MALARIA, HOOKWORMS AND SCHISTOSOMIASIS COINFECTION AND ITS EFFECT ON ANAEMIA AMONG SCHOOL CHILDREN IN ZAMBEZI DISTRICT OF ZAMBIA

By

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This dissertation of Mr. DAVID GATSINZI RUTAGWERA is approved as fulfilling part of the requirements for the award of the Degree of Master of Science in Medical Parasitology by the University of Zambia.

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ABSTRACT

Introduction: Malaria, hookworm and schistosomiasis are wide spread in most parts of Zambia and often occur in same communities at high frequencies particularly in schoolchildren thereby making co-infection of these parasites very likely. In this study we determined the co-infection rate of malaria, hookworm and schistosomiasis and investigated the effect this co-infection has on anaemia in children living in Zambezi district of Zambia.

Methodology: This was a cross sectional study conducted in 263 school going children sampled using a random multistage sampling method from school going children in Zambezi district. Kato-Katz technique was used to screen stool samples for soil transmitted helminthes and *Schistosoma mansoni*. Urine filtration microscopy was use to screen urine samples for *Schistosoma haematobium*. Geimsa thick blood smear microscopy was used for malaria testing and HemoCue photometer used to measure haemoglobin concentration. Association between parasitic infections and anaemia were measured using odds ratios calculated by both bivariate and multivariate logistic regression. 8 of the study participants did not return stool samples while 2 participants did not return urine samples however all participants were included in the analysis.

Results and discussion: District anaemia prevalence rate was 46% (n = 263, 95% CI: 40.4% - 52.06%) and the overall district prevalence rates of parasites, namely; Malaria, Hookworm, *S. haematobium*, *S. mansoni*, and *Enterobius* were 50.6% (n = 263; 95% CI: 44.5% – 56.6%), 42.4% (n = 255; 95% CI: 36.4% – 48.5%), 29.5% (n = 261; 95% CI: 24.2% – 35.2%), 14.5% (n = 255; 95% CI: 10.6% – 19.2%), and 1.2% (n = 255; 95% CI: 0.3% - 3.2%) respectively. Both *Ascaris lumbricoides and Trichiura Trichuris* had a prevalence rate of 0.0% (n = 255; 95% CI: 0.0% - 1.2%). The majority of parasitic infections (55.7%) detected were multiparasitic in nature. The overall prevalence rate of malaria, hookworm and *S. haematobium* co-infection was 8.9% (n = 258; 95% CI: 5.9% - 12.9%) while that of malaria, hookworm and *S. mansoni* co-infection was 2.7% (n = 255; 95% CI: 1.2% - 5.3%). Other significant co-infections detected included malaria and hookworm co-infection, malaria and *S. haematobium* co-infection, hookworm and *S haematobium* co-infection, Malaria and *S. mansoni* co-infection and hookworm and *S. mansoni* co-infection with prevalence rates of 11.8% (n = 255; 95% CI: 8.2% – 16.2%), 10.7% (n = 262; 95% CI: 7.4% - 14.9%), 4.3% (n = 255; 95% CI: 2.3% - 7.4%), 2.7% (n = 255; 95% CI: 1.2% - 5.8%) and 2.0% (n = 255; 95% CI: 0.7% – 4.3% respectively. Several other detected co-infections had prevalence rates below 1%.This study did not detect significant association between anaemia and co-infection with malaria, hookworm and schistosomiasis. However we have shown that co-infection with malaria and hookworm (n = 255; OR: 5.0; 95% CI: 1.7 -14.1) doubles the anaemia odds ratio in malaria (n = 263; OR: 2.2, 95% CI: 1.2 - 4.3) alone.

Conclusion and recommendations: There is high prevalence of anaemia, malaria, hookworm and schistosomiasis and high levels of multiparasitism in schoolchildren in Zambezi District. The main predictors of anaemia were age, being underweight, location, and parasitic infections. We therefore recommend integration in the School Health Nutrition programme of anti helminth, anti malaria and iron status improvement activities to reduce morbidity of anaemia, parasitic infections and co-infections and accelerate the reduction in the prevalence of parasitic infections.

ACKNOWLDGEMENTS

I would like to extend sincere gratitude to my supervisor, the late Dr Cecilia J. Shinondo (May her soul rest in eternal peace) for her valuable guidance, mentorship and support throughout this study. Many thanks also go to Dr C. Kankansa and my father Dr J. M. V. Rutagwera for financial support that allowed smooth running of the study. I would also like to thank the field and technical staff especially laboratory personnel from the University Teaching Hospital, parasitology laboratory for their collaboration and hard work during data collection. Sincere thanks also go to the schoolchildren, teachers, parents and Zambezi district officials specially the District Education Board Secretary office for their participation and assistance in conducting field work. Lastly but not the least, I would like to wholeheartedly express my gratitude to my wife, Angela C. K. Rutagwera for all sacrifices made and enduring this process. May the Heavenly Father richly reward you all, for without your contribution this study would have not been successfully completed.

DEDICATION

This dissertation is dedicated to my mother, Mrs. Agnes Ntamabyariro. Though many miles apart for what seems like eternity, thoughts of you and memories of you still brings joy to my heart. My the peace of God and the fellowship of the Holy Spirit be with you as we wait on the Lord Jesus to fulfill his promise of justice and freedom.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABBREVIATION

AIDS : Acquired Immune Deficiency Syndrome

BMI : Body Mass Index

CI : Confidence Interval

EPG : Eggs per gram

GCP : Good Clinical Practice

HB : Haemoglobin

HIV : Human Immunodeficiency Virus

IBM : International Business machine

MIS : Malaria Indicator Survey

OR : Odds Ratio

SES : Socioeconomic Status

SHN : School Heath Nutrition

SPSS : Statistical Package for Social Sciences

TV : Television

UNCF : United Nation Children's Fund

UNU : United Nation University

UTH : University Teaching Hospital

WHO : World Health Organization

ZBCP : Zambia Bilharzia Control Programme

CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND INFORMATION.

It is estimated that over a third of the world's population, especially those living in the tropics and sub-tropics, are infected with helminth or malaria parasites (de Silva et al., 2003). Traditionally parasites belong to the helminth, protozoa, and arthropod families and according to the 54th WHO's World Health Assembly, parasitic infections are major causes of morbidity and mortality in Africa.

Of the three parasitic infections of interest in this study, malaria causes the largest disease burden with 40% of the world population at risk and 90% of the infection occurring in Africa (Greenwood and Mutabingwa, 2002), followed by schistosomiasis infecting 187 million people worldwide the majority of whom lives in Sub Saharan Africa (de Silva et al., 2003) then hookworms which infects about 740 million people worldwide but mainly in sub-Saharan Africa, the Americas, China and East Asia (de Silva et al., 2003).

The wide and overlapping distribution of parasites in endemic areas often results in high co-infection rates (Patney and Andrew, 1998). This phenomenon in which a single individual is infected by several parasites is referred to as multiparasitism or polyparasitism. Generally, multiparasitism in a population results from several conditions. These include high frequencies

of parasites in the same population, similar geographical distribution of parasites, shared risk factors, common transmission methods (Petney and Andrew, 1998) and genetic and immunological predisposition (Cox, 2001 and Ellis et al., 2007). The geographical distribution of malaria and helminthes is largely determined by climate (Brooker and Michael, 2000), poverty, environmental contamination, water bodies, and lack of effective preventative measures (Booth, 2006). Since several parasites are endemic in Zambia and the above mentioned predictors of multiparasitism are common in most parts of the country, it is plausible that some individuals in Zambia may be infected with two or more parasites.

Malaria, hookworm and schistosomiasis are widespread in Zambia and often occur in high frequencies in schoolchildren (Clement, 2005). Three species of *Plasmodium*, namely; *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale* cause malaria in Zambia (Wolfe, 1968) but almost all (95%) of malaria cases in Zambia are caused to *P. falciparum*. Schistosomiasis or Bilharzia in Zambia is caused by two *Schistosoma* species; namely *Schistosoma haematobium* which is the causative agent of urinogenital schistosomiasis and *Schistosoma mansoni* which is the causative agent of intestinal schistosomiasis. Two species of hookworms, namely *Ancylostoma duodonale* and *Nector americanus* infect humans. *A. duodonale* predominates in Middle East, North Africa, India and formerly in Southern Europe. *N. americanus* predominates in the Americas, Sub Saharan Africa, Southeast Asia, China and Indonesia.

Independent surveys by Zambia Bilharzia Control Programme (ZBCP) on behalf of the Ministry of Health have found high frequencies of malaria, hookworm and schistosomiasis in all

provinces of Zambia with a combined prevalence rate of these parasites exceeding 100% (ZBCP 2008suvery report). This means that it is highly probable that co-infection with malaria, hookworm and schistosomiasis occurs in Zambia but it is largely ignored as most studies consider each parasite independently and rarely look at co-infections despite increasing evidence form research that it may have considerable health and epidemiological consequences. Therefore, this study seeks to determine the co-infection rate of malaria, hookworm and schistosomiasis in a population of schoolchildren in Zambia's Zambezi District who are exposed to high infection rates of malaria, hookworm and schistosomiasis. Association between single and concurrent parasitic infections with anaemia will also be established.

1.2. STATEMENT OF THE PROBLEM

According to literature, studies conducted in Zambia on malaria and helminth infections often do not consider concurrent infections with these parasites and only consider each parasite separately. However since these parasites occur in school children at high frequencies, their co-infection certainly occur in some children but has not been studied extensively. Therefore no local data is available on co-infections with malaria and these helminthes and implications of these co-infections on morbidity, epidemiology and pathology of these parasites are still poorly understood.

Furthermore, control activities for both malaria and helminth infections have been scaled up in Zambia. However these activities are conducted independent and irrespective of each other.

None considers what effect this approach has on the overall success of control activities and the epidemiology of other parasites that are not targeted in vertical control activities.

1.3. STUDY JUSTIFICATION.

Malaria, hookworm and schistosomiasis are the major parasitic infections found in Zambia, particularly in rural and peri urban areas. Surveys by ZBCP indicate a wide and overlapping distribution of these parasites thought out the country. This means that concurrent multiple infection with these parasites is highly probable (Patney and Andrew, 1998) and therefore its importance should be clearly understood.

Results from this study gives an insight on the prevailing malaria and helminth multiparasitism and its effect on anemia burden, haematuria and school children well-being in Zambezi district therefore providing caregivers, educators and policy makers with additional information for better understanding of public health importance of parasitic infections.

Understanding the co-distribution of parasites and morbidity implications of multiparasitism could also provide valuable information to healthcare providers who must decide how to appropriately screen and treat children in resource limited setting. The information is vital in the formulation of parasite control strategies and would indicate the possible benefits of integrated parasite control strategies.

CHAPTER TWO

2.0. LITERATURE REVIEW

In absolute numbers, the majority of malaria and helminthic infections occur in Asia but their largest clinical disease burden is found in sub-Sahara Africa (de Silva et al., 2003; Snow et al., 2005). Analysis of Geographical Information System data collected in Africa suggests that of the three main soil-transmitted helminthes, hookworm is more geographically widespread throughout the sub Saharan Africa while *Ascaris lumbricoides* and *Trichuris trichiura* are typically restricted to equatorial regions (Brooker et al., 2006). Schistosomiasis on the other hand is characterized by focal distribution throughout Africa.

Consequently, since it is well established that malaria is widespread in sub-Saharan Africa, the overlap in the distribution of *P. falciparum* and helminth infections is greatest for hookworm (Brooker et al., 2006). Multiparasitism is common in populations exposed to different parasites with high prevalence rates. This phenomenon has been known for decades (Stoll, 1947) and has been studied mainly in Africa but has also been reported from continental America and East Asia (Patney and Andrew, 1998). Most studies have found multiparasitism to be common and widespread (King and Bertino, 2008).

In Zambia the prevalence and distribution of schistosomiasis, soil-transmitted helminth and malaria has been studied in several provinces. One study conducted in Southern, Eastern and Lusaka provinces reported that *S. haematobium* infection was widespread with the highest

prevalence in schools located in Itezhi-tezhi and Kalomo districts. It was also reported in the same study that *S. mansoni* was more restricted in its distribution with high prevalence in schools located in small pockets in Siavonga, Sinazongwe and Itezhi-tezhi districts. Furthermore, in this same study, hookworm infection was reported to be widespread with high prevalence rates evident in most districts while *A. lumbricoides* and *T. trichiura* infections were highly restricted in their distributions with high prevalence in schools mainly located in Choma, Mazabuka and Lusaka districts (Clements, 2005).

Unpublished survey reports of ZBCP show similar trends in prevalence and distribution of schistosomiasis and soil-transmitted helminth in the remaining Provinces of Zambia. Generally, these surveys indicate that malaria, hookworm and *S. haematobium* are widespread in Zambia, whereas *S. mansoni* shows a focal distribution in Zambia. Both *A. lumbricoides* and *T. trichiura* are highly restricted mainly to peri-urban settlements where there is high population density and poor sanitation. Furthermore, the same surveys show that treatment levels are very low in all provinces and that morbidity due to *S. haematobium* and *S. mansoni* is high in several districts in Luapula, Southern, Eastern and North Western provinces.

According to the Zambia national malaria indicator survey (MIS) conducted in 2010, Malaria is endemic throughout Zambia with a national overall prevalence rate of 16% among children less than five years of age (under-five) and is still a major public health problem. In the past, malaria endemicity was relatively uniform in Zambia, but with a sustained and robust malaria control program, the 2010 MIS showed consistent decline in malaria prevalence and divided Zambia into three malaria epidemiological zones basing on the prevalence of malaria among under-five

children at the peak of transmission season. In zone one (Lusaka city and surroundings), malaria prevalence has been reduced to less than 1%. In zone two (Central, Copperbelt, North-Western, Southern and Eastern provinces), malaria prevalence has been reduced to less than 10%. And in zone three (Luapula and Northern provinces), malaria prevalence exceed 20%. This survey reported that the prevalence of malaria in this age group is significantly higher in rural areas (20.4%) than in urban areas (5.2%) and reaches its peak at age three years. The survey also reported a provincial malaria prevalence of 6.1% among under-five children for the province in which this study will be conducted, namely North-Western province (MIS 2010 report).

Helminth infections are most prevalent and intense among school-aged children (Bundy and Medley, 1992). Maximum prevalence and intensities of *A. lumbricoides* and *T. trichiura* are achieved in children aged five to fifteen years of age while hookworm and schistome infections are at pick in late childhood to early adulthood (WHO, 2002). In contrast malaria infections are generally concentrated among young children. However, age patterns of malaria depend on severity, transmission intensity and seasonality. In all settings however, severe malaria cases are concentrated in under-five children but this change with increasing transmission intensity which shifts the cases to younger children (Carneiro et al., 2010).

Because of these differences in the age pattern distributions, co-infection and co-morbidity of malaria and helminthes may not be found in the same individual. At young age, when malaria is so intense, helminth infections are generally infrequent and relatively of light intensity (Brooker et al., 1999). Among school-aged children, severe malaria is rare whatever the level of malaria transmission. However, mild malaria episodes do occur although incidence is lower in area of

high malaria transmission, while in areas of low malaria transmission, mild clinical malaria episodes occur among school-aged children at a time when helminth infections are most prevalent and intense (Mungwi et al., 2005). Asymptomatic malaria infections are however highest in school-aged children (Kimbi et al., 2005). Malaria and hookworms are also prevalent in pregnant women, especially so in primigravidae (Shulman et al., 1999). Thus, it is among school-aged children and pregnant women that helminth and malaria co-infection is likely to be found. Analysis of spatial congruence of P. falciparum and various helminthes suggest that a quarter of African school-aged children are at risk of *P. falciparum* and hookworm co-infection (Booker et al., 2006).

In humans few epidemiological studies on helminth and malaria interactions have been conducted and have yielded conflicting results. It is well documented that helminth infections and *P. falciparum* malaria independently or as co-infections cause reduced school attendance (Nokes and Bundy, 1993), reduced school performance (Partnership for child development, 2002), impair child growth (Stephenson et al., 1993) and impair intellectual development (Nokes et al., 1999).

Helminth infections are also associated with protection against cerebral malaria (Nacher et al., 2000) therefore suggesting a protective effect on malaria severerity. Similarly, Briand and others reported the protective effect of schistosomiasis on malaria in a study conducted in Senegal which showed that children lightly infected with *S. haematobium* had lower *P. falciparum* densities than those not infected suggesting a negative interaction between both parasites (Briand et al., 2005). In contrast, a study in Thailand by Nacher and others showed that intestinal

helminth infections are associated with increased incidence of *P. falciparum* malaria (Nacher et al., 2002) suggesting a positive effect on malaria incidence.

As far as clinical consequence of co-infections is concerned, emphasis has been put on how helminthes affect the clinical patterns of malaria. However, malaria may also exacerbate the consequences of helminth infection. An important consequence of both malaria and helminth infection is anaemia. Anaemia refers to a reduced ability of red blood cells (RBC) to deliver adequate oxygen to various tissues in the human body. This is mainly due to reduced number of RBCs, reduced concentration of haemoglobin, reduced volume of blood, and genetic disorders. Beside micronutrient deficiency, parasitic infections are also major causes of anaemia (WHO/UNICEF/UNU, 2001).

It is well recognized that malaria is a significant contributor to anaemia both in young children and pregnant mothers, operating in a number of mechanisms, including haemolysis, phagocytosis (Hotez et al., 2004) and dyserythropoiesis (Menendez et al., 2000). The link between hookworm and anaemia is also well known (Booker et al., 1999) and it has been demonstrated that hookworm infections cause anaemia mainly by intestinal blood loss (Hotez et al., 2004).

Schistosomes cause anaemia by iron deficiency arising from chronic blood loss either in the intestinal tract or urinary tract depending on the causative agent, splenic sequestration, autoimmune haemolysis and anaemia of inflammation (Torentino & Friedman, 2007). Anaemia in schistosomiasis can also arise from red blood cell destruction and dyserythropoiesis (Friedman, 2005). A recent study conducted in school children in Kenya, has suggested an

association between *S. mansoni* intensity and both anaemia and low haemoglobin concentration (Koukounari et al., 2008).

Based on these distinctive mechanisms by which malaria, hookworms and schistosomes cause anemia, it was speculated that coinfection with these parasites can worsen anaemia. In 2006 Brooke and others demonstrated that hookworm and malaria are additive in their effect in reducing haemoglobin concentrations among East African school children (Brooker et al., 2006). A year earlier, Freidman and others had reported that schistosomes also can contribute to anaemia (Freidman et al., 2005) potentially exacerbating anaemia arising from malaria.

However, in a study conducted in Rwanda, Muphasoni and others found no significant association between any malaria and helminthes co-infections and higher odds of anaemia than in single infections (Muphasoni et al., 2009). These results should be treated with caution as both anaemia and malaria have low incidences in that region of Rwanda. Co-infections of hookworm and schistosome are also thought to exacerbate anaemia. One study conducted in the Philippines by Ezeamama and others found that the risk of anaemia is amplified beyond the sum of risks for individual children co-infected with hookworms and schistosomiasis (Ezeamama et al., 2008).

An illustration of the broader health impact of anaemia arising from co-infections has been provided by a hospital based study in Nigeria which reported lower birth weights of babies born from women co-infected with *P. falciparum* and helminthes than those infected with *P. falciparum* alone (Egwunyenga et al., 2001). Co-infections with plasmodia and schistosomes may also have synergistic effects on organ pathology due to infection. For example, malaria may

exacerbate hepatosplenic morbidity associated with schistosome infection (Fulford et al., 1991p; Booth et al., 2004).

With regard to control strategies, it has been shown that integrated school based anti helminthic and sustained prompt malaria treatment has the potential to reduce significantly the prevalence rates of helminthes and malaria. In a longitudinal study conducted in Zimbabwe, Midzi and others concluded that two rounds of combined praziquantel and albendazole treatment at six months intervals and sustained prompt malaria treatment significantly reduced the overall prevalence of *S. haematobium*, *S. mansoni*, soil transmitted helminthes and *P. falciparum* malaria in school children by 73.5%, 70.8%, 67.3% and 58.8% (Midzi et al., 2011).

Owing to such studies and considering that funding for helminth control programmes is rare, Hotez and others suggested integrating a rapid impact package for helminthes with programmes for HIV/AIDS, Tuberculosis and Malaria that have attracted major funding support for control (Hotez et al., 2006).They argue that coordination of major and minor global health partnerships and their associated research communities would give a significant thrust to the efforts now underway to reduce disease and poverty worldwide.

CHAPTER THREE

3.0. METHODOLOGY

3.1. Objectives

3.1.1. Main objective

The main objective of this study was to determine the prevalence rate of malaria, hookworms and schistosomiasis co-infection and its effect on anaemia among school children in Zambezi District of Zambia.

3.1.2. Specific objectives

The specific objectives of the study were:

a. To determine prevalence rates of soil-transmitted helminthes, schistosomiasis and malaria in school children.
b. To determine Nutritional status of school children participating in the study using body mass index (BMI).
c. To establish an association between anaemia and malaria, hookworm and schistosomiasis co-infections.

3.2. METHODS

3.2.1. Study design and population

This was a cross sectional survey conducted in school children in Zambezi District of the North-Western Province of the Republic of Zambia. The study was conducted from the 17th to the 19th of May 2011. School children were drawn from grade three to grade seven. According to the 2010 National Census, Zambezi District has a population of 88, 841. The estimate of the number of school children in the district were obtained from FHI 360 Education Policy and Data Centre website monitored on 09.02.2013, which stated that in 2008 the total number of pupils in basic schools was 20, 789 of which 9, 608 were females and 11, 174 were males. In the same year, the total number of basic schools was 94 (http://www.epdc.org/sites/default/files/documents/Zambia_subnatz_Zambezi.pdf).

3.2.2. Study site

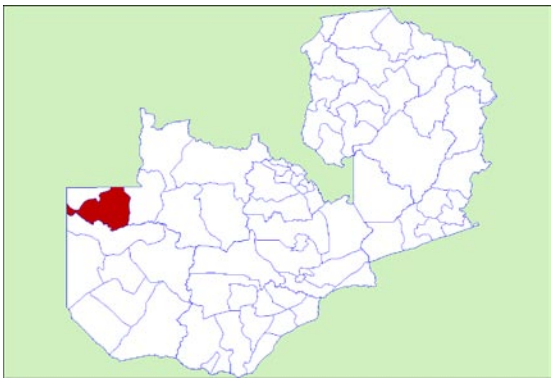


Figure 1: Map of Zambia adopted from Google maps

Zambezi District is situated in North-Western Province of Zambia between longitude 22 degrees and 24 degrees East and latitude 13 degrees to 14.30 degrees South. It has a surface area of about 18,300 Km^2 and its altitude varies from 914 m to 1100 m above sea level. The district was named after the Zambezi River which traverses it in a North to

South direction thus dividing it's terrain into vast flood plains on the West bank and a plateau on the East bank. Like the rest of Zambia, the District has two main seasons; a rainy season (November to April) and a dry season (May to October). The dry season is further divided into a cold dry season (May to August) and a hot dry season (September to October). Temperatures range between 15^{o}C to 32^{o}C with an average annual rainfall of about 1000 mm. It is a remote rural district in which agriculture and fishing are the main economic activities.

3.2.3. Sampling techniques

The sample frame was the list of primary and community schools in Zambezi Districts as kept by District Education Board Secretary's Office. Schools were excluded if they were inaccessible by car and if they were located far from the Zambezi Boma (20 km or more away from the Boma). This was mainly due to the sand terrain and bad roads that made most basic schools inaccessible by car throughout the year and budget considerations. A multistage random sampling procedure described by WHO (Montresor et al., 1998) was used to select study participants as follows; five schools were randomly selected from a list of eligible Basic Schools in Zambezi District. The schools thus selected were Lwantembu East, Lwitadi, Kawumbu, Chilenga and Mapachi Basic Schools. Four of the schools were East bank of the Zambezi River along the Mutanda – Chavuma road while Mapachi Basic School was on the West bank.

After selection of schools, the heads of each school were communicated to and asked to sensitize parents and teachers on the importance of the study and obtain consent for their school's participation in the study from the Parents and Teachers Association. In each school, using class

registers, 10 pupils (five girls and five boys) were randomly selected from grade three to grade seven to participate in the study. Selected pupils who refused to take part in the study were replaced by other pupils in the same class.

3.2.4. Sample size

The minimum **s**ample size required to determine the prevalence rate of malaria, hookworm and schistosomiasis in a population was calculated using openepi software version 2, at 95% confidence level as shown in table 1. Because we did not come across data indicating coinfection rate of malaria, hookworm and schistosomiasis, we estimate a 20% expected prevalence rate of malaria and helminthes co-infection basing on 25% rate of school children at risk of malaria and hookworm coinfection in sub-Saharan Africa, reported by Booker and others (Booker at al., 2006). The sample size thus calculated was 243.

3.2.5. Inclusion criteria

To participate in this study one had to be enrolled in grades one, two, three, four, five, six and seven in one of the basic school in Zambezi District selected to participate in this study and both the grade and the pupil must have been in school at the time the study team visited the school. He/she had to be without a history of deworming for at least the past 12 months, to be willing to give verbal assent to participate in the study and be willing to provide required samples. The head teacher of school, on behalf of the parents and teachers association had also to sign and date an informed consent form.

TABLE 1: Sample Size calculation for Frequency in a Population

Population size(for finite population correction factor or fpc)(*N*):	**20782**
Hypothesized % frequency of outcome factor in the population (*p*):	**20%+/-5**
Confidence limits as % of 100(absolute +/- %)(*d*):	**5%**
Design effect (for cluster surveys-*DEFF*):	**1**

Sample Size(*n*) for this study

Confidence level (%)	Sample Size
95%	**243**

Equation

Sample size $n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$

3.2.6. Exclusion criteria

Pupils with a history of deworming in the past 12 months were excluded from the study. Other pupils that were excluded from the study were those in grades higher than grade seven, those who did not attend the selected schools or were absent on the day of data collection and those who refused to participate in the study.

3.2.7. Sample collection and processing

A study team comprising of three laboratory technicians, one nurse and the investigator was set up to collect and analyze samples. The laboratory technicians came from the University Teaching Hospital (UTH), Parasitology laboratory. Two of the three technicians had more than 10 year experience on the bench while the other had more than five years on the bench.

A mobile study laboratory was set up in three schools of the five selected schools on different dates. Thus, on 17th May 2011 a mobile lab was set up at Lwantembu East Basic School, on the 18th May 2011 the mobile lab moved to Kawumbu Basic School and lastly on the 19th May 2011 the mobile lab was at Mapachi Basic School. Participants from the remaining two schools were transported to the nearest school in which a laboratory was set up for sample collection and analysis as follows: participants from Lwitadi Basic School were taken to Lwantembu East Basic School while those from Chilenga Basic School were taken to Kawumbu Basic School. A four steps process was used to collect samples. The steps were; step 1: participants selection, registration, height and weight measurement; step 2: stool and urine collection; step 3: finger prick, Haemoglobin (HB) measurement and malaria smear preparation; and step 4: questionnaire. This process was done in each of the schools a mobile lab was set up. To complete the process each child was expected to pass through all the steps.

Three types of biological samples were collected from each participant, namely one stool sample, one urine sample and two drops of capillary blood. To collect stool, one stool collection container with scoop was given to each participant. Each participant was asked to go to the toilet

and fill the container with fresh stool and submit the stool sample immediately. To collect urine, one urine collection container was given to each participant. Each participant was asked to fill the container with urine and submit the sample immediately.

Capillary blood was collected by way of a finger prick. To do a finger prick, a finger was selected, warmed, swabbed using alcohol pads and the alcohol allowed to dry. A sterile, disposable 2mm lancet was then used to puncture the skin. The first drop of blood was wiped off using a sterile dry cotton wool and then, the following two drop were collected, one in a microcuvette for Haemoglobin measurement and the other on a microscope slide for a malaria thick smear preparation. After collection of the two drops, bleeding was stopped by applying light pressure using a sterile dry cotton wool for few seconds. All samples were collected in the morning from 09:00 to 13:00.

3.3. MATERIALS AND PROCEDURES

3.3.1. Parasitological examination of stool

One stool sample was collected from each participant to test for soil transmitted helminthes and *S. mansoni*. The stool was first examined physically for consistency and appearance. Then two stool Kato-Katz (Katz et al., 1972 and Louis et al, 2003) thick smears were prepared on microscope slides immediately after collection of stool samples and examined within an hour for helminth ova under X10 objective of a standard light microscope. Results were reported as eggs/g of stool. Since a 24 mg template was used to prepare stool Kato-Katz slides, to calculate

eggs/g a factor of 24 was multiplied with the average number of eggs per slide. Stool samples were tested within one hour

3.3.2. Urine analysis for helminth ova

One urine sample was collected from each participant to test for *S. haematobium* and haematuria. Upon receipt, the volume of the sample submitted for testing was approximated. Using a haemastix dip stick rapid testing method, the samples were first tested for haematuria. To do this, the dip stick was placed in urine, allowed to stand for one minutes and then the developed color on the stick compared with a reference chart provided with the test kit. Then 10ml of the urine were aspirated using a 10 ml syringe and filtered through a membrane holder assembly containing membrane filter (urine filtration kit for schistosomiasis, Vastergaard Fandsen A/S, Denmark). The membrane was then examined for helminth ova under X10 objective of a standard light microscope. *S. haematobium* eggs were counted and reported as eggs/10ml. In cases where less than 10ml of urine were submitted for testing, all the urine was filtered as above and results in eggs/10ml calculated. Urine samples were tested within one hour to two hours after collection.

3.3.3. Thick smear malaria microscopy

One blood thick smear slide was prepared from each participant to test for malaria. The slides were stained with 10% Geimsa stain for 15 minutes. Stained slide securely stored in closed slide racks away from dust and light and were examined in Lusaka at UTH by the parasitology

laboratory for malaria parasites under X100 oil immersion objective of a standard light microscope. An area covering 200 white blood cells was examined and parasitaemia reported as parasites/µl. Malaria slides were tested within two weeks after collection

3.3.4. Haemoglobin concentration measurement

Capillary blood collected in a microcuvette was analyzed using the Hemocue blood hemoglobin photometer (HemoCue AB, Angelholm, Sweden) (von Schenck *et al*. 1986) to measure Hb as per manufacturers' instructions. Results were reported in g/dl.

3.3.5. Haematuria (blood in urine) detection

Dip sticks for urinalysis was used to detect haematuria as per manufacturer's instructions

3.3.6. Anthropometric measurements

Height was measures using a stadiometer while weight was measured using a digital weighing balance. Children were asked to remove shoes before height and weight measurement.

3.4. DATA COLLECTION AND ANALYSIS

Lab results were entered on a lab form and both anthropometric results and individual characteristics were collected a questionnaire. Data was entered in an excel sheet and Statistical

analysis was performed using IBM SPSS Statistics 20. Pearson Chi-square test was used to test whether prevalence rates measured were significantly different. Association between anaemia and independent variables was measured in terms of odds ratios calculated using binary logistic regression. A multivariate logistic regression was used to adjust odds ratios for confounders

3.5. ETHICAL AND ADMINISTRATIVE CONSIDERATION

Good Clinical Practice (GCP) principles were strictly followed in the course of this study. Ethical approval was obtained from the University of Zambia's Biomedical Research Ethics Committee. Approval was also obtained from the University of Zambia, School of Medicine Postgraduate Committee. Permission to conduct the study was obtained from the Ministry of Health, the Ministry of Education and other relevant authorities such as the District Education Board Secretary office. Prior to commencement of this study signed and dated informed consent forms were obtained from school managers. And verbal assent was obtained from each child before sample collection.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1. Population characteristics

A total of 263 children in primary school were recruited to participate in this study. Of these children, 8 failed to provide stool samples while 2 failed to provide urine samples. However all children provided samples for HB measurement and malaria testing. Selected Basic Schools, namely; Chilenga, Kawumbu, Lwantembu East, Lwitadi, and Mapachi contributed 19%, 20.2%, 21.3%, 19% and 20.5% of the study population respectively. All the 263 children were included in the analysis. The general characteristics of the study population are shown in table 2.

TABLE 2: Characteristics of study participants

Variable	Number	Minimum	Maximum	Mean	Standard deviation
Age	263	7.0	19.0	11.8	2.6
Years of residency	263	7.0	19.0	11.8	2.6
Height (Cm)	263	113.3	170.2	138.7	12.3
Weight (Kg)	263	19.0	69.0	33.8	9.0
HB levels	263	6.5	15.4	11.6	1.6
BMI	263	13.2	25.1	17.2	2.0

Participants were aged 7 to 19 years old, with a mean age of 11.8 (n = 263, SD = 2.6) years and were all born and permanently lived in areas around the schools in which they were recruited from. Table 3 below shows the age distribution of study participants.

TABLE 3: Age distribution of study participants

Age group (years)	Female	Male	Total
7 to 10	45	42	87
11 to 14	81	57	138
15 to 19	14	24	38
Total	140	123	263

As can be seen from table 3, 87 children or 33.1% of study participants were between 7 and 10 years old while 138 children or 52.5% of study participants were between 11 and 14 years old. Lastly 38 children or 14.4% of study participants were between15 and 19 years old. Overall 53.2% of study participants were females.

The general health well-being of study participants was characterized by self reported symptoms experienced by participants within two weeks prior to the study as shown in table 4. Common *S. haematobium* symptoms, namely; pain when urinating and visible blood in urine (haematuria), had the highest prevalence rates in the study population at 19.4% and 21.2% respectively.

Other significant symptoms included headache, abdominal pain and dizziness with 17.5%, 16% and 9.1% respectively. These are general symptoms often associated with malaria. Abdominal pain can also be associated with many other conditions including hookworm, schistosomiasis.

TABLE 4: Symptoms experienced by participants within two weeks prior to the study

Symptoms	Prevalence rate (%) n = 263
Pain when urinating	19.4
Visible blood in urine	21.2
Headache	17.5
Vomiting	2.3
Abdominal pain	16
Blood in vomits	0
Diarrhea	4.9
Dizziness	9.1
Difficulty in breathing	1.1
Face/body swelling	0.4
Rash	0.8
Blood in stool	4.6
Bloody diarrhea	3.1

For this study, no significant association was found between any of the symptoms in table 3 and infection with either hookworm or *S. mansoni*. However children who had seen blood in their urine within two weeks prior to the study were 9 times (n = 263; P < 0.001; OR: 9.4; 95% CI: 4.8-18.8) and almost 3 times (n = 263; P = 0.006; OR: 2.8; 95% CI: 1.3-6) more likely to be infected with *S. haematobium* and malaria respectively than children who had not seen blood in their urine during the same period. These children were also 7 times (n = 263; P < 0.001; OR: 7.5; 95% CI: 3.8- 14.70 more like to have blood detected in their urine by Haemastix.

Children who had abdominal pains within two weeks prior to the study were 4 times more likely to be infected with *S. haematobium* (n = 263; P < 0.001; OR: 3.7; 95% CI: 1.9-7.3) and 3 times more likely to be infected with malaria (n = 263; P = 0.012; OR: 2.5; 95% CI: 1.2-5.3) than children who did not have a abdominal pain in the same period. Headache was only associated with malaria and children who had a headache within two weeks prior to the study were 3 times more likely to be infected with malaria than those who did not have a headache in the same

period. Dizziness and pain when urinating were only associated with *S. haematobium* and children who had pain when urinating within two weeks prior to this study where 5 times more likely to be infected with S. haematobium (n = 263; P < 0.001; OR: 5.5, 95% CI: 2.9-10.6) while those who had been dizzy in the same period were 3 times more likely to be infected with *S. haematobium* than children who had neither pain when urinating nor were dizzy within two weeks prior to this study. No association was found between any of these to five self reported symptoms with anaemia.

The social economic indicators of participants' families are shown in table 5. Using these indicators, children were classified into three social economic status (SES), namely low, middle and high social economic status.

TABLE 5: Social economic indicators

Variable	Prevalence rate (%) n =263
Own a mosquito nets	82.1
Has shoes	90.8
Own cell phone	72.2
Own a fridge	6.8
Own motorcycle/scooter/car/truck	6.5
Own a TV	20.9
Own bicycle	70.7
Own a radio	66.9
Own cattle/goats	52.5
Own chicken/ducks	79.8
Low SES	35.4
Middle SES	42.6
High SES	22.1

The high SES included children from households which owned one of the following: truck, car, motorcycle, fridge and TV. The middle SES included children who came from households that

owned one of the following: cattle and goats but did not own any of those items used to define the high SES. The low SES included children from households that did not own any of the items used to define the high SES and middle SES. The prevalence of various SES in schools is shown in table 6.

TABLE 6: Prevalence of various SES in schools

	Prevalence in Schools (%)				
SES	Chilenga (n=50)	Kawumbu (n=53)	Lwantembu East (n=56)	Lwitadi (n=50)	Mapachi (n=54)
Low	48.0	30.2	39.3	32.0	27.8%
Middle	34.0	45.3	53.6	60.0	20.4%
high	18.0	24.5	7.1	8.0	51.9%

In this study we did not find any association between low SES with being underweight, sex, age, haematuria and parasitic infections of hookworms, *S. haematobium* and *S. mansoni*. A strong association was however found between socioeconomic status and malaria and location. Children in the low SES were 3 times more likely to have malaria than those from the high SES (P = 0.005; OR: 3.2; 95% CI: 1.4 – 7.3). On the other hand we found association between middle SES with malaria and *S. haematobium*. Children in the middle SES were 4 times more likely to have either malaria (P = 0.001; OR: 3.7; 95% CI: 1.7 – 8.6) or *S. haematobium* (P = 0.031; OR: 3.9; 95% CI: 1.1 – 13.5) than those from the high SES.

4.1.2. Nutritional status

Anthropometric measurements were used to calculate Body Mass Index (BMI) as an indicator of the nutritional status of study participants. BMI was calculated by “dividing weight in Kilograms

by height in meters squared" and categorized as follows; normal weight: 18-5-24.9, underweight: <18.5, overweight: 25-29.9 and obese: >30. The distribution of study participants in different BMI categories by schools is shown in table 7. Being underweight was assumed to be an indicator of nutritional deficiency.

Overall, the study observed that 79.1% (n = 263, 95% CI: 73.7 – 83.7) of the participants were underweight. In schools, the prevalence of being underweight were; 77.8% (n = 54, 95% CI: 65.3% – 87.4%) in Mapachi Basic School, 78% (n = 50, 95% CI: 65% - 87.5%) in both Chilenga and Lwitadi Basic Schools, 80.4% (n = 56, 95% CI: 68.4% - 89.2%) in Lwantembu East Basic school and lastly 81.1% (n = 53, 95% CI: 68.9% - 90%) in Kawumbu Basic School. The prevalence of being underweight in schools did not differ significantly (P = 0.989).

TABLE 7: BMI status of study participants by school (n = 263)

	Prevalence in Schools (%)				
SES	Chilenga (n=50)	Kawumbu (n=53)	Lwantembu East (n=56)	Lwitadi (n=50)	Mapachi (n=54)
Normal weight	22.0	18.9	19.6	22.0	20.4
Underweight	78.0	81.1	80.4	78.0	77.8
Overweight	0.0	0.0	0.0	0.0	1.9

Significant associations were found between sex, age and being underweight. As a result, male children were twice more likely to be under weight than females (P = 0.032; OR: 2.3; 95% CI: 1.1 – 5.0). Our data also shows that the younger you are the more likely to be underweight you are. Therefore children between 11 and 14 years are 5 times more likely to be underweight than their counterparts above 15 years (P < 0.001; OR: 5.2; 95% CI: 2.2 – 12.2) and children between 7 and 10 years are 47 times more likely to be underweight than their counterparts above 15 years

(P < 0.001; OR: 47.0; 95% CI: 11.5 – 192.3). We also found an almost significant association between malaria and being underweight (P < 0.074; OR: 2.0; 95% CI: 0.9 – 4.2). On the other hand we did not find any association between S. haematobium, S. mansoni, Hookworm, SES, location and being underweight.

4.1.3. Prevalence of Anaemia

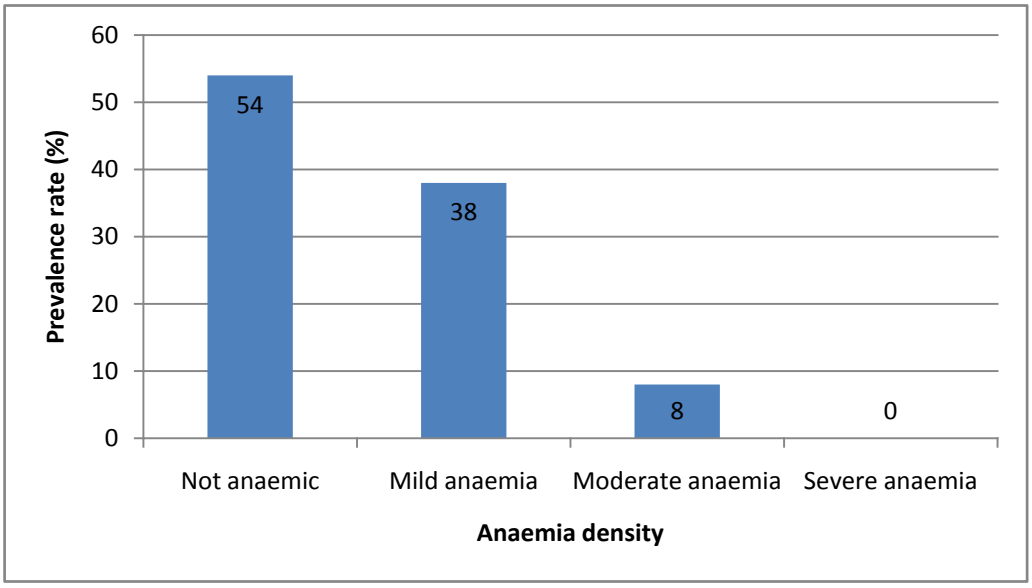
Anaemia was defined as having an HB measurement of less than 12.0 g/dl according to WHO guidelines (WHO/UNICEF/UNU, 2001). As has already been shown in table 3, the mean hemoglobin level in school children in Zambezi district was 12.1 (n = 263, SD = 1.56, 95% CI: 11.9 – 12.3) g/dl with a range of 7.0 - 15.9 g/dl.

The study observed that the District prevalence rate of anaemia in school children was 46% (n = 263, 95% CI: 40.4% - 52.06%). The prevalence of anaemia in schools sampled from were 7.4% (n= 54, 95% CI: 2.4 – 16.9) in Mapachi Basic School, 43.4% (n = 53, 95% CI: 30.6% – 56.9%) in Kawumbu Basic School, 56% (n = 50, 95% CI: 42.1% – 69.2%) in Chilenga Basic School, 57.1% (n = 56, 95% CI: 44% - 69.6%) in Lwantembu East Basic School and lastly 68% (n= 50, 95% CI: 54.2% – 79.8%) in Lwitadi Basic School. The prevalence of anaemia in schools differed significantly (P < 0.001).

Anaemia was categorized into mild anaemia (10.0g/dl ≤ Hb ≤ 11.9 g/dl), moderate anaemia, 7 ≤ Hb ≤ 9.9 and severe anaemia (Hb <7). Anemia density in the study participants is shown in figure 2. The prevalence of mild anaemia was 38% (n = 263, 95% CI: 32.3% - 44%) while that

of moderate anaemia was 8.0% (n = 263, 95% CI: 5.1% - 11.7%). None of our study participants had severe anaemia.

FIGURE 2: Anaemia density in study participants (n = 263).

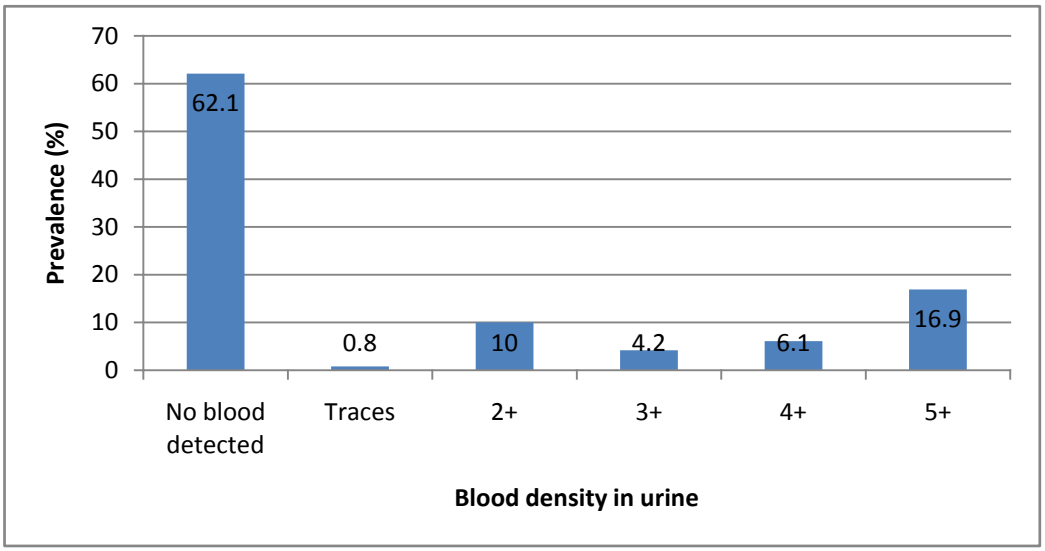


4.1.4. Prevalence of haematuria

Haemastix results in urine are shown in figure 3. 71.7% of the participants in whose blood urine was detected had 3+ and above concentrations of blood in urine. At this concentration blood is very visible in urine. The overall prevalence haematuria was 37.9% (n = 261, 95% CI: 32.2% - 43.9%) in Zambezi District. In schools, the haematuria prevalence was 0% (n = 54, 95% CI: 0% - 5.4%) in Mapachi Basic School, 20% (n = 50, 95% CI: 10.6% – 32.8%) in Chilenga Basic School, 29.4% (n = 51, 95% CI: 18.2% - 42.9%) in Kawumbu Basic School, 54% (n = 50, 95% CI: 40.1% - 67.4%) in Lwitadi Basic School and lastly 83.9% (n = 56, 95% CI: 72.5% - 91.9%) in Lwantembu East Basic School. The prevalence of haematuria in schools differed significantly

(P < 0.001). No association was found between sex, age, SES, being underweight, *S. mansoni*, hookworm and haematuria. However significant associations were found between *S. haematobium* (P < 0.001; OR: 4.4; 95% CI: 1.9 – 10.0), malaria (P = 0.034; OR: 2.2; 95% CI: 1.1 – 4.6), Lwitadi area (P = 0.025; OR: 3.3; 95% CI: 1.2 – 9.3) and Lwantembu area (P < 0.001; OR: 8.2; 95% CI: 2.5 – 26.4).

FIGURE 3: Blood concentration in urine (n = 261)



4.1.5. Prevalence of parasites

The observed prevalence rates of parasitic single infections, and co-infections observed in this study are shown in table 8. Malaria had the highest prevalence rate (50.6%, n = 263, 95% CI: 44.5% - 56.6%) in the District, followed by hookworm (42.4%, n = 255, 95% CI: 36.4% - 48.5%), then *S. haematobium* (29.5%, n = 261, 95% CI: 24.2% – 35.2%), then *S. mansoni* (14.5%, n = 255, 95% CI: 10.6% – 19.2%) and lastly *Enterobius vermicularis* (1.2%, n = 255, 95% CI: 0.3% - 3.2%). Both *Ascaris lumbricoides* and *Trichiura trichuris* were not observed

(0%, n = 255, 95% CI: 0% - 1.2%). Two species of *Plasmodium* were found to cause malaria in Zambezi district. *P. falciparum* accounts for the majority (94.7%) of malaria infections whereas *P. malariae* accounts for 3% malaria infections and 2.3 % of malaria infections are mixed infections of *P. falciparum* and *P. malariae*.

Only 20.6% (n = 253, 95% CI: 15.5% - 25.9%) of children tested negative to all parasites detectable by the diagnostic tests used in this study. About 44.3% (n = 253, 95% CI: 38.2% - 50.4%) had concurrent infections with parasitic infections. The overall prevalence rate of malaria, hookworm and *S. haematobium* co-infection was 8.9% (n = 258, 95%CI: 5.9% - 12.9%) while that of malaria, hookworm and *S. mansoni* co-infection was 2.7% (n = 255, 95% CI: 1.2% - 5.3%). Several other co-infections were detected as shown in table 6.

The prevalence rate of Malaria in schools ranged from 36% to 67.9% while those of hookworm, *S. haematobium*, *S. mansoni* and enterobius ranged from 32.7% to 56.6%, 1.9% to 78.6%, 0% to 57.7% and 0% to 3.8% respectively as shown in Table 9. Among schools, Lwantembu East Basic School had the highest prevalence rates of both *S. haematobium* and Malaria at 78.6% and 67.9% respectively. The highest prevalence rates of hookworm (56.6%) and *E. vermicularis* (3.8%) were found at Kawumbu Basic School, while that of *S. mansoni* (57.7%) was found at Mapachi Basic School. The prevalence rates of malaria ($P<0.001$), *S. haematobium* ($P<0.001$), *S. mansoni* ($P<0.001$) and hookworm ($P = 0.005$) differed significantly between schools. For the rest of the parasites, the differences in the prevalence rates were not significant

TABLE 8: District prevalence rates of parasitic infections and co-infections

	n	Prevalence (%)	95% CI (%)
Single infections			
Ascaris lumbricoides	255	0	0 - 1.2
T. Trichuris	255	0	0 - 1.2
Enterobius	255	1.2	0.3 - 3.2
Hookworm	255	42.4	36.4 - 48.5
S. Mansoni	255	14.5	10.6 - 19.2
S. Haematobium	261	29.5	24.2 - 35.2
Malaria	263	50.6	44.5 - 56.6
Co-infections			
Malaria-hookworm-*S. haematobium*	258	8.9	5.9 - 12.9
Malaria-hookworm-*S. mansoni*	255	2.7	1.2 - 5.3
Malaria-hookworm-*enterobius*	255	0.4	0 - 1.9
Malaria-*S. haematobium-enterobius*	255	0.4	0 - 1.9
Hookworm-*S. haematobium-S. mansoni*	255	0.4	0 - 1.9
Malaria-hookworm	255	11.8	8.2 - 16.2
Malaria-*S. haematobium*	262	10.7	7.4 - 14.9
Malaria-*S. mansoni*	255	2.7	1.2 - 5.8
Hookworm-*S. haematobium*	255	4.3	2.3 - 7.4
Hookworm-*S. mansoni*	255	2	0.7 - 4.3
Hookworm-*enterobius*	255	0.4	0 - 1.9
S. haematobium-S. mansoni	255	0.8	0.1 - 3.1
Number of infections per child			
Zero	253	20.6	15.5 - 25.9
One	253	35.2	29.5 - 41.2
Two	253	31.2	25.7 - 37.1
Three	253	13	9.3 - 17.6

Table 9: Prevalence of blood in urine, anemia, underweight and parasitic infections in schools.

	Prevalence rate in schools (%)				
	Chilena (n;95% CI)	Kawumbu (n;95% CI)	Lwantembu East (n;95% CI)	Lwitadi (n;95% CI)	Mapachi (n;95% CI)
Parasites					
Ascaris lumbricoides	**0** (49 ; 0 - 5.9)	**0** (53;0 - 5.5)	**0** (52 ; 0 - 5.6)	**0** (49 ; 0 - 5.9)	**0** (52 ; 0 - 5.6)
T. Trichuris	**0** (49 ; 0 - 5.9)	**0** (53;0 - 5.5)	**0** (52 ; 0 - 5.6)	**0** (49 ; 0 - 5.9)	**0** (52 ; 0 - 5.6)
Enterobius	**0** (49 ; 0 - 5.9)	**3.8** (53 ; 0.6 - 11.9)	**1.9** (52 ; 0.1 - 9.1)	**0** (49 ; 0 - 5.9)	**0** (52 ; 0 - 5.6)
Hookworm	**51** (49 ; 37.2 - 64.8)	**56.6** (53 ; 43.1 - 69.4)	**32.7** (52 ; 21 - 46.2)	**46.9** (49 ; 33.3 - 60.9)	**25** (52 ; 14.7 - 38)
S. Mansoni	**6.1** (49 ; 1.2 - 15.8)	**7.5** (53 ; 2.4 - 17.2)	**0** (52 ; 0 - 5.6)	**0** (49 ; 0 - 5.9)	**57.7** (52 ; 44 - 70.5)
S. Haematobium	**18** (50 ; 9.1 - 30.5)	**13.7** (51 ; 6.2 - 25.3)	**78.6** (56 ; 66.4 - 87.8)	**32** (50 ; 20.2 - 45.8)	**1.8** (54 ; 0.1 - 8.8)
Malaria	**36** (50 ; 23.6 - 49.9)	**37.7** (53 ; 25.5 - 51.3)	**67.9** (56 ; 54.8 - 79.1)	**66** (50 ; 52 - 78.1)	**44.4** (54 ; 31.6 - 57.8)
Conditions					
Anaemia	**56** (50 ; 42.1 - 69.2	**43.4** (53 ; 30.6 - 56.9)	**57.1** (56 ; 44 - 69.6)	**68** (50 ; 54.2 - 79.8)	**7.4** (54 ; 2.4 - 16.9)
Haematuria	**20** (50 ; 10.6 - 32.8)	**29.4** (51 ; 18.2 - 42.9)	**83.9** (56 ; 72.5 - 91.9)	**54** (50 ; 40.1 - 67.4)	**0** (54 ; 0 - 5.4)
Underweight	**78** (50 ; 65 - 87.5)	**81.1** (53 ; 68.9 - 90)	**80.4** (56 ; 68.4 - 89.2)	**78** (50 ; 65 - 87.5)	**77.8** (54 ; 65.3 - 87.4)

4.1.6. Parasite intensity

Hookworm infection intensity results are shown in figure 5. The infection intensity was categorized according to WHO guidelines (WHO, 1998) as follows: light infection: 1–1,999epg, moderate infection: 2,000–3,999epg, heavy infection: >4,000epg. The study found that 95.4 % of

all hookworm infections were light infections, 3.7% were moderate infections and 0.9% was heavy infections.

S. haematobium infection intensity results are shown in figure 6. The infection intensity was categorized according to WHO guidelines (WHO, 1998) as follows: light infection: <50 eggs/10 ml urine and heavy infection: >50 eggs/10 ml urine. The study found that 61% of S. haematobium infections were light infections while 39% were heavy infections. *S. mansoni* infection intensity results are shown in figure 7. The infection intensity was categorized according to WHO guidelines (WHO, 1998) as follows: light infection: 1–99egs epg, moderate infection: 100–399epg, heavy infection: >400epg. The study found that 56.7% of S. mansoni infections were light infections, 27% were moderate infections and 16.2% were heavy infections.

FIGURE 4: Hookworm intensity in study participants

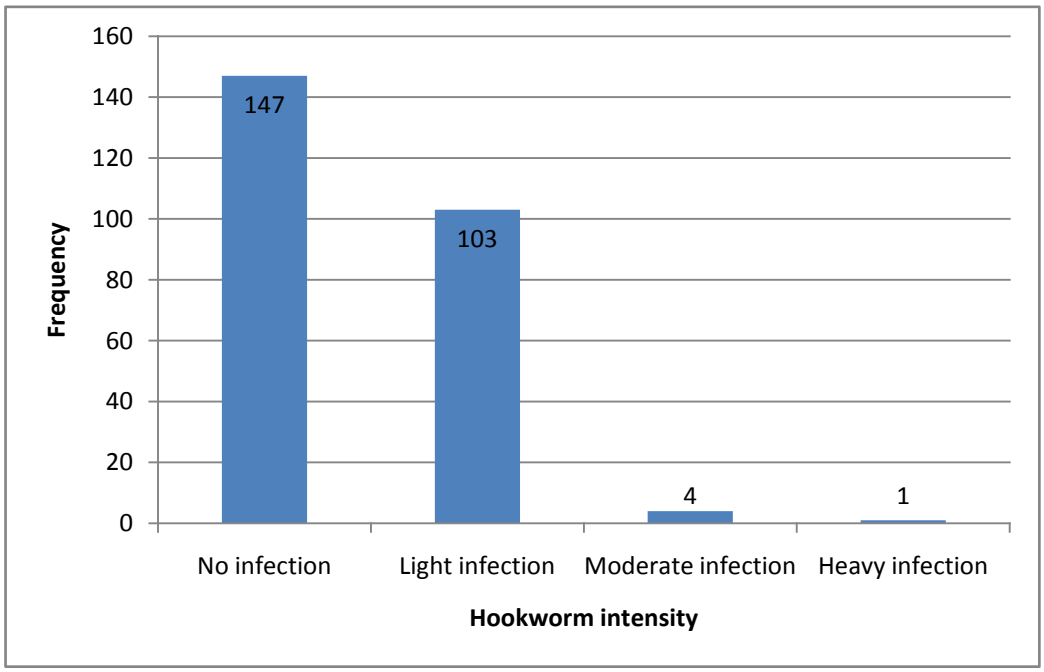


FIGURE 5: *S. haematobium* intensity in study participants

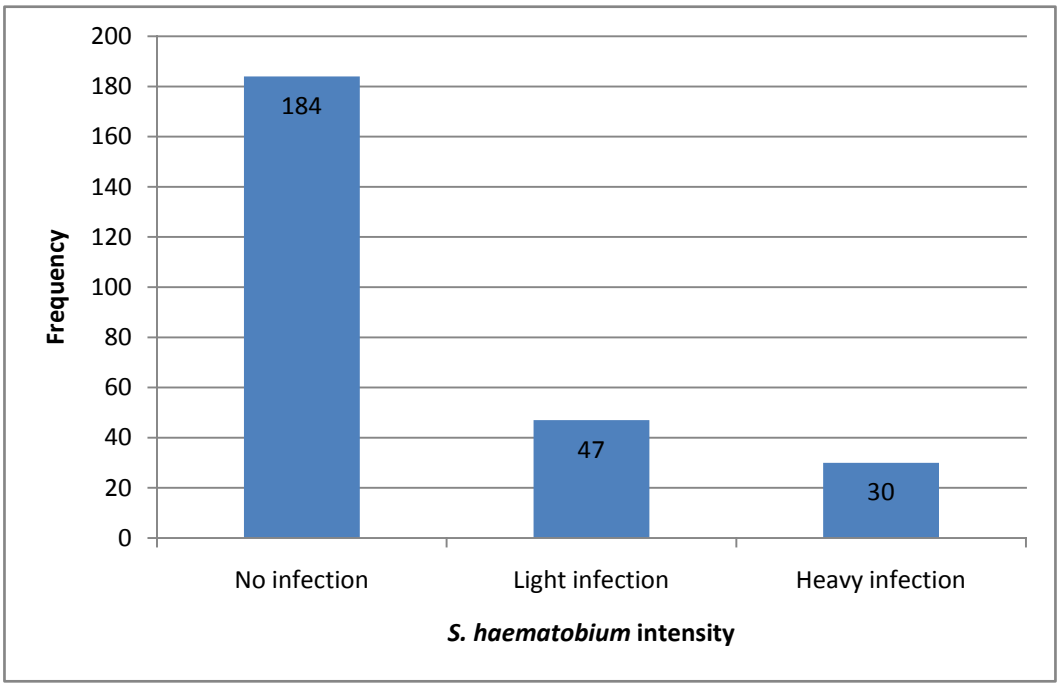
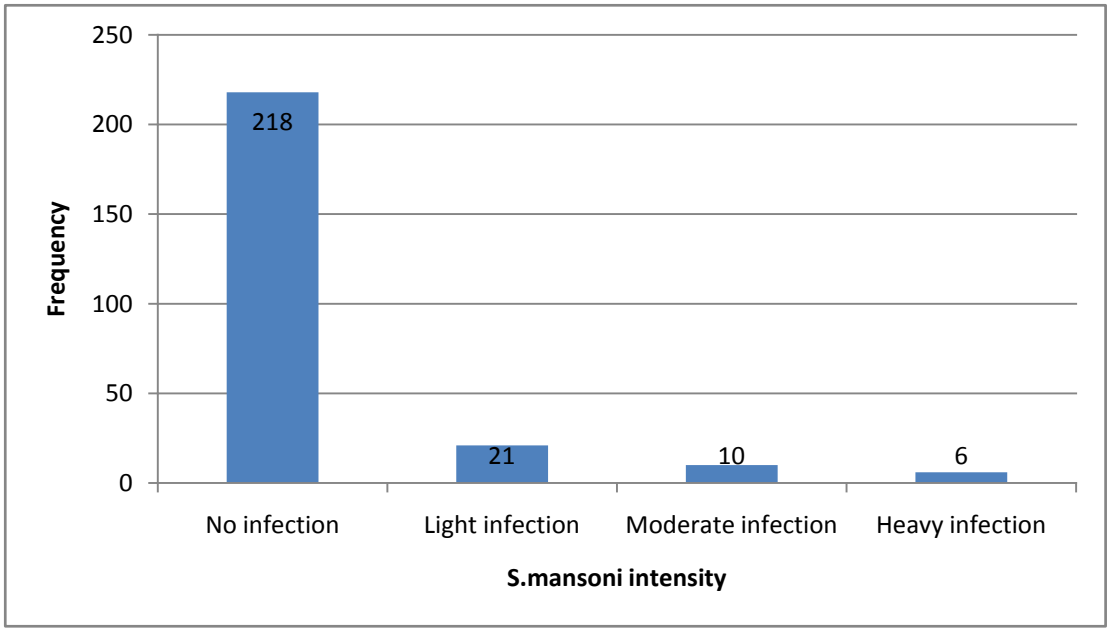


FIGURE 6: *S. mansoni* intensity in study participants



4.1.7. Association between anemia and parasitic infections

Both bivariate and multivariate logistic regression analysis were used to determine the association between anaemia and single parasitic infections. Bivariate logistic regression was used to identify potential confounders while multivariate logistic regression was used to determine the association between anaemia and parasitic infections controlling for confounders.

Potential confounders in the association between anaemia and each single parasitic infection were those independent variables with significant association in bivariate regression analysis. In this way age, underweight, socioeconomic status, location, and haematuria were identified as confounders.

All parasitic infections under consideration in this study were included in one multivariate logistic regression model together with confounders. Sex was also included in the multivariate logistic regression model because the association between sex and anaemia was almost significant in bivariate analysis and a large sample size would have probably detected the association. Results for both bivariate and multivariate regression analysis are shown in table 10.

Using the multivariate regression model discussed above, it was determined that of the parasitic infections of interest in this study, only malaria was significantly associated with anaemia and children infected with malaria were twice more likely to be anaemic than those who were not infected with malaria (OR: 2.2; 95% CI: 1.1 – 4.3).

TABLE 10: Association between anemia and single parasitic infections

			Bivariate analysis		Multivariate analysis	
Variable	n	% with Anaemia	OR	95% CI	OR	95% CI
Age group	**263**	46				
7 to 10 years	87	69	6.2	2.6 - 14.6	**4.7**	**1.5 - 14.1**
11 to 14 years	138	37	1.6	0.7 - 3.6	1.6	0.6 - 4.3
15 and above	38	26.3	Ref		Ref	
Sex	**263**	**46**				
Female	140	40.7	Ref		Ref	
Male	123	52	1.6	0.97 - 2.7	1.8	0.97 - 3.4
Underweight	**263**	**46**				
Yes	208	52.9	4.5	2.2 - 9.2	**3.1**	**1.3 - 7.3**
No	55	20	Ref		Ref	
Socioeconomic status	**263**	**46**				
Low	93	51.6	2.4	1.2 - 4.7	1.1	0.4 - 2.7
Middle	112	49.1	2.1	1.1 - 4.2	1.0	0.4 - 2.6
High	58	31	Ref		Ref	
Location	**263**	**46**				
Mapachi	54	7.4	0.1	0.03 - 0.3	**0.1**	**0.03 - 0.5**
Chilenga	50	56	1.7	0.8 - 3.6	1.5	0.6 - 3.7
Lwantembu East	56	57.1	1.7	0.8 - 3.7	2.0	0.7 - 5.7
Lwitadi	50	68	2.8	1.2 - 6.2	**3.5**	**1.3 - 9.1**
Kawumbu	53	43.4	Ref		Ref	
Haematuria	**261**	**45.6**				
Yes	99	57.6	2.2	1.3 - 3.6	0.9	0.4 - 1.9
No	162	38.3	Ref		Ref	
S. mansoni	**255**	**45.5**				
Positive	37	13.5	0.1	0.06 - 0.4	1.1	0.3 - 4.0
Negative	218	50.9	Ref		Ref	
Hookworm	**255**	**45.5**				
Positive	108	52.8	1.7	1.0 - 2.7	1.3	0.7 - 2.6
Negative	147	40.1	Ref		Ref	
S. haematobium	**261**	**45.6**				
Positive	77	58.4	2.1	1.2 - 3.6	0.9	0.4 - 2.1
Negative	184	40.2	Ref			
Malaria	**263**	**46**				
Positive	133	55.6	2.2	1.3 - 3.6	**2.2**	**1.1 - 4.3**
Negative	130	36.2	Ref			

Deleterious associations were also found between age, being underweight, Lwitadi area and anaemia. As a result, children aged between 7 and 10 years were 5 times more likely to anaemic than their counterparts aged above 15 years while underweight children were 3 times more likely

to be anaemic than those who were not underweight (OR: 3.1; 95% CI: 1.3 – 7.3) and those leaving around Lwitadi basic school were 3 times more likely to be anaemic (OR: 3.5; 95% CI: 1.3 – 9.1) than those who did not.

Contrary, our data indicate that there is a protective association between anaemia and Mapachi area. As a result, children living around Mapachi basic school were less likely to be anaemic (OR: 0.1; 95% CI: 0.03 – 0.5) than those who do not.

4.1.8. Association between anaemia and co-infections

Multivariate regression analysis was conducted as above to determine the association between anaemia and malaria-hookworm-schistosoma species co-infections except that in this case single infections were not included in the multivariate regression analysis. Results obtained in this analysis are shown in table 11. Of all possible co-infections of malaria and/or hookworm and/or schistosoma species, only malaria – hookworm co-infection was significantly associated with anaemia and children who had this co-infection were 5 times more likely to be anaemic than their counterparts who did not have the co-infection (OR: 5.0; 95% CI: 1.7 – 14.3). Having either one infection or two infections was also significantly associated with anaemia. As a result, children who had one parasitic infection were 3 times more likely to be anaemic than those who tested negative for all parasites in this study (OR: 3.2; 95% CI: 1.3 – 8.0) while children who had two concurrent infections were 4 times more likely to be anaemic than those who tested negative for all parasites in this study (OR: 3.8; 95% CI: 1.5 – 9.9).

TABLE 11: Association between anaemia and co-infections

Variable	n	% with Anaemia	Bivariate analysis		Multivariate analysis	
			OR	95% CI	OR	95% CI
Malaria-hookworm-S. haematobium	**258**	**45.3**				
Positive	23	65.2	2.4	0.998 - 6	1.1	0.4 - 3.0
Negative	235	43.4	Ref		Ref	
Malaria-hookworm-S. mansoni	**255**	**45.5**				
Positive	7	14.3	0.2	0.02 - 1.6	1.3	0.1 - 13.1
Negative	248	46.4	Ref			
Malaria-hookworm	**255**	**45.5**				
Positive	30	73.3	3.8	1.6 - 9	**5.0**	**1.7 - 14.3**
Negative	225	41.8	Ref		Ref	
Malaria-S. haematobium	**262**	**45.8**				
Positive	28	57.1	1.7	0.7 - 3.7	0.9	0.3 - 2.5
Negative	234	44.4	Ref		Ref	
Malaria-S. mansoni	**255**	**45.5**				
Positive	7	0				
Negative	248	46.8				
Hookworm-S. haematobium	**255**	**45.5**				
Positive	11	36.4	0.7	0.2 - 2.4	0.4	0.1 - 1.5
Negative	244	45.9	Ref			
Hookworm-S. mansoni	**255**	45.5				
Positive	5	0				
Negative	250	46.4				
S. haematobium-S. mansoni	**255**	**45.5**				
Positive	2	50				
Negative	253	45.5	Ref			
Number of infections per child	**253**	**45.1**				
Zero	52	30.8	Ref	Ref		
One	89	43.8	1.7	0.8 - 3.6	**3.2**	**1.3 - 8.0**
Two	79	53.2	2.5	1.2 - 5.3	**3.8**	**1.5 - 9.9**
Three	22	51.5	2.4	0.97 - 5.9	3.0	0.96 - 9.2

This study failed to find any statistically significant associations between anaemia and any co-infection that included either *S. haematobium* or *S. mansoni*. Interestingly none of the children who had malaria and *S. mansoni* co-infection was anaemic (n = 7) and only one child who had malaria, hookworm and *S. mansoni* co-infection was anaemic (n = 7).

4.1.9. Association between malaria and helminth

Only the association between *S. haematobium* and malaria was significant (P<0.001) and children infected with *S. haematobium* were almost 3 times more likely to be infected with malaria than those who were not infected with *S. haematobium* (OR: 2.7, CI: 1.6-4.7). This study also showed that malaria increases the likely hood of haematuria in *S. haematobium* infection. After adjusting for age group, sex, socioeconomic status, being underweight and location. Adjusted haematuria OR associated with *S. haematobium* was 4.4 (n = 262; 95% CI: 1.9 – 10.0). However adjusted OR associated with *S. haematobium*-malaria co-infection increased to 7.3 (n = 262; 95% CI: 1.6 – 34.1). Surprisingly our data also shows that there is a significant association between malaria and haematuria as a result, adjusted haematuria OR associated with malaria was 2.2 (n = 262; 95% CI: 1.1 – 4.6).

4.2. DISCUSSION

This study reports high prevalence of parasitic infections in schoolchildren in Zambezi district. To be specific, 79.4% (n = 253; 95% CI: 74.1% - 84.1%) of the children tested positive at least to one parasitic infection. Such high prevalence of parasitic infections in schoolchildren has also

been reported by others. In North western, one study found that Tanzania for example, one study reported that 83.5% of school children were infected by at least one parasite (Mazigo et al., 2010). The parasites detected in this study were malaria, hookworm, *S. haematobium*, *S. mansoni* and *Enterobius*. At 95% confidence level, we estimate that in the target population (schoolchildren), the prevalence rates of these parasites lay between 44.5% and 56.6% for malaria, 36.4% and 48.5% for hookworm, 24.2% and 35.2% for *S. haematobium*, 10.6% and 19.2% for *S. mansoni*, and 0.3% and 1.2% for *Enterobius*.

We did not however detect *A. lumbricoides* and *T. trichiura* in our study population. However this does not mean that these parasites do not exist in Zambezi district. On the contrary, at 95% confidence level, the prevalence of these parasites could be between 0.0% and 1.2%. Failure to detect *A. lumbricoides* and *T. trichiura* in studies rural areas is common (Mazigo et al., 2010). This I probably because of less human clouding observed in most African rural area. Malaria, hookworm and *S. haematobium* were detected in all schools sampled indicating a wide distribution of these parasites in the district. *S. mansoni*, however, was detected in 3 of the 5 schools sampled from and most of the *S. mansoni* infections (81.1%) were found in one school (Mapachi basic school), indicating a focal distribution of *S. mansoni* in the district. This pattern of distribution has been reported previously in Southern and Eastern provinces of Zambia (Clement, 2005).

The prevalence rate of malaria in schoolchildren in Zambezi district (50.6%; 95% CI: 44.5% to 56.6%) differ significantly from the predictions of the Malaria control centre which puts Zambezi district in zone two of malaria endemicity in Zambia with a malaria prevalence rate of

less than 10%. This is probably because of the different methods used in determining malaria prevalence rates and differences in target populations. Whereas the Malaria Control Centre targets children less than five years (under fives), this study targeted schoolchildren (7 to 19 years). This clearly demonstrates the danger of extrapolating results to the general population other population groups. Therefore large well stratified population based surveys are needed to determine accurately malaria prevalence in the country.

Like many other studies (Stoll, 1947; Petney and Andrew, 1998; King and Bertino, 2008), concurrent multiple parasitic infections (multiparasitism) were found to be common in our study population. Of the 253 children who provided all the samples required in this study, 52 tested negative to all parasitic infections, 89 tested positive to one parasitic infection, 79 tested positive to two parasitic infections and 33 tested positive to three parasitic infection. This implies that 44.3% of the children tested in this study were infected by two or more parasites and that the majority (55.7%) of the parasitic infections detected in this study were concurrent multiparasitic infections in nature.

The highest number of parasites found in a child in this study was 3 found in 16.4% of the infected children. However this is not the highest number of parasites ever reported in a Zambian school child, unpublished survey reports by the Zambia Bilharzias Control Program have found up to 4 parasites in schoolchild in Northern and Luapula provinces. Elsewhere in the region, up to 4 in northern Rwanda (Mupfasoni et al, 2009) and up to 3 parasites in a child were reported in north western Tanzania (Mazigo et al., 2010). Our findings confirm the high prevalence and wide distribution of multiparasitism in Zambian schoolchildren. Unfortunately, multiparasitism

is still ignored in many programmes, policies and research in Zambia. This tendency to focus on single parasitic infections and ignore co-infections is common in many places (Petney and Andrew, 1998; King and Bertino, 2008) and as a result multiparasitism is ill understood. Studies are therefore needed to highlight the public health importance of this phenomenon.

The overall district prevalence rate of anaemia in schoolchildren was estimated to be between 40.4% and 52.1% (n = 263; rate: 46%; 95% CI: 40.4% - 52.06%), indicating that anaemia is a problem in schoolchildren in Zambia. In this study, the predictors of anaemia were age, location, being underweight, malaria, malaria – hookworm co-infection and the number of parasites harbored. Surprisingly SES was not a predictor of anaemia in our target population probably because though some households may be better off than others in the district, overall Zambezi is a poor district compared to urban centers such that the majority of the people in the district are poor by Zambian standards. Indeed the main economic activity in the district is peasant farming and fishing.

Being underweight was considered an indication of nutritional deficiency in Zambezi District. Nutritional deficiency is a known cause of anaemia (nutritional anaemia) in many parts of the world. Nutritional anaemia is caused by micronutrient deficiency of folic acid, vitamin B12 and iron. Micronutrient deficiency of public health importance is a consequence of poor diet often associated with poverty and poor absorption of nutrients associated with chronic infections. With 73.7% to 83.7% (n = 263; 79.1%; 95% CI: 73.7% – 83.7%) of the school children being underweight in Zambezi District, it is clear that nutritional deficiency is a huge problem in the district and a major contributor to anaemia as reflected in the fact that underweight children are

three times more likely to be anaemic (n = 263; OR: 3.1; 95% CI: 1.3 – 7.3) than their counterparts who are not underweight. Therefore, Nutritional deficiency requires urgent attention in Zambezi district. Nutritional deficiency is mainly a consequence of poverty but parasitic infections especially malaria, hookworm and schistosomiasis are also known to exacerbate both nutritional deficiency and anaemia (WHO/UNICEF/UNU, 2001). Our failure to detect association between these parasites and nutritional deficiency could be in part be explained by the fact that almost all school children in Zambezi District (up to 83.7%) are underweight.

Tackling nutritional deficiency and improvement of the nutritional well-being in general requires involvement of a wide range of sectors and organization (WHO/UNICEF/UNU, 2001). Focus should be put on improving iron status through poverty reduction, access to a balanced diet, access to better health services, clean water and sanitation. Food-based approaches, such as promotion of dietary improvement and food fortification, are more sustainable and are preferable over supplementation in the improvement of access to a balance diet. School-based programs like SHN and the School Feeding Programmes are a good starting point but to be effective, they must be supplemented by community-based programs to lift families out of poverty and improve living standards.

Although Malaria, hookworm and schistosomiasis are thought to be major causes of anaemia in Africa (Kakounari et al., 2008), in this study we failed to detect any association between hookworm and anaemia (n = 255; OR: 1.3; 95% CI: 0.7 – 2.6) and between *S. haematobium* and anaemia (n = 261; OR: 0.9; 95% CI: 0.4 – 2.1). Similar results were reported by Mazigo et al., 2010 in North Western Tanzania. The lack of association between anaemia and hookworms in

this study is most likely due to the fact that the majority of hookworm infections in our study population (95.4%) were light infections therefore hookworm intensity in our population were lower than the threshold required to cause anaemia.

For *S. haematobium* however the reasons why we failed to detect association with anaemia are not clear. This is because we had fairly a good number of heavy infections (39% of *S. haematobium* infections) and widespread haematuria (haematuria prevalence 37.9%; n = 261, 95% CI: 32.2% - 43.9%) which has been reported to be the main mechanisms by which *S. haematobium* causes anaemia (Hotez et al., 2004). Excluding location in the multivariate logistic regression model actually indicate that *S. mansoni* might have a protective effect against anaemia (n = 253; OR: 0.3; 95% CI: 0.1 – 0.8).

Our data however shows a strong association between malaria and anaemia. Children infected with malaria were twice more likely to be anaemic (n = 263; OR: 2.2; 95%CI: 1.4-3.3) than children who were not malaria infected.

Several studies have reported that malaria, hookworm and schistosomiasis are additive in their effect in reducing haemoglobin concentration (Mungwi et al., 2006; Brooker et al., 2006; Freidman et al., 2005). Contrary to these studies, we did not find any significant association between, malaria, hookworm and *S. haematobium* co-infection (n = 258; OR: 1.1; 95% CI: 0.2 – 3.0) and anaemia nor was there a significant association between malaria, hookworm and *S. mansoni* co-infection (n = 255; OR: 1.3; 95% CI: 0.1 – 13.1) and anemia. This is despite having found strong associations between malaria and anemia (n = 263; OR: 2.2; 95% CI: 1.1 – 4.3),

and between malaria – hookworm co-infection and anaemia (n = 255; OR: 5.0; 95% CI: 1.7 – 14.3).

Interesting all co-infections that included schistosomiasis were not associated with anaemia indicating that schistosomiasis might have a protective effect and can in part explain why leaving in Mapachi, where schistosomiasis is the dominant parasite, is protective against anaemia. This can be explained by the factor that schistosomiasis is associated with lower P. falciparum parasite density in children (Briand et al., 2005).

Failure to detect association between multiparasitic infections and anaemia has been observed in several places in Africa such as northern Rwanda (Muphasoni et al., 2009) and North West Tanzania (Mazigo et al., 2006). This is often attributed to the fact that in most studies, this one included, few individuals infected with each multiparasitic infection and the majority of anaemia cases are mild.

However, comparing the odds of anemia in single infections of malaria (n = 263; OR: 2.2; 95%CI: 1.4-3.3), hookworm (n = 255; OR: 1.3; 95%CI: 0.7 – 2.6), and in malaria and hookworm co-infection (n = 255; OR: 5.0; CI: 1.7 – 14.3), it is clear that malaria and hookworms are additive in their deleterious effect on anaemia in our study population. This synergy has also been reported by several studies. One such studies was done in Eastern African school children and demonstrated that hookworm and malaria are additive in their effect in reducing haemoglobin concentrations (Brooker et al., 2006b). This is probably because, malaria and hookworm cause anemia in different ways. Malaria mainly cause anemia mainly by hemolysis

and phagocytosis (Hotez et al., 2004) while hookworm infection causes anaemia by chronic intestinal blood loss (Hotez et al., 2004).

This study also demonstrates that *S. haematobium* and malaria are additive in their effect on haematuria. Adjusted odd ratio of haematuria in *S. haematobium* – malaria co-infection (OR: 7.3; 95% CI: 1.6 – 34.1) was equivalent to the sum of adjusted odds ratio of haematuria in *S. haematobium* alone (OR: 4.4; 95% CI: 1.9 – 10) and malaria alone (OR: 2.2; 95% CI: 1.1 – 4.6). Although it has been suggested that co-infection of malaria and schistosome may aggravate organ pathologies associated with schistosomiasis (Fulford et al., 1991p; Booth et al., 2004), here we also show that malaria is associate with haematuria and it increases the likelihood of haematuria in *S. haematobium*. Studies are therefore needed to elucidate the mechanisms by which this peculiar association between malaria and haematuria operate

Our findings suggest that parasitic infections particularly, malaria, hookworm *S. haematobium* are major causes of morbidity in schoolchildren in Zambezi district. As already alluded to, malaria is a major cause of anaemia in schoolchildren and hookworm worsens this anaemia, thereby contributing significantly to morbidities associated with iron deficiency in growing up children such as impaired child growth, impaired intellectual development and reduced school performance. Additionally top 5 symptoms most commonly experiences by schoolchildren within two weeks prior to this study are commonly associated with malaria and/or *S. haematobium*. These top five symptoms were visible blood in urine, pain when urinating, headache, abdominal pain and dizziness with prevalence rates of 21.2%, 19.4%, 17.5%, 16% and 9.1% respectively. Children with malaria or urinary schistosomiasis were several times more

likely to experience these symptoms than their counterparts without these infections. Symptoms like these may not be life threatening but for sure will not allow a child participates actively in school activities and may result in absenteeism therefore resulting in poor grade for infected children.

The co-distribution and co-infection of Malaria and helminthic infections observed in Zambezi district indicate that the integration of helminth control, malaria control and iron deficiency control would be mutually beneficial to individual control programs. Helminth control programs in Zambia focus on schoolchildren who are also the main reservoir of malaria because they harbor most of the asymptomatic malaria (Kimbi et al., 2005) which remains untreated. Therefore schoolchildren provide a continuous source of malaria parasites to mosquitoes which in turn infect under-five children in whom morbidity and mortality of malaria is most prevalent. These same schoolchildren also represent a large chunk of the population bearing anaemia burden.

Provision of intermittent preventive treatment (IPT), long lasting insecticide treated nets (LLINs) in schools, health education for prevention and prompt malaria treatment combined with regular treatment with albendazole and praziquantel would offer a cost effective strategy for control of several parasites in this set up as has been reported in set ups similar to this one (Booker et al., 2007). Furthermore such a strategy would reduce morbidity arising from both single infections and co-infections as requested by WHO (WHO, 2006) and contribute to the overall reduction in the prevalence rates of several parasites (Midzi et al., 2011). Including dietary improvement information, hygiene practices and promotion of better care and feeding practice in the health

education would make such a package better suited to improve school health thereby contributing to the achievement of the Millennium Development Goal number two (universal primary education) and accelerating the achievement of Goal number six (disease eradication).

There are a number of limitations in this study. We did not consider other factors that are associated with anaemia such as iron deficiencies, sickle-cell and thalassaemia. Furthermore due to financial constraints, the study only collected one stool and one urine sample from each participant instead of collecting several successive samples. This could have resulted in under detection of helminthes as egg shedding in helminthes is not constant. Because of our study was localized and only a portion of the population considered, extrapolating results to other regions of populations groups may not be possible.

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

Malaria, hookworm and schistosomiasis occur in high frequencies but mainly in low intensity among schoolchildren in Zambezi district. Two types triple co-infection with malaria, hookworm and schistosomiasis occur in Zambezi district with a prevalence rates of 8.9% (n = 258; 95% CI: 5.9 – 12.9) and 2.7% (n = 255; 95% CI: 1.2 – 5.3) for malaria, hookworm and *S. haematobium* and malaria, hookworm and *S. mansoni*. Therefore we conclude that the overall prevalence rate of malaria, hookworm and schistosomiasis in Zambezi district is 11.6%. The prevalence of anaemia in schoolchildren is 46.0% (n = 263; 95% CI: 40.4% - 52.6%).

We could not detect association between malaria, hookworm and *S. haematobium* co-infection and anaemia (n = 258; OR: 1.1; 95% CI: 0.2 – 3.0) nor was there an association between malaria, hookworm and *S. mansoni* co-infection (n = 255; OR: 1.3; 95% CI: 0.1 – 13.1). We have however demonstrated that, malaria (n = 263; OR: 2.2; 95%CI: 1.4-3.3), malaria-hookworm co-infections (n = 255; OR: 5.0; 95% CI: 1.7 – 14.3), being underweight (n = 263; OR: 3.1; 95% CI: 1.3 – 7.3), being 10 years or below (n = 263; OR: 4.7; 95% CI: 1.4 – 14.1) and location are major predictors of anaemia and in schoolchildren in Zambezi district. We also conclude that that hookworm doubles the likelihood of anaemia associated with malaria.

5.2. RECOMMENDATIONS

We therefore recommend an urgent inclusion of iron status improvement activities and intermittent malaria treatment in the School Health and Nutrition program in Zambezi district to supplement regular deworming and disease prevention strategies implemented in this program and mitigate the high prevalence of parasitic infections and anaemia in Zambezi district. This could easily be done by consolidating and intensifying efforts to mobilize decision makers, donors and communities surrounding schools.

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APPENDICES

APPENDIX 1

APPENDIX 1

Laboratory results – stool and urine examination **Enter details for each child in 1 column; do not leave gaps**

District ☐☐☐☐☐☐☐☐ **School/community** ☐☐☐☐☐☐☐☐☐☐

Date (DD/MM/YYYY) ☐☐/☐☐/☐☐☐☐ **Observer Initials** ☐☐

ID	1.	2.	3.	4.	5.	6.	7.	8.
Name								
1. Blood visible in stool? *Enter 1 for 'Yes, 0 for 'No'*								
2. Consistency of stool? *1=formed,2=loose,3=watery*								
3. *S. mansoni* egg count (Epg)	1.	1.	1.	1.	1.	1.	1.	1.
Read 1 smear A	3.	3.	3.	3.	3.	3.	3.	3.
Enter 0 to 99999; ;nm=not measured								
4. *S mansoni* egg count (Epg)	2.	2.	2.	2.	2.	2.	2.	2.
Read 1 smear B	4.	4.	4.	4.	4.	4.	4.	4.
Enter 0 to 99999; nm=not measured								
5. Hookworm egg count (Epg)								
Enter 0 to 99999; nm=not measured								
6. Ascaris egg count (Epg)								
Enter 0 to 99999;nm=not measured								
7. Trichuris egg count (Epg)								
Enter 0 to 99999; nm=not measured								
8. Malaria								
Level of parasitaemia								
9. Visible blood in urine? *Enter 1 for 'Yes', 0 for 'No'*								
10. Haemostix result *0=none, 1=trace haemolysed, 2=trace non-haemolysed, 3=+, 4=++, 5=+++*								
11. Approximate volume of urine sample *Enter volume (ml); nm=missing*								
12. Volume of filtered urine sample *Enter volume (ml); nm=missing*								
13. *S haematobium* egg count *Enter 0 to 99999; nm=not measured*								

Individual data collection, SCI longitudinal survey

1 Personal details

1. Observer initials
2. District
3. School/community name
4. Today's date (DD/MM/YYYY) / /
5. ID (see guidelines)
6. Name
7. Sex 1= male, 2=female
8. Age † years
9. How long have you lived here?† years months

2 Clinical picture and pathology

10. Height . cm
11. Weight . kg
12. Haemoglobin g/litre
13. Mid-upper-arm circumference* . cm
14. Triceps skinfold thickness* mm

† baseline survey only *community survey only

3.Personal questionnaire

15. Initials of person asking questions

Have you had any of these symptoms in the last 2 weeks ? (read through list)

16. Blood in stool 0=No, 1=Yes, 2=Don't know
17. Blood in urine 0=No, 1=Yes, 2=Don't know
18. Bloody diarrhoea 0=No, 1=Yes, 2=Don't know

Have you had any of these symptoms today or yesterday? (read through list)

19. Headache 0=No, 1=Yes, 2=Don't know
20. Vomiting 0=No, 1=Yes, 2=Don't know
21 Abdominal pain 0=No, 1=Yes, 2=Don't know
22. Blood in vomit 0=No, 1=Yes, 2=Don't know
23. Diarrhoea 0=No, 1=Yes, 2=Don't know
24. Dizziness 0=No, 1=Yes, 2=Don't know
25. Difficulty breathing 0=No, 1=Yes, 2=Don't know
26. Face/body swelling 0=No, 1=Yes, 2=Don't know
27. Rash 0=No, 1=Yes, 2=Don't know
28. Pain when urinating 0=No, 1=Yes, 2=Don't know
29. Do you know what schistosomiasis is? 0=No, 1=Yes, 2=Don't know
30. Have you ever been treated for schistosomiasis? 0=No, 1=Yes, 2=Don't know
31. If 'yes' did you have pills or injection? 1=pills, 2=injection, 3=both 4=traditional medicine

32 – 40 Does your family have any of the following at home? (read through list)

32. Chickens or ducks? 0=No, 1=Yes
33. Cattle or goats? 0=No, 1=Yes
34. Radio 0=No, 1=Yes
35. Bicycle 0=No, 1=Yes
36. Television? 0=No, 1=Yes
37. Motor cycle/scooter or motor car/truck? 0=No, 1=Yes
38. Refrigerator? 0=No, 1=Yes
39. Mosquito nets? 0=No, 1=Yes
40. Telephone or cell phone? 0=No, 1=Yes
41. Is the child wearing shoes? 0=No, 1=Yes

APPENDIX 2

APPENDIX 3

COINFECTION OF MALARIA HOOKWORM AND SCHISTOSOMIASIS IN ZAMBEZI DISTRICT IN ZAMBIA

PARTICIPANTS INFORMATION SHEET

Invitation and information sheet for school managers together with PTA members whose schools have been selected to take part in the coinfection of *P. falciparum* (malaria), Hookworms and *Schistosoma* species (Bilharzia) in Zambezi district of Zambia study. It must be discussed prior to administering of the informed consent form. This form must also be used to give sensitize the pupils and invite them to take part in the study

INVITATION

Your school is among five schools selected to take part in coinfection of Malaria, Hookworms and Bilharzia in Zambezi district of Zambia study. Therefore we would like to invite you and your school to take part in the above mentioned study to study the coinfection rates of P. *falciparum*, Hookworms and *Schistosoma* species in Zambezi district and what effect this coinfection has on the levels of haemoglobin in children's blood.

WHAT IS THE STUDY ABOUT?

The coinfection of P. *falciparum,* Hookworms and *Schistosoma* species in Zambezi district of Zambia study will help to answer important questions about the prevalence and distribution of malaria, Hookworm and Bilharzia in Zambezi district.

Information at the ministry of health in Lusaka shows that each of the above mentioned diseases is common in Zambezi district. Previous studies have shown that malaria, hookworms and bilharzias independently may lead to anaemia. In this study we would like to find out if there are children harboring the parasites that cause the parasitic infections. Beside that we would also like to determine the effect the presence of the three parasitic infections in one individual has on anaemia levels.

To do this, we will collect stool, urine and few drops of blood from selected children to test for hookworms, bilharzias and malaria in a makeshift lab set up in one or the classrooms. We will also measure haemoglobin levels and study the blood picture.

WHAT IS INVOLVED?

If you agree to take part in this study, you will be asked to sign a consent form for the study. After signing the consent form you will be given one week to convene a PTA meeting in which you will inform the PTA member about this project and sick their consent. The research team leader will also attend this meeting to answer any questions. If the PTA gives consent, the following will then be done:

1. A research team will visit you school with laboratory equipments for one. During this visit, the team will collect one stool sample, one urine sample and two drops of blood

from each child selected to take part in the study and examine them on site. The laboratory tests for malaria, hookworms, bilharzias and anaemia will be done. The waste will be disposed of on site.

2. You and your teacher will select randomly 10 pupils (five boys and five girls) from each grade to participate in the study.
3. The study team will give you results of the tested children. All children will be given deworming tablets. Severely anaemic children and those with malaria will be referred to the nearest health centre for treatment.

WHAT ARE THE POSSIBLE BENEFITS AND RISKS OF TAKING PART IN THE STUDY?

Benefits

Benefits from the study to both participating pupils and communities include;

- On site deworming of all pupils and its associated benefits
- On site diagnosis of malaria and severe anaemia and referral to the district hospital for treatment
- Health education
- Provision of back up Albendazole, praziquantel, iron, and folic acid to the district hospital.

Risks

Although safe, there may be side effects associated with administration of praziquantel especially if it is administered on an empty stomach. The side effects are rare and include

dizziness, vomiting and hypoglycemia. To minimize the occurrence of these side effects, a snack will be provided to all pupils that will be treated. Furthermore, treated pupils will be observed for an hour to make sure there are no major side effects.

Compensation

This study will be undertaken at no cost on the part of the participants. Therefore no compensation will be given to participants. The study however will bear the cost of treatment that any participant may incur due to injury or side effects related to the study

WHAT ELSE DO I NEED TO KNOW?

Ethical approval has been sought and obtained from the University of Zambia Ethics Committee. Dissertation approval has been obtained from the University of Zambia, School of Medicine. Permission to conduct the study has been obtained from the ministry of Health, Ministry of education and other relevant authorities such as the District Health Management Team and the District Education Board Secretaries.

You and the teachers that will participate in this study will receive a lunch allowance at the government rate.

All slides prepared will be stored properly and sent to the University Teaching Hospital in Lusaka for quality assurance. The slides will not be identified with names (anonymous) and these will only be used within this study.

CONFIDENTIALITY

All information collected in this study will be kept confidential. Participants will only be identified by study codes, initials, age and date of birth on forms, documents and samples that are completed.

FINALLY

Thank you for taking part in this study. Please ask any questions and let us know if there are things that you do not understand, or would like more information about.

CONTACT DETAILS

If you have any concerns or other questions about the study or the way it has been carried out, please contact:

- Mr David rutagwera, University of Zambia, School of medicine, department of biomedical sciences, P.O.Box 50110 Lusaka. Phone number:+260 979 342832
- Dr Cecilia Shinondo University of Zambia, School of medicine, department of biomedical sciences, P.O.Box 50110 Lusaka. Phone number:+260 979 751313
- Biomedical Ethics Committee, Ridgeway campus P.O.Box 50110 Lusaka, phone: 256067 (for any ethical issues.)

APPENDIX 4

COINFECTION OF MALARIA HOOKWORM AND SCHISTOSOMIASIS IN ZAMBEZI DISTRICT OF ZAMBIA

CONSENT FORM

Participant Information must be provided as given on the participants information sheet prior to the administration of the informed consent form provide

YOUR PARTICIPITATION IS VOLUNTARY

This consent form gives you information about the study, which will be discussed with you. Once you have been told about this study, and if you agree that your school and the pupils in your care should to take part, you will be asked to sign this consent form. You will be given a signed copy to keep.

Before you learn about the study, it is important to know the following:

- Your participation is entirely voluntary
- You or any of your pupils may decide not to take part or to withdraw from the study at any time.

CONSENT PROCEDURE

Please sign tick appropriately:

I have read/been read and understood the information sheet on the study. I understand the benefits and possible risks of the pupils participating in this study. The details of this study have been explained to me by ……..…… and my questions have been answered satisfactorily.	❍ yes ❍ no
I know that the school and any pupils can withdraw from the study at any time without it affecting our care.	❍ yes ❍ no
I understand that information may be reviewed by properly authorised individuals as part of the study and that such information will be treated as strictly confidential.	❍ yes ❍ no
I agree that blood, stool and urine samples will be taken from the participating pupils once during the study team visit to the school	❍ yes ❍ no
I agree that the slides prepared from samples collected in this study will be taken to the university teaching hospital for quality assurance	❍ yes ❍ no

Name school manager	Signature (or thumbprint) of school manager	Date
		D D M M M **2 0** *Y Y*

Name witness (if thumbprint above)	Signature (or thumbprint) of witness	Date
		D D M M M **2 0** *Y Y*

Name principal investigator	Signature of principal investigator	Date
		D D M M M **2 0** *Y Y*

IMPORTANT: one signed original to be kept in the researchers file and one copy to be left at the school