rates have been documented including 39.2% among antenatal clients attending primary health care facilities in Kano state (Gajida *et al*., 2010), 29.4% of blood donors in Zaria, Kaduna State (Oche & Aminu, 2012) and 26% among pregnant women in Port Harcourt, Rivers State (Michael *et al*., 2013). Prevalence of 35% was reported among long distance truck drivers in Delta State (Erhabor *et al*., 2012), 42.3% in Otukpo, Benue state (Jombo *et al*., 2010), 36.1% and 36.6% in Abia and Plateau States, respectively (Noland *et al*., 2014) and 41.6% among pregnant women in a semi-urban community of Argungu, Kebbi State North-western Nigeria (Fana *et al*., 2015).

In other sub-saharan countries, prevalence of malaria were documented as 48.2%, 47.8%, 49.3%, and 42·9% from Democratic Republic of Congo (Mvumbi *et al*., 2016), Mozambique (Temu *et al*., 2012), Burkina Faso **(**Geiger *et al*., 2013), and Sierra Leone **(**National Malaria Control Programme Sierra Leone, 2013**),** respectively. Likewise, 42.0% of 2346 schoolchildren living in a high transmission setting in western Kenya were positive for *P. falciparum* (Kepha *et al*., 2016). Leading by Nigeria, these countries were considered the top six countries most affected by malaria (WHO, 2015).

Our findings showed a similar prevalence rate among the male and female participants, which concurred with previous studies from Nigeria, Mozambique and

Kenya (Noland *et al*., 2014; Temu *et al*., 2012; Brooker *et al*., 2004). However, males were found to be at higher risk of malaria infection due to exposure, inherent and cultural determinants (Loha & Lindtjørn, 2012; Al-Mekhlafi *et al*., 2011; Winskill *et al*., 2011; Bates *et al*., 2004). High and repeated exposure to malaria among males may result in development of partial immunity that makes them at lower risk of clinical malaria compared to females (Kepha *et al*., 2016; Smith *et al*., 1999).

With regards to location selected in this study, there was no significant difference in the prevalence of malaria dominated *P. falciparum*. However, we found an obvious compatibility in Shanono community that had the lowest history of recent malaria (24.2%) and the highest insecticide treated nets (ITN's) utilization (62.6%). This may be due to populations' specific characteristics that facilitated human–mosquito contacts, thus malaria risk could vary widely between the districts, villages or even households (Carter *et al*., 2000). A recent study among children in Zambezi province, Mozambique showed that characteristics such as age, household monthly income, ITN utilization and type of toilet were the significant risk factors associated with the prevalence of *P. falciparum* and were varied (ranging from 22.1% to 87.2%) between villages within the same province (Temu *et al*., 2012).

The prevalence of *P. falciparum* was also significantly higher among respondents within age groups > 10-18 years (66.2%) and > 18 years (61.7%) as compared to > 5 - 10 years (41.7%) and ≤ 5 years old (37.5%). This difference may be due to the ITN usage where usage was higher among younger respondents. Moreover, individuals aged > 10 years are expected to have a higher exposure to mosquito bites due to their more frequent outdoor activities as compared to younger children, particularly during night-time. These findings were found to be consistent with recent reports that ITNs utilization rates was significantly higher among youngest children, particularly those

aged below 5 years, and heads of the family (Ferrari *et al*., 2016). Likewise, another study revealed that adolescent boys were found to be the least likely group to use ITNs (Garley *et al*., 2013). In accordance, a recent study in Abia and Plateau States found that prevalence of *Plasmodium* infection was significantly associated with age although in their study, prevalence was highest among children 5-9 years (Noland *et al*., 2014). In contrast, a recent study among Kenyan schoolchildren found that *P. falciparum* infection reduced with increasing age, with those aged 11-15 years had 0.78 odds of infection compared to those aged 5-10 years (Kepha *et al*., 2016).

This study also found that low household monthly income (< NGN32,000) increases the odds of malaria by about 1.6 times among the participants. This is in agreement with previous reports from other African countries that showed malaria was more common among people of lower socioeconomic status who often live in poor housing conditions that increase their exposure to infection (Loha & Lindtjørn, 2012; Kepha *et al*., 2016; Pullan *et al*., 2010; Noor *et al*., 2009). Malaria and poverty are intimately connected as malaria primarily affects low and middle income countries where the poorest communities were the most severely affected with malaria due to poor socioeconomic and environmental status, and inadequate services for prevention, diagnosis and treatment (WHO, 2015). Hence, these endemic communities are trapped in a vicious cycle of poverty, underdevelopment and disease.

Despite high IRS coverage and equitable ITNs distribution (63.4%), poverty at both the community and household level were significant risk factors for malaria in North West Tanzania (West *et al*., 2013). Use of ITNs was considered as one of the most cost-effective interventions against malaria in highly endemic areas, and it was associated with significant reductions in malaria morbidity and mortality particularly among pregnant women and children aged below 5 years (Gamble *et al*., 2006). The present

study showed that not using ITNs is a significant risk factor of malaria among the studied population and this is consistent with previous studies from Nigeria and Ethiopia (Loha & Lindtjørn, 2012; Fana *et al*., 2015).

Despite the high ITNs possession rate (79.5%) in this study, only half of the participants declared and were found using the ITNs (42.8%). This unsatisfactory compliance was in agreement with previous studies from Nigeria (Morwell *et al*., 2014; Noland *et al*., 2014; Kilian *et al*., 2013; Oyeyemi *et al*., 2010; Afolabi *et al*., 2009) and other countries like Kenya and Yemen (Atieli *et al*., 2011; Al-Taiar *et al*., 2009). A recent national-based study from Nigeria has demonstrated a notable gap between the ownership and use of ITN and showed that at national level only 28.7% of the population had access to an ITN within the household and this rate increased to 50.0% in areas with a recent campaign (this include Kano state) (Kilian *et al*., 2013). In addition, the study highlighted a considerable gap in the intra-household possession of ITNs, as about two-thirds of the households covered by ITN distribution did not have enough ITNs for every household member. On the other hand, we observed that up to 30% of the ITNs were misused as blankets, window curtains, door curtains and as fishing nets. Moreover, some participants declared that they have washed the ITNs before use to remove the chemicals that were either smelly or allergic to some individuals. In addition, some of the ITNs were old, not re-treated or in poor condition.

The misused ITNs characteristics were reported from many malaria-endemic African countries including Nigeria, Uganda, Tanzania, Kenya, Zambia, Sierra Leone and Democratic Republic of Congo (Adebayo *et al*., 2015; Kilian *et al*., 2015; Lowassa *et al*., 2012; Eisele *et al*., 2011; Baume *et al*., 2009; Minakawa *et al*., 2008). Some individuals sold off the free-given ITNs in open markets and refused to use due to a misconception that the ITN is a purely Western intervention that has been forced on

African communities, with little regard to local norms or cultures (Eisele *et al*., 2011) as well as having a perception that malaria or mosquitoes are not a serious problem. Subsequently, participants that live in houses with pit or ground dug latrines were 1.4 times more likely to be infected with malaria compared to those who have toilets with pour flush system in their houses. Unavailability of in-house toilets has been reported as a significant predictor of malaria in Ethiopia and Yemen (Ayele *et al*., 2012).

With regards to respondents' knowledge attitude and practices (KAP), this study indicated that general awareness about malaria was high among Hausa communities in Kano State and almost all of them (95.6%) have heard about malaria. This characteristic was expected as malaria is considered as a major health problem in these communities. Some respondents did not comprehend the cause, symptom and prevention but 77.8% of them recognized fever as a detrimental consequence of malaria. Some did not mention mosquito as the cause of infection directly, but mentioned environmental hygiene or stagnant water, which are all mosquito-breeding sites. Majority of them had information about malaria prevention, using bed nets with or without insecticide, improving personal hygiene and taking medication, which were the three main preventive measures. These characteristics concurred with previous studies from other parts of Nigeria (Singh *et al*., 2014b; Erhun *et al*., 2006), and other malaria-endemic countries (Kimbi *et al*., 2014; Kinung'hi *et al*., 2010; Isah *et al*., 2007).

However, a previous study among pregnant women from Ogun State in southwest Nigeria showed that knowledge of malaria transmission and prevention was generally poor in which only 36.3% of them mentioned the association of malaria infection with mosquito bites and none of them mentioned the use of ITNs as a preventive measure (Idowu *et al*., 2008a). Likewise, a previous study among rural farming communities in Oyo State (south-western Nigeria) reported low level of

knowledge about malaria transmission and symptoms in which only 12.4% indicated the role of mosquito bite in transmitting the disease and less than half (46.7%) were able to mention at least one symptom of malaria (Oladepo *et al*., 2010). Similarly, recent studies from Southwest Nigeria revealed that knowledge of malaria was still low among under-five caregivers, pregnant women and mothers (Bello & Rehal, 2014; Adebayo *et al*., 2015; Runsewe-Abiodun *et al*., 2012).

In this Study, majority of the respondents (91.5%) indicated that malaria causes serious disease, which is in agreement with previous studies from Nigeria and other countries (Singh *et al*., 2014b; Adedotun *et al*., 2010; Isah *et al*., 2007). Only a very small number of respondents (3.3%) considered malaria as not harmful and claimed that "malaria is not so serious because everyone has it, and cannot be cured". Some said "It sometimes causes fever, when one takes medication the fever goes away but the malaria does not, we are born with it". To others "malaria cannot be cured, it always comes back" and "malaria is in our blood, it is always there, just avoid going down with fever by avoiding stress and always eat good food". Few were indifferent in thinking that nuisance of mosquito was only the bite, if can be avoided better but otherwise has no consequences. However, our findings were contradictory with a recent study from Osun State, Southwest Nigeria that reported majority of mothers perceived malaria as a simple disease and they were comfortable and can be cured using home based remedies (Bello & Rehal, 2014).

Despite majority (91.5%) of the participants realized the seriousness of malaria infection, yet about 19.9% of the respondents preferred to start treatment at home and only seeked help from hospitals when their home based remedies failed. The home treatment is usually self-medication or unqualified prescription by family members, friends and unrecognized chemists. The common practice of self-treating with analgesic

and then antimalarial drugs (when symptoms persisted) have been reported from other parts of Nigeria (Okeke & Okeibunor, 2010; Oladipo *et al*., 2015; Adedotun *et al*., 2010; Idowu *et al*., 2008b) and Africa (Deressa *et al*., 2003; Thera *et al*., 2000). Intriguingly, a previous study from Oyo state (southwest Nigeria) showed that about 90% of suspected malaria cases were first self-treated at home with traditional herbs or drugs purchased from medicine stores (Adedotun *et al*., 2010). This characteristic gave a significant difference in treatment-seeking behaviour between urban and rural mothers in southeast Nigeria where two-thirds of urban mothers preferred private/government health facilities while two-thirds of their rural counterparts preferred self-treatment with drugs bought from medicine vendors (Okeke & Okeibunor, 2010).

Notwithstanding, few of the respondents believed that sunlight contributed to the occurrence and severity of malaria. Moreover, misconception about the cause of malaria was documented and expressed as excessive heat, stress and unbalanced diet (Mbanugo & Emenalo, 2004; Idowu *et al*., 2008b; Oguonu *et al*., 2005). In other African and Asian countries, malaria was attributed to the sun, drinking alcohol, eating sour foods, playing in rain, eating cold foods, forest, lack of sanitation, body contact with infected person, bedbugs, and witchcraft (Obol *et al*., 2011; Kinung'hi *et al*., 2010; Njama *et al*., 2003).

Characteristics such as age, gender, educational level, employment status and household monthly income also have great influence on having adequate knowledge about malaria. Younger respondents showed better level of knowledge about malaria transmission and prevention than adults (aged ≥15), and this could be attributed to schools or media especially television. Likewise, males had substantially higher level of knowledge about malaria transmission, symptoms and prevention and this might be due to their higher exposure as well as by some behavioural and cultural factors of not

educating a daughter. In Nigeria, prevalence of malaria was highest among pregnant women compared to other groups (Gunn *et al*., 2015; Oladeinde *et al*., 2012; Ohalete *et al*., 2011). Hence, targeting those women with a health education programme about the disease could result in a significant reduction in the malaria incidence in the country.

Similarly, higher levels of knowledge about malaria transmission, symptoms and prevention were also reported among the employed respondents and those having household monthly income of ≥ NGN32,000 compared to their counterparts, and this was compatible with the higher prevalence reported among the unemployed and low monthly income families. This finding is in agreement with previous studies in Nigeria (Bello & Rehal, 2014; Adebayo *et al*., 2015; Oladepo *et al*., 2010). Obviously, poverty hinders the efforts of malaria control programme in the study areas and other parts of Nigeria. These groups of people may have limited access to media and other information sources as well as do not have enough income to use in terms of preventing malaria infection, for example buying ITN, insecticide or seeking for treatment at hospital. Implimentation of the free distribution of Long Lasting Insecticide-treated Net (LLIN) by the government and other non-governmental organizations reduced the market price of the ITNs [e.g. from NGN650 (US$4.00) to NGN250 (US$1.50)] to a more affordable price. Educated people give credence to buying and using bed nets (either treated or not) and insecticide sprays than non-educated even if they have sufficient family monthly income.

Previous studies from Nigeria showed that level of education was a strong predictor of positive malaria-related KAP (Russell *et al*., 2015; Morwell *et al*., 2014; Adedotun *et al*., 2010). Similarly, the choice of going to hospital at the appropriate time also depends on the level of education, with the non-educated respondents patronizing more of the unauthorized practitioners or resolved to use medication from vendors on the street or

shops. This practice coupled with poor quality of antimalarial drugs, propagates the emergence and widespread of chloroquine-resistant malaria among the population in Africa (Oladipo *et al*., 2015; Sawadogo *et al*., 2011). Therefore, policy makers should realize that implementing training of medical and paramedical personnels on malaria treatment and management must include "shop operators" since they are operating at the closest point to the population and is an attitude that cannot be stopped immediately.

In the same vein, health education was documented to be an imperative tool to improve knowledge, promote appropriate behaviour and reduce the incidence of malaria infection (Amoran, 2013; Ayi *et al*., 2010; Brooker *et al*., 2008). Previous studies have assessed the effect of health education intervention on the KAP about malaria amongst mothers of children under-five years old in a rural area of Ogun State, Nigeria. The report gave a significant improvement in the knowledge of relevant signs and symptoms, indoor spraying, window and door nets usage as well as usage of correct dose of artemisinin-based combination therapy in the home management of malaria, correct use of ITNs and maintenance of environment cleanliness (Amoran, 2013; Amoran *et al*., 2012; Fatungase *et al*., 2012).

Health education interventions improved knowledge of people regarding malaria transmission and control, induced changes in behaviour of early malaria diagnosis and implementation of vector control that subsequently decreased the prevalence of malaria in Colombia (Rojas *et al*., 1992). Similarly, educating Kenyan schoolchildren on how mosquitoes breed and transmit malaria resulted in changes in their attitudes and behaviours thereby reducing mosquito breeding sites by 69% as compared with only 1% in the control not-educated group (Ogutu *et al*., 1992). Another study from Thailand also showed that schoolchildren changed their behaviour positively towards malaria prevention due to health education programme (Okabayashi *et al*., 2006).

5.5 Prevalence, infection risk as well as knowledge, attitude and practices of respondents against Schistosomiasis

The total prevalence rate of *Schistosoma* infection was 17.8%, which included the urogenital (*S. haematobium*, 8.3%) and intestinal (*S. mansoni*, 8.9%) schistosomiasis. This prevalence is in accordance with prevalence rates reported by previous studies including 11.5% in Adamawa state (Nale *et al*., 2007), 15.3% in Ebonyi state (Ivoke *et al*., 2014), 15.7% (only *S. haematobium*) in Anambra state (Ugochukwu *et al*., 2013), 17.4% in Ibadan Oyo state (Okoli & Odaibo, 1999), and 18.7% in Plateau and Nassarawa states (Evans *et al*., 2013). Higher prevalence rates were reported from Kano state itself with 44.2% among 493 schoolchildren in Minjibir, infected with *S. haematobium* (Duwa *et al.*, 2009). Further 50.3% children aged 5-17 years, were infected with *S. mansoni* (Bassey & Umar, 2004) and 42.7% of 6600 individuals from 132 towns/villages of Kano state were positive for *S. haematobium* (Abdullahi *et al.*, 2009).

High prevalences of *Schistosoma* infections were noted from several areas in Nigeria. In Osun State, the prevalence of *S. haematobium* was up to 58.1% in 167 preschool children (Ekpo *et al*., 2010) and 55% among 300 children (Amuta & Houmsou, 2014). Therefore, our result showed lower prevalence in Kano state which is most probably due to the integrated and cost-effective approaches that have been implemented by the Federal Ministry of Health for the purpose of eliminating the multiple Neglected Tropical Diseases (NTDs) in Nigeria including lymphatic filariasis, onchocerciasis, schistosomiasis, human African trypanosomiasis and leprosy by the year 2020 (Hotez *et al*., 2012).

At the community level, the present study revealed a significantly varying prevalence from the highest 30.9% (Gwarzo), 21.2% (Shanono), 15.7% (Kura), and 11.9%

(Minjibir) to the lowest 11.8% (Bebeji). The variations of *Schistosoma* prevalence rates were noted from different communities and locations in Nigeria (Bigwan *et al*., 2012; Abdullahi *et al.*, 2009; Ekpo *et al*., 2008b). Despite reports of high prevalence, several communities of Nigeria announced lower prevalence rates such as 2.3% in Ogun (2.3% *S. mansoni* and 0.6% *S. haematobium*) (Agbolade *et al*., 2007) and 6% in Yobe (10% *S. haematobium* and 2% *S. mansoni*) (Bigwan *et al*., 2012).

Regarding gender, our findings showed that the prevalence was significantly higher among male participants as compared to females and this is in agreement with many previous reports (Ivoke *et al*., 2014; Bigwan *et al.*, 2012; Abdullahi *et al*., 2009; Duwa *et al*., 2009). This could be attributed to the religious and cultural beliefs. For instance, in Islamic communities, females are not allowed to go for swimming or bathing in the open water sources and also do not participate in fishing and irrigation activities. Moreover, males were more likely to be knowledgeable of the existence of an open water source in their area compared to females (Kapito-Tembo *et al*., 2009).

In our analysis, we found that the prevalence was significantly higher among participants in age group below 18 years compared to those aged ≥ 18 years; the highest prevalence rate was seen among those aged 10-18 years (27.8%). Previous studies have shown the age-dependency of *Schistosoma* occurrence, which indicated that the prevalence peaked in adolescence and decreases slowly with increasing age (Ugochukwu *et al*., 2013; Abdullahi *et al*., 2009; Nmorsi *et al*., 2005; Ekwunife *et al*., 2004). The excessive mobility of adolescents was due to swimming, bathing and playing in open water that could explain the higher prevalence rate among this age group. Moreover, previous studies from Nigeria, Kenya and Malawi reported an increasing trend of infection among children aged 6-13 years and declining from 14 years onwards (Kapito-Tembo *et al*., 2009; Satayathum *et al*., 2006; Nduka *et al*.,

1995). There was also suggestion that age-acquired immunity against re-infection may contribute to the lower prevalence among young-adults aged ≥ 15 years (Etard *et al*., 1995).

The potential factors associated with *Schistosoma* infection in this study were age < 18 years, presence of infected family member and having history of past *Schistosoma* infection. These findings are in agreement with previous studies from Nigeria (Amuta & Houmsou, 2014; Ekpo *et al*., 2008b; Nmorsi *et al*., 2007) and other countries including Yemen, Malawi and Egypt (Sady *et al*., 2013; Kapito-Tembo *et al*., 2009; El-Khoby *et al*., 2000). Individuals residing in these communities with the presence of other infected family members were more than 3-folds higher risk of acquiring *Schistosoma* infection. It was suggested that infected family member served as a source of infection. The presence of an infected family member may contribute to the transmission of infection among other family members who may have similar water contact, exposure and behaviour (Sady *et al*., 2013). Similarly, our findings showed that participants who had history of *Schistosoma* infection were 2.87 times more likely to be infected compared with individuals that are not at risk. This could be partially attributed to the clustering of communities with high infection rates around infested water sources putting the residents at high risk of re-infection (de Souza Gomes *et al*., 2014; Kapito-Tembo *et al*., 2009).

Schistosoma infection was also found to be associated with gender and employment status of the participants in which higher prevalence was seen among males and not working participants. However, this significant association was not retained in the multivariate analysis. Previous reports documented varied association between gender and *Schistosoma* infection. Some studies found no association between gender and infection (Sady *et al*., 2013; Opara *et al*., 2007; Satayathum *et al*., 2006) but some

documented that male gender was a significant risk factor of *Schistosoma* infection in Nigeria (Okoli & Odaibo, 1999; Evans *et al*., 2013), Malawi (Kapito-Tembo *et al*., 2009), Zanzibar (Rudge *et al*., 2008) and South Darfur (Deribe *et al*., 2011). In contrast, there was a study from Nigeria that revealed a significant association with female gender (Ogbeide *et al*., 1993).

Subsequently, we also found no significant association between infections and the participant's educational level, household monthly income, sources of drinking and household water. However, schistosomiasis was documented as a poverty-related disease; with a higher prevalence among participants belonging to families with low household income (Sady *et al*., 2013). Moreover, higher educational level of the household head (leader) was identified as a protective factor against schistosomiasis in Nigeria and Cote d'Ivoire (Ugbomoiko *et al*., 2010; Matthys *et al*., 2007). Hence, our findings suggest that improving socioeconomic status alone may not contribute to a significant reduction of schistosomiasis prevalence rate in these communities and integrated control measures should be implemented.

With regards to knowledge, attitude and practices (KAP) towards schistosomiasis, our findings showed that the respondents were conversant with especially urinary schistosomiasis. About three-quarters of the respondents had prior knowledge on schistosomiasis. This could be attributed to the fact that schistosomiasis is endemic in Nigeria and there have been intensive efforts to control the disease throughout the country. Consequently, the high percentage of self-reported history of *Schistosoma* infection among the participants supports the endemicity in these communities. Accordingly, previous reports showed variation in the level of awareness among Nigerian population including 33.8% - 42% in Delta state, southeastern Nigeria (Ukwandu & Nmorsi, 2004; Onyeneho *et al*., 2010) and 64.4% in Ogun and Niger

states along the middle belt and southwestern region (Akinwale *et al*., 2004). Poor knowledge on schistosomiasis and its causes were reported in Malawi and Kenya (Odhiambo *et al*., 2014; Poole *et al*., 2014) while high level of awareness (80%) was reported in Zimbabwe (Ndamba *et al*., 1998).

In this study, majority of the respondents did not know the causes, mode of transmission, signs and symptoms, and preventive measures of schistosomiasis. This indicates lack of health education among the targeted populations, which should be provided during mass chemotherapy campaigns. With regards to knowledge on the causes of schistosomiasis, 44.7% (168/376) of the respondents who have heard about schistosomiasis mentioned worms as the cause of infection. Even though half of those respondents recognized polluted water bodies as infection foci, yet only 6.1% (23/376) considered avoiding contaminated water as a preventive measure. A previous study in Anambra state observed that most of the subjects understood that water bodies transmit the infection and they desist from drinking but still use it for domestic activities (Ekwunife *et al*., 2004). Correspondingly, this characteristic was also noted in other endemic areas of Africa and Brazil (Gazzinelli *et al*., 1998; Chimbari *et al*., 1992; Kloos, 1995). This indicates that awareness merely does not necessarily result in behavioural changes which is often more difficult to be achieve, requiring long periods of time to ensure compliance with healthier practices.

With regards to knowledge about signs and symptom, our findings showed that 59% of the respondents mentioned haematuria while one-third of them could not associate the infection with any symptom, and yet none of the respondents mentioned itching, rashes or fever. Conversely, a previous study from Brazil reported diverging information where the subjects were able to associate these symptoms with the infection (Uchoa *et al*., 2000). It is also worth noting that respondents' knowledge about the

symptoms of intestinal schistosomiasis was negligible as only 14.9% mentioned blood in stool. This is in agreement with previous studies in Western Cote d'Ivoire Senegal and Egypt (Acka *et al.*, 2010; Sow *et al.*, 2003; Mehanna *et al*., 1997). This could be due to the disease has been frequently confused with other diseases exhibiting similar symptoms. The local name for schistosomiasis in Hausa language "*Tsargiya*" is synonymous to urinary schistosomiasis, meaning blood in urine, and this may also explain the better knowledge about haematuria.

Subsequently, two-thirds of the respondents did not know the transmission mode of *Schistosoma* and one quarter (27.9%) of them mentioned contaminated water bodies. Similarly, three quarters of them did not know how to prevent the infection and 23.9% of them mentioned medication. Only 6.1% of the respondents mentioned that the disease is associated with contacting contaminated water and none of them indicated the role of snails in the transmission of *Schistosoma*. Similarly, poor knowledge about transmission mode and prevention were noted from Zimbabwe **(**Midzi *et al*., 2011) as well as Ogun and Niger States in Nigeria (Akinwale *et al*., 2004).

Public health information on schistosomiasis from clinics, hospitals, or media is lacking and majority of the respondents knew about the disease from their family members or neighbours. Therefore, misconceptions and diverging views about causation, transmission and symptomatology of the disease prevail. Up to 18.9% of the respondents believe that eating salty or sour food causes the infection and sharing toilet with an infected person can spread the disease. Moreover, a community leader believed that the disease is God's wishes and cannot be prevented. Referring to haematuria, an elderly lady said 'it is just something that children especially boys do but will outgrow later'. Misconceptions such as eating too much salt, stepping in witches place, jumping over fire or eating green mangoes could cause schistosomiasis were noted in school

children from Zimbabwe (Midzi *et al*., 2014). Likewise, a previous study among 207 household heads in Cote d'Ivoire found that a large proportion of them believed that avoiding unripe fruit consumption is a preventive measure of intestinal schistosomiasis (Acka *et al*., 2010). Interestingly, a study in Delta state, southern Nigeria reported that some respondents mentioned maturity and witchcraft as cause, and sexual contact with infected person as the transmission mode for schistosomiasis (Onyeneho *et al*., 2010).

In this study, the respondents' knowledge about schistosomiasis and its causes (worms and polluted water), transmission (contaminated water), symptomatology (haematuria) and prevention (taking medication) were significantly higher among males compared to females. Similarly, knowledge about causes (worms and polluted water) and prevention (taking drugs) was significantly higher among respondents aged below 18 years compared to those aged ≥18 years. In addition, we also found that this knowledge varies considerably among the communities aligned with their respective prevalence rates. Despite high prevalence of *Schistosoma* infection a previous study in western Kenya found that most respondents stated having heard about schistosomiasis but very few had knowledge about signs and symptoms, causes, transmission and prevention (Odhiambo *et al*., 2014).

On the hand, educated respondents had significantly better knowledge of haematuria as a sign, contaminated water as a mode of transmission, and taking medication as a preventive measure for schistosomiasis as compared to their non-educated counterparts. Likewise, working respondents had significantly better knowledge on avoiding contaminated water as a preventive measure of schistosomiasis compared to those who were not working. These findings are in agreement with a previous study from China that educational level, occupation and higher income were significant predictors of knowledge of schistosomiasis (Zeng *et al*., 2011). Moreover, a study among 908

household heads in Uganda reported significant association between knowledge of schistosomiasis and gender, age and educational level of the respondents (Kabatereine *et al*., 2014).

Attitude about the disease revealed that two-thirds of the respondents agreed that schistosomiasis is a serious disease. As expressed by one respondent 'Yes it is a serious disease. From experience, I know it is very painful and who knows how much blood one loses daily?' Besides the pain experienced during urination and defecation, high perception on the devastating nature of this disease was probably due to the fact that it is customary for people in these communities to associate anything that results in blood coming out of the body as very serious. Higher percentage (83.7%) of such positive attitude was reported among 3,000 participants from the mountainous region of China (Liu *et al*., 2014). In contrast, none of the women from Cote d'Ivoire who participated in a KAP survey was able to give an accurate description of signs of schistosomiasis (Acka *et al*., 2010). Besides, our findings also showed that males, aged ≥ 18 years, educated and working respondents had significantly higher positive attitude about the seriousness of the disease compared to their counterparts. The association between water contact and risk of *Schistosoma* transmission was well documented (Mwanga & Lwanbo, 2013; Baruun & Aagaard- Hansen, 2008; Dalton & Pole, 1978; Farooq & Mallah, 1966). However, age and gender were the only two significant factors that help in predicting the level of *S. haematobium* infection but an individual's water contact pattern was not significant (Barbour, 1985).

Swimming and domestic activities were found to be the main reason for participants to get in contact with water body. Among the boys, group swimming was an important recreational activity especially during the midday school break or after school hours. Males under the age of 21 years were documented to be responsible for up to 77% of

water contamination with *Schistosoma* eggs in Northern Nigeria and suggested that serious attention on group swimming by young males should be considered by any of the control strategy body (Tayo *et al*., 1980). However, there was no significant association between water contact and the prevalence of infection in our study. Furthermore, the findings showed that the proportion of those who had water contact due to swimming was significantly higher among males and those aged <18 years compared to females and those aged ≥18 years. On the other hand, working respondents had significantly lower water contact particularly swimming as compared to those not working who may have more leisure time for such activities. Likewise, the proportion of those who practiced swimming in the water bodies was significantly higher among educated respondents compared to their non-educated counterparts. This may indicate the need for schistosomiasis-related health education among endemic communities to help people understand that their own behaviour is a key factor in the transmission of *Schistosoma*.

All the respondents admitted to having a toilet facility at their households (either pit or pour flush), yet they still practice open or indiscriminate urination/defecation along the watersides and nearby bushes. A teenager argued that 'it is not feasible to go back home just to answer nature's call' while another claimed 'why burden ourselves when there is water around to clean'. Similar findings were reported by previous studies in Nigeria (Akinwale *et al*., 2004; Ekwunife *et al*., 2004). Based on these findings, it can be deduced that provision of toilets at home alone would not prevent this undisciplined manner and public education on the importance of using the toilets in controlling schistosomiasis and other parasitic infections should be provided to targeted population.

Another important attitude was the tendency of seeking medication where majority of the respondents preferred self-medication with drugs bought from shops (called

chemist) or from drug hawkers on the streets. Only one third of them sought treatment for haematuria at the nearest hospital/clinic. A similar habit was observed among residents of Ogun and Niger states who testified to going to hospitals only after failed attempt of other options (Akinwale *et al*., 2004). Some of the respondents claimed going to the 'chemist' were cheaper, easier and no time-consuming queue. Up to 12.7% of them claimed that they sought traditional medicine practitioners for treatment and conversely claimed to obtain faster cure. Besides, they feel more comfortable explaining their health problems to the much familiar traditional practitioners. However, an elder respondent stated "traditional medicine was what we used in our time when we had blood in urine, but nowadays it sometimes takes long time to work or does not at all". Another young mother said "we take our children to the traditional medical practitioner for medication because with hospital treatment the disease comes back after few months". A previous study among schoolchildren in southwestern Nigeria documented that more than 80% of the children claimed that urinary schistosomiasis is a serious disease, yet did not compliment with the appropriate treatment seeking behaviour (Onayede *et al*., 1996). Perhaps the inclination on self-medication or traditional medicine was sometimes due to poverty or inaccessibility of functioning hospitals/clinics.

The association of treatment seeking behaviour with the respondents' characteristic revealed that seeking treatment from hospitals/clinics was significantly higher among males compared to females and among older respondents aged ≥ 18 years compared to those aged < 18 years. On the other hand, self-medication was significantly lower among those aged ≥ 18 years compared to their younger counterparts. Likewise, significantly higher proportions of non-educated respondents do nothing to treat haematuria or blood in stool as compared to educated counterparts. A previous study in Uganda found that level of education did not impact on schistosomiasis treatment

seeking behaviour (Kabatereine *et al*., 2014). In Hausa rural communities, females are prohibited from going outside without permission of the head of household (husband/father) even to hospitals. Moreover, women are less self-sufficient, thus they depend on the males for financial support to seek treatment. Previous studies found similar association between water contact and age and gender regardless of employment status (Barreto, 1993; Firmo *et al*., 1996).

5.6 Prevalence and distribution of *Blastocystis* subtypes

Blastocystis was documented to be the most common unicellular eukaryote in human faecal samples (Osman *et al*., 2015; El Safadi *et al*., 2014; Silberman *et al*., 1996). Prevalence of *Blastocystis* in humans may exceed 50% in developing countries and lesser (up to 20%) in industrialized countries (El Safadi *et al*., 2014; Scanlan *et al*., 2014). In Nigeria, detection of *Blastocystis* is not routinely carried out due to inexperience in diagnosis and less awareness as compared to many other endemic parasites. To our knowledge, only three previous studies were reported on *Blastocystis* among Nigerians, two of which employed the direct faecal smear technique reporting prevalence of 2.83% and 0.94% among HIV-positive and HIV-negative patients, respectively (Ochigbo *et al*., 2011) while Adekunle (2002) found only in a child out of 1,273 subjects. More recently, Alfellani *et al*., (2013a) discovered a higher prevalence (49%) by PCR among clinical samples from Lagos, Nigeria. Thus, the present study is the first study conducted to explore the prevalence and subtype (ST) of *Blastocystis* in the healthy Nigerian population.

In this study, prevalence of *Blastocystis* was 29.2% based on stool direct smear and *in vitro* cultivation technique. *In vitro* cultivation was claimed to be the most suitable method for the diagnosis of *Blastocystis* and was extensively used in most of the

laboratories (Santos & Rivera, 2013; Abdulsalam *et al*., 2012; Dogruman-Al *et al*., 2010, Suresh & Smith, 2004). Due to the long journey during transportation from Nigeria to Malaysia, we believed many *Blastocystis* isolates died and were undetectable, thus the actual prevalence is most probably higher than currently detected. However, the current result concurred with the findings from many other countries including Africa. In Libya, *Blastocystis* were respectively reported 21.2% and 28% among the outpatients from Sebha hospital (Abdulsalam *et al*., 2013a; Alfellani *et al*., 2013a), as well as Egypt 22% **(**El-Shazly *et al*., 2006), Colombia 22.4% (Boeke *et al*., 2010), Malaysia 25.6% (Abdulsalam *et al*., 2012) and Iran 28.2% (Daryani *et al*., 2006).

Higher prevalence exceeding 40% were from communities in Venezuela (Velasco *et al*., 2011), Peru (Machicado *et al*., 2012), Colombia (Ramirez *et al*., 2014), and in school children or orphanage in Indonesia (Pegelow *et al*., 1997), Thailand (Saksirisampant *et al*., 2003) and Lebanon (El Safadi *et al*., 2014). Likewise high prevalence was reported among immigrants in Naples, Italy (Gualdieri *et al*., 2016), Qatar and UAE of which the majority of whom were from African and Asian countries (AbuOdeh *et al*., 2016; Abu-Madi *et al*., 2015). High prevalence of 100% was reported from Northern Senegalese children (6-10 years old) (El Safadi *et al*., 2014). Besides, lower prevalence has been reported among school-aged children from Thailand, Turkey, Indonesia and India (Yaicharoen *et al*., 2005; Aksoy *et al*., 2007; Idris *et al*., 2010; Rayan *et al*., 2010) respectively. Thus *Blastocystis* sp. is cosmopolitan to human population.

In this study, prevalence of *Blastocystis* was notably higher among male respondents than in females but slightly more among children < 15-years-old than in adults. Similarly, Abdulsalam *et al*. (2013a) observed male participants were more prone to acquire the infection than females though adults' ≥ 18-years-old were more likely to be

infected than younger participants in Libya. This may be due to extra exposure among males with involvement in unhygienic activities such as consumption of foods and drinks from street food stalls that have been associated to facilitating transmission of faecal-oral and water borne infections. Children are carefree and do not mind eating, drinking or playing in polluted environments. Beside, Abdulsalam *et al*. (2013a) highlighted the association of infection with gender or age, might be driven by environmental rather than physiological factors. Abu-Madi *et al*. (2015) expressed that apparently the most pronounced risk factors of *Blastocystis* transmission includes poor personal and community hygiene, culture, and lifestyle of a population.

Nevertheless other studies observed contrasting association of infection with gender and age of participants. Results from UAE and Qatar showed comparable prevalence of *Blastocystis* infection among immigrants irrespective of gender or age (AbuOdeh *et al*., 2016; Abu-Madi *et al*., 2015) respectively. Anuar *et al*. (2013) and Abdulsalam *et al*. (2012) also reported homologous prevalence of *Blastocystis* infection according to age and gender of the participants in Malaysia. However Abu-Madi *et al*. (2010) from Qatar, reported prevalence of infection peaked among the youngest (less than 20-years-old) and the eldest (above 70-years-old) participants. Li *et al*. (2007) demonstrated utmost prevalence of *Blastocystis* sp. among adults (60 years and above) in Shanghai, China. In disparity, a study in Thailand reported significantly higher prevalence in younger children (Pipatsatitpong *et al*., 2012).

In this study, difference in prevalence at each of the study sites may be due to availability of treated water supply. Most respondents from Bebeji testified to living without safe water supply but actually residents of Minjibir experienced the least supply of treated water from a rather small water treatment plant at Tomas Dam. The well-known Wase Dam is also situated in Minjibir and is regarded as a highly polluted water

body, which receives both industrial and all the domestic effluents from Kano city (Bichi, 1993). Duwa *et al*. (2009) attested to the dam as a source of parasitic infections to the rural popullace of Minjibir when using the water for domestic purposes. Furthermore, both Bebeji and Kura are dry season irrigation areas, which are supplied by Lake Tiga and it's tributaries (Kadawa and Bagauda) that run down from River Niger. The residents use the water for domestic purposes when there is treated water shortage, and majority are farmers. It is not ruled out that this water body does not aid in transmission of water borne parasites including *Blastocystis*. In Gwarzo, residents receive safe water supply from a recently renovated Guzu-Guzu treatment plant however, it does not prevent people from engaging in activities like swimming at the polluted river.

From the 29.2% (161/551) of *Blastocystis* positive samples, only 54 (32.3%) or (54/161) were successfully grown in complete Jones medium. The remaining samples were unable to grow probably due to lost potency or death during transportation from Nigeria to Malaysia. However, DNA extraction and amplification were successfully carried out against all the 54 cultured isolates. The phylogenetic tree revealed four well-defined clades that identified and classified the isolates into four different subtypes. The isolates clustered under ST1, ST2, ST3 and ST4, each with well-supported bootstrap values. Isolates belonging to ST4 were clustered into two groups in a well-supported clade (64 - 99%) while all others were on an individual clade. Despite ST5-ST9 were not discovered in our study, yet the topology and branch order of the tree in the present results showed ST1 and ST2 are closely related. The tree also revealed that ST4 and ST8, as well as ST6 and ST9 are closely related. Other researchers have obtained comparable tree topology and branch order (Abdulsalam *et al*., 2013b; Whipps *et al*., 2010; Noël *et al*., 2005). The close relationship between ST1 and ST2, ST4 and ST8 as

well as ST6 and ST9 were previously noted in several phylogenetic studies (Abdulsalam *et al*., 2013b; Santín *et al*., 2011; Whipps *et al*., 2010; Stensvold *et al*., 2009a; 2007).

Blastocystis ST1 was the most prevalent (40.7%, 22/54), followed by ST3 (33.3%, 18/54), ST4 (14.8%, 8/54), and ST2 (11.1% 6/54). ST1–ST4 isolates have been postulated as most dominant in all human populations worldwide while ST5 - ST9 are sporadically found (Meloni *et al*., 2011). Subtypes ST10–ST17 were exclusively reported in animals and ST1 was the most dominant in four African countries (Alfellni *et al*., 2013b). Correspondingly, ST1 was also reported dominant by other workers from Libya and Egypt (Abdulsalam *et al*., 2013a; Hussein *et al*., 2008) respectively, as well as Qatar that included equally African immigrants and local samples (Abu-Madi *et al*., 2015). Consequently, ST1 was the second most prevalent reported from Senegal, Egypt, UAE, Turkey and India (El Safadi *et al*., 2014; Souppart *et al*., 2010; AbuOdeh *et al*., 2016; Özyurt *et al*., 2008; Pandey *et al*., 2015) respectively.

Relatively, ST3 is highly host specific and its infections are primarily human-to-human (Abdulsalam, 2013; Yoshikawa *et al*., 2000). Prevalence of ST3 in the present study might be explained by the low living standards and poor personal hygiene prevailing in Nigeria particularly rural areas. Circumstances as such tranquil human-to-human transmission with ease. The third most prevalent subtype was ST4 among the Nigerian samples agreeing with comparative study of Alfellani *et al*. (2013a), although they did not detect ST2 among Nigerians. However, ST2 has the least prevalence in our finding. Subtype ST2 was the least detected and ST4 was not found among African immigrants in UAE (AbuOdeh *et al.*, 2016). Discrepancies in the specific and relative proportions of STs observed between countries and within the same country reflect true differences between communities. Thus denote the divergent epidemiological milieus that abet transmission most likely pertaining to local living conditions and customs,

rather than differences in susceptibility to the infection (Souppart *et al*., 2010; Stensvold *et al*. 2009b; Li *et al*. 2007).

As previously suggested, ST1 and ST2 are zoonotic infections from domestic animals and rodents are the main animal reservoir for ST4 (Tan, 2008; Alfellni *et al*., 2013a; Stensvold *et al*., 2012; 2011; 2009a; Noël *et al*., 2005). Hence hypothetically, the high prevalence of ST1 in this study might imply that contacting animal faeces could be a potential source of infection. Several families in Nigeria especially in rural areas keep animals at home, including cows, goats, sheep, poultry, cats, dogs, donkeys and horses, while rodents were unavoidable nuisance. However, studies on the distribution of *Blastocystis* subtypes in animals in Nigeria are required to substantiate.

In our findings, majority of the infections was single subtype infection and only 5.6% were mixed infections. The prevalence of mixed infections observed in the present study was roughly similar to that described in other countries such as Senegal (8.6%; El Safadi *et al*., 2014), Libya (6%; Abdulsalam *et al*., 2013a), Egypt (5%; Souppart *et al*., 2010), Turkey (4.3%; Dogruman- Al *et al*., 2008), France (7.5%; Souppart *et al*., 2009), Italy (13.3%; Meloni *et al*., 2011) and China (2.6%; Yan *et al*., 2006). The prevalence also corresponds to the calculated (6.4%) subtype isolates from all epidemiological studies published to date from Africa (17 of 266 samples) listed by Alfellani *et al*., (2013a). Research has established that most *Blastocystis* infections were of single subtypes however, Scanlan *et al*. (2015) have recently demonstrated that mixed infections in healthy individuals were under-estimated. Using a recently developed sub-typing assay, they confirmed 22% of previously classified, as single-subtype infections were actually mixed-subtype infections**.**

In this study, DNA sequences were found with identical nucleotide sequences (clones a, b, c, and d) and still 8 isolates had less identical sequences in their clones.

Similar observation was made in *Blastocystis* clones of the same isolates which showed sequence variation in the SSU rDNA gene (Abdulsalam, 2013; Meloni *et al*., 2011; Souppart *et al*., 2009; Noël *et al*., 2005; 2003; Arisue *et al*., 2003). Differences among groups of clones of the same isolate were linked to co-infection of two variants within same subtype or sequence variations of the SSU rDNA gene copies within the same isolate (Santín *et al*., 2011; Souppart *et al*., 2009; Scicluna *et al*., 2006). Although in this instance, further studies such as multilocus sequence typing (MLST) are required to highlight the explicit situation.

CHAPTER 6: CONCLUSIONS

6.1 Conclusions

Parasitic infections are prevalent in most of the tropical and sub-tropical regions in the world and polyparasitism is the norm rather than the exception in these communities. Multiple parasitic infections or polyparasitism has long been acknowledged though it was seldom reported. Recently, there are growing interest focused on polyparasitism especially on determining the predictors of the infections, global burden in terms of mortality and morbidity. Particularly that it is cumbersome to accurately interpret burden of polyparasitism based on data generated from a single infection. More efforts are now under way in improving diagnostic tools that could buttress challenges faced. In this study, we focused on polyparasitism in correlation with socioeconomic and environmental influences amid perception and mannerism in a developing population of Nigeria. Our findings patronise the significance of understanding predictors of parasitic infections and transmission dynamics of individual communities. The major findings in our study includes:

i. There were 15 parasitic species / genus morphologically recovered from 3 types of samples (faecal, urine and stool), which resulted in a high prevalence of 84% parasitic infections and poses a public health challenge to the rural Hausa communities.

ii. The most prevalent parasitic infection was malaria (60.6%) followed by intestinal protozoan (45%), soil transmitted helminths (22%) and blood flukes (19.2%). Poor environmental hygiene, unfavourable socioeconomic conditions and culture promote survival and transmission of parasites in these

communities. Particularly, the availability of mosquito breeding sites, inadequate treated water supply and sewage treatment throughout Nigeria.

iii. Multiple parasitic infections (60.9%) were found more prevalent than the single infection (39.1%). As many as eight parasitic species were detected in a single host signifying the chronic nature of parasitic infections in Hausa people of Northern Nigeria.

iv. Among the multiple infections, double infections (24%) were driven by *P. falciparum* / *Entamoeba* complex or *Blastocystis* sp. Almost all the houses in the communities have in-house water storage and stagnant domestic open sewage that supports breeding of mosquitoes and enhances transmission of protozoan parasites.

v. The infections were of light intensity, but could result in chronic infections, thus may influence increased morbidity. Instances of increase rate of bladder cancer were associated to chronic *S. haematobium* infections in the state.

vi. The risk of polyparasitism was associated with the presence of an infected family member and not wearing proper footwear outside home. Having poor hygiene and infected family member predisposes other members, promote transmission through contamination of food/water and serve as reservoir to malaria. Factors such as inappropriate footwear, indiscriminate defecation in farms and along walkways within communities, use of untreated human/animal faeces as manures and poverty driven scavengers would aid the transmission of STH especially hookworms.

vii. The prevalence was high among male residents of Kura (adult) and Minjibir (children) with *Plasmodium, Blastocystis* and hookworm species. Adult residents of Kura were mostly farmers that used untreated manures and walk bare footed in the farmlands polluted with indiscriminate defecation. While in

Minjibir, young boys were seen actively playing in the dirty playgrounds especially in the early evening hours. The streets were characterized with open drainages that sustained the constant breeding of mosquitoes. The untreated water used by the communities could introduce the water borne parasites to this population.

viii. Malaria was found to be endemic with high prevalence among males (61.2%) and females (59.7%), which may be due to exposure, inherent and cultural determinants in the studied communities. Respondents above 10-years-old have higher malaria prevalence as compared to those below 10-years-old (>60%). Least prevalence was among ≤5 years old who mostly used the distributed ITN's. Predictors of malaria in the study were multi-characteristic including age (P = 0.008; OR = 2.68), household monthly income (P = 0.008; OR= 1.61), proper usage of ITN (P = 0.004; OR = 1.66) and availability of in-house toilet facility (P = 0.155; OR = 1.44).

ix. The study subjects had good levels of knowledge (95.6%) and attitude (91.5%) regarding transmission, symptoms and prevention against malaria. However, these did not translate into improved preventive and treatment seeking behaviours. Despite, high level of ITNs ownership (79.5%), usage (42.8%) fall below the national target. In addition, fewer usage of insecticide residual spray (36.8%) jointly translates into the high malaria prevalence.

x. *Schistosoma* infection was found lower (17.8%) than previously reported (44.2% and 50.3%) in Kano state. This may indicate the success of the integrated and cost effective approaches implemented by the Federal Ministry of Health with the view to eliminate multiple NTD's in Nigeria. *Schistosoma* infection was detected higher among male participants (20.6%) as compared to females (13.3%), which may be due to religious and cultural beliefs of the

Hausa community in limiting females from outdoor activities. The infection was commonly detected among <18-years-old (27.8%), which attributed to the excessive mobility of adolescents in terms of swimming, bathing and playing in open water body.

xi. The potential risk factors associated with schistosomiasis in this study were age < 18-years-old (P = 0.012; OR = 1.76), having an infected family member (P < 0.001; OR = 3.36) and having history of previous *Schistosoma* infection (P < 0.001; OR = 2.62). Gender (P = 0.029; OR = 1.70) and employment status (P = 0.014; OR = 1.75) were also found associated with the infection though were not retained after multivariate analysis.

xii. Respondents' knowledge about the cause, transmission, symptoms and prevention of *Schistosoma* infection were still inadequate and subsist misconceptions still exist. This could be an obstacle to the control and elimination of the infection in the communities. Though attitude towards *Schistosoma* infection was positive but it did not elicits appropriate behavioural changes. Participants continue to have contact with unsafe water bodies (50.9%) and prefer self-medication (47.7%) as first line of treatment.

xiii. To the best of our knowledge, this is the first study conducted to explore the prevalence and *Blastocystis* subtype distribution among apparently healthy Nigerian population. It was detected up to 29.2% in the study population and was notably higher among male participants (114; 33.5%) than in females (53; 25.1%) and among young (≤ 15-years-old; 62; 36.3%) than adults (> 15-years-old; 105; 27.6%). The differences were also most probably environmental rather than physiological factors.

xiv. Four *Blastocystis* subtypes were recovered including ST1 the most prevalent (40.7%), followed by ST3 (33.3%), ST4 (14.8%) and ST2 (11.1%). Mixed

infections among subtypes were least (5.6%) recovered. The high prevalence of ST1 might imply zoonotic transmission as participants were living in close contact with animals but needs further studies to substantiate. The prevalence of ST3 (human to human transmission) in the study demonstrated the low living standards and poor personal hygiene prevailing in Nigeria, particularly in rural areas.

6.2 Recommendations

Based on the above-mentioned conclusions of this study, the following recommendations are suggested:

i. Parasitic infections are considered as major public health problems in the country. Therefore, implementation of integrated approach aimed at controlling or eliminating the infections with emphasis on educating the population about parasitic infections as the bedrock for further control measures.

ii. Improvement of the poor environmental sanitation, personal hygiene, ignorance and provision of basic amenities that influence parasitic infections.

iii. Mass deworming programmes should be revived, complemented with health education both community-based and as part of school curriculum, imparting not only good hygiene and sanitary practices but also information about causes, transmission and preventive routes of parasitic infections.

iv. There is an urgent need to investigate and implement integrated control measures to significantly reduce malaria prevalence in the country. Besides, efforts to increase ITN's ownership, as well as monitoring to ensure consistent and proper use of the ITN in the communities.

v. More studies should be carried out to determine the prevalence of *Blastocystis* sp. in both human and animals in Nigeria. To establish epidemiological data, clinical significance and to substantiate or otherwise possible zoonotic transmission in the country.

vi. Encourage advanced molecular diagnosis of parasitic infections.

vii. Provision of public conveniences in both urban and rural areas is advocated.

6.3 Limitations

A limitation of this study was that single stool and urine samples were collected thus prevalence of parasitic infections could be higher than reported. Parasites egg-output varies day-to-day, hence collection of multiple samples would increase diagnostic sensitivity. Estimation of multiple infections might be under estimated due to many more parasites that could be detected such as filaria (lymphatic filariasis and onchocerciasis).

In this study, another constraint was collaboration with officials to reach the population was challenging. Thus one out of the six local government areas initially proposed study site was excluded due to political unrest in the southwestern part of the State.

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List of Publications and Papers Presented

A. Publications from this thesis:

1. **Salwa Dawaki**, Hesham M. Al-Mekhlafi, Init Ithoi, Jamaiya Ibrahim, Awatif M. Abdulsalam, Abdulhamid Ahmed, Hany Sady, Nabil A. Nasr, Wahib M. Atroosh. **The menace of schistosomiasis in Nigeria: Knowledge, attitude, and practices regarding schistosomiasis among rural communities in Kano State. *PLoS ONE*.** 2015, 10(11): e0143667. Doi: 10.1371/journal.pone.0143667

2. **Salwa Dawaki**, Hesham M. Al-Mekhlafi, Init Ithoi, Jamaiya Ibrahim, Awatif M. Abdulsalam, Abdulhamid Ahmed, Hany Sady, Wahib M. Atroosh, Mona A. Al-Areeqi, Fatin N. Elyana, Nabil A. Nasr, Surin J. **Prevalence, and risk factors of schistosomiasis among Hausa communities in Kano State, Nigeria**. ***Journal of the São Paulo Institute of Tropical Medicine*.** 2016, 58(54). Doi:Org/10.1590/S1678-9946201658054

3. **Salwa Dawaki**, Hesham M. Al-Mekhlafi, Init Ithoi, Jamaiya Ibrahim, Wahib M. Atroosh, Awatif M. Abdulsalam, Hany Sady, Fatin N. Elyana, Ado U. Adamu, Saadatu I. Yelwa, Abdulhamid Ahmed, Mona A. Al-Areeqi, Lahvanya R. Subramaniam, Nabil A. Nasr and Yee-Ling Lau. **Is Nigeria winning the battle against malaria? Prevalence, risk factors and KAP assessment among Hausa communities in Kano State.** ***Malaria Journal.*** 2016, 15(351). Doi:10.1186/s12936-016-1394-3

B. Publications related but not directly arising from this thesis

1. Hany Sady, Hesham M. Al-Mekhlafi, Wahib M. Atroosh, Ahmed K. Al-Delaimy, Nabil A. Nasr, **Salwa Dawaki**, Mona A. Al-Areeqi, Init Ithoi, Awatif M. Abdulsalam, Kek Heng Chua and Johari Surin. Knowledge, attitude, and practices towards schistosomiasis among rural population in Yemen. ***Parasites & Vectors*.** 2015, 8:436. Doi: 10.1186/s13071-015-1015-8
2. Hany Sady, Hesham M. Al-Mekhlafi, Romano Ngui, Wahib M. Atroosh, Ahmed K. Al-Delaimy, Nabil A. Nasr, **Salwa Dawaki**, Awatif M. Abdulsalam, Init Ithoi, Yvonne A. L. Lim, Kek Heng Chua and Johari Surin. Detection of Schistosoma mansoni and Schistosoma haematobium by Real-Time PCR with High Resolution Melting Analysis. ***International Journal of Molecular Sciences*.** 2015, 16, 16085-16103. Doi: 10.3390/ijms16716085
3. Wahib M. Atroosh, Hesham M. Al-Mekhlafi, Adel Al-Jasari, Hany Sady, Ahmed K. Al-Delaimy, Nabil A. Nasr, **Salwa Dawaki**, Awatif M. Abdulsalam, Init Ithoi, Yee Ling Lau, Mun Yik Fong and Johari Surin. Genetic variation of pfhrp2 in Plasmodium falciparum isolates from Yemen and the performance of

HRP2-based malaria rapid diagnostic test. ***Parasites & Vectors*****.** 2015, 8:388. Doi: 10.1186/s13071-015-1008-x

4. Wahib M. Atroosh, Hesham M. Al-Mekhlafi, Adel Al-Jasari, Hany Sady, **Salwa S. Dawaki**, Fatin N. Elyana, Mona A. Al-Areeqi, Nabil A. Nasr, Awatif M. Abdulsalam, Lahvanya R. Subramaniam, Meram Azzani, Init Ithoi, Yee Ling Lau and Johari Surin. Different patterns of pfcrt and pfmdr1 polymorphism in Plasmodium falciparum isolates from Tehama region, Yemen. ***Peer Journal.*** 2016, 4:e2191. Doi: 10.7717/peerj.2191

5. Fatin Nur Elyana, Hesham M. Al-Mekhlafi, Init Ithoi, Awatif M. Abdulsalam, **Salwa Dawaki**, Nabil A. Nasr, Wahib M. Atroosh, Mohamad Hafiz Abd-Basher, Mona A. Al-Areeqi, Hany Sady, Lahvanya R. Subramaniam, Tengku Shahrul Anuar, Yee Ling Lau, Norhayati Moktar and Johari Surin. A tale of two communities: intestinal polyparasitism among Orang Asli and Malay communities in rural Terengganu, Malaysia. ***Parasites & Vectors*****.** 2016, 9:398. Doi: 10.1186/s13071-016-1678-z

6. Al-Areeqi Mona A., Sady Hany, Al-Mekhlafi Hesham M., Anuar T. S., Al-Adhroey H. A., Ithoi Init, Lau Yee L., Atroosh Waheeb M., **Dawaki Salwa S**., Elyana Fatin N., Nasr Nabil A. and Surin Johari. First molecular epidemiology of *Entamoeba histolytica*, *E. dispar*, *E. moshkovskii* infections in Yemen: Different species-specific associated risk factors. ***Tropical Medicine & International Health*****.** 2017, 22(4): 493-504.

C. Conference presentations

1. **Salwa Shehu Dawaki, Init Ithoi, Jamaiya Ibrahim and Hesham M. Al-Mekhlafi.** Ameabiasis, giardiasis and *Blastocystis* infections among the Hausa-Fulanis of northern Nigeria. **Poster presentation** at the 50th Golden Jubilee of Malaysian Society of Parasitology and Tropical Medicine (**MSPTM)** and 6th ASEAN Congress of Tropical Medicine and Parasitology **2014**. Kuala Lumpur, 5-7 March 2014.

2. **Salwa Shehu Dawaki, Jamaiya Ibrahim, Init Ithoi and Hesham M. Al-Mekhlafi.** Prevalence of parasitic helminths among Hausa communities in Kano, Nigeria. **Poster presentation** at the 50th Golden Jubilee of Malaysian Society of Parasitology and Tropical Medicine **(MSPTM)** and 6th ASEAN Congress of Tropical Medicine and Parasitology **2014**. Kuala Lumpur, 5-7 March 2014.

3. Dawaki S.S., Init I., Jamaiah I., Hesham M. Al Mekhlafi, Awatif M. and Yelwa S. Multiple parasites infections among the Hausa community of Kano, Nigeria and associating risk factors. **Oral presentation** at the 51st Annual Scientific Conference of the Malaysian Society of Parasitology and Tropical Medicine (**MSPTM**). Kuala Lumpur, 3-4 March **2015**.