The effect of improved water and sanitation on the prevalence of schistosomiasis and soil transmitted helminths (STH) amongst female primary school aged children in Ugu District of KwaZulu-Natal, South Africa.

DISCIPLINE OF PUBLIC HEALTH MEDICINE, SCHOOL OF NURSING AND PUBLIC HEALTH, COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF KWAZULU-NATAL, DURBAN, SOUTH AFRICA

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by

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ABSTRACT

BACKGROUND. Inadequate water supply and sanitation adversely affect the health and socio-economic development of communities and place them at risk of contracting *S. haematobium* and soil transmitted helminths (STH).

AIM. The aim of the study was to determine if improved water and sanitation infrastructure has had an impact on the prevalence and intensity of schistosomiasis and soil transmitted helminths in female pupils aged 10-12 years attending primary schools in Ugu district, KwaZulu-Natal.

METHODS. A descriptive cross-sectional study was conducted in Ugu district amongst primary school pupils from 18 randomly selected. Kato-Katz and urine centrifugation techniques were used to analyze stool and urine samples respectively. A structured questionnaire was used to collect water contact information, and one stool sample and three consecutive day’s urine samples, were collected from each participant. Information on sanitation and water infrastructure in communities was obtained through interviews with community ward councillors. Same analysis were done on the data from 1998 Parasite Control Programme (PCP) and findings used to compare with current study’s findings.

Results. Amongst the 1057 pupils interviewed, prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* was 25% and 26% respectively, and corresponding mean intensities of infections were 21 and 26 eggs per gram. The prevalence of *Schistosoma haematobium* was 32.2% and the mean intensity of infection was 60
When asked whether pupils knew about schistosomiasis, whether they had had red urine in the past week and if they had ever had dysuria, 60%, 9% and 22% respectively, answered in the affirmative. The 15 Ugu ward councillors reported improved access to safe water and sanitation.

**CONCLUSION.** Improved service delivery is likely to have contributed to reduced prevalence of STHs. However, a third of the study samples and a quarter of the study sample was infected with *S. haematobium* and STHs respectively.
DECLARATION

This Master of Medical Sciences dissertation is my own work and all primary and secondary sources have been appropriately acknowledged. The dissertation has not been submitted to any other institution as part of an academic qualification.

This dissertation is prepared in partial fulfilment of the requirement of the Master of Medical Sciences degree at the School of Nursing and Public Health, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa.

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ACRONYMS AND ABBREVIATIONS

BREC – Biomedical Research Ethics Committee
CI – Confidence Interval
DoE – Department of Education
DoH – Department of Health
DPLG – Department of Provincial and Local Government
HIV – Human Immuno-deficiency Virus
IDP – Integrated Development Plan
KZN – KwaZulu – Natal
MDGs – Millenium Development Goals
PCP – Parasite Control Programme
PI – Principal Investigator
RDP – Reconstruction and Development Programme
SD – Standard Deviation
STD – Sexually Transmitted Diseases
STH – Soil Transmitted Helminth
SPSS 18 – Statistical Package for Social Sciences
UDSP – Ugu District HIV and AIDS Strategic Plan
UKZN – University of KwaZulu – Natal
UN – United Nations
VIP – Ventilated Improved Pit Latrine Toilets
WC – Water Closet
WHO – World Health Organisation
WWTP – Waste Water Treatment Plant
WWTW – Waste Water Treatment Works
Table of Contents

ABSTRACT ................................................................................................................................. 2
DECLARATION .......................................................................................................................... 4
ACKNOWLEDGEMENTS ........................................................................................................... 5
ACRONYMS AND ABBREVIATIONS ......................................................................................... 7
List of Tables ............................................................................................................................ 11
List of Figures .......................................................................................................................... 12
Chapter One: Introduction ........................................................................................................ 13
1.1 BACKGROUND ................................................................................................................... 13
  1.1.1 Introduction to Schistosomiasis (parasitic disease caused by flatworms) ................... 13
  1.1.2 Epidemiology of schistosomiasis ............................................................................. 14
  1.1.3 Introduction to Soil Transmitted Helminth (STH) .................................................... 15
  1.1.4 Epidemiology of STHs ............................................................................................ 16
1.2 Rationale for study ............................................................................................................. 17
1.3 Aim of research .................................................................................................................. 19
1.4 Specific objectives of research ......................................................................................... 19
1.5 Assumptions underlying the study ................................................................................... 19
1.6 Operational Definitions Used ........................................................................................ 20
1.7 Organisation of the report ................................................................................................ 21
Chapter Two: Literature Review ............................................................................................... 23
2.1 Introducing the Problem ................................................................................................... 23
  2.1.1 Problem of STHs and Schistosomiasis .................................................................. 23
  2.1.2 Children as Targets ............................................................................................... 24
  2.1.3 Impact of helminth infections on children ............................................................... 25
2.2 Comparisons of prevalence of helminth infections (STHs) and schistosomiasis between
    Boys and Girls .................................................................................................................... 27
2.3 Findings from 1998 Parasite Control Programme (PCP) in Ugu District, KwaZulu-Natal
    ............................................................................................................................................. 28
  2.3.1 Prevalence ................................................................................................................ 28
  2.3.2 Intensity .................................................................................................................... 29
  2.3.3 Water Contact reported by pupils in PCP survey .................................................... 29
2.4 Water and Sanitation in Ugu District ................................................................................. 30
2.5 Accessibility to Water by community of Ugu district ......................................................... 31
2.6 Sanitation status in Ugu .................................................................................................... 33
  2.6.1 Rural backlogs in Sanitation in Ugu District ............................................................ 33
  2.6.2 Sanitation and Water in Schools ............................................................................. 35
2.7 Unemployment and income level in Ugu District ............................................................. 36
2.8 Millenium Development Goals (MDGs) ........................................................................... 36
Chapter Three: Materials and Methods .................................................................................... 39
Chapter Four: Results

3.1 Introduction ................................................................................................................. 39
3.2 Recruitment of Staff ................................................................................................. 39
3.3 Type of Research ........................................................................................................ 39
3.4 Study Design ............................................................................................................... 40
3.5 Study Area .................................................................................................................. 40
3.6 Study Population ........................................................................................................ 45
3.7 Recruitment of Study Subjects .................................................................................. 45
3.7.1 Pupils ..................................................................................................................... 45
3.8 Study Sample .............................................................................................................. 46
3.9 Exclusion Criteria ....................................................................................................... 46
3.10 Data Collection .......................................................................................................... 46
3.10.1 Urine Collection, Packing and Transportation ................................................... 46
3.10.2 Urine Examination Method and Parasitology .................................................... 47
3.10.3 Stool Collection, Packing and Transportation .................................................. 48
3.10.4 Stool Examination and Parasitology ................................................................. 48
3.11 Water and Sanitation in the study area .................................................................... 49
3.12 Water contact information – Questionnaires ......................................................... 49
3.13 Data Management .................................................................................................... 50
3.14 Comparison of data from 1998 and 2010 ............................................................... 50
3.15 Data Analysis ............................................................................................................ 51
3.16 Ethical Considerations ............................................................................................. 52

Chapter Four: Results ................................................................................. 53

4.1 Introduction ................................................................................................................. 53
4.2 Description of Study Sample ................................................................................... 53
4.3 Sample Analysis of Schistosomiasis and Soil Transmitted Helminths ...................... 55
4.3.1 Analysis of urine samples for Schistosomiasis ..................................................... 55
4.3.2 Analysis of stool sample for S. mansoni and STHs (A. lumbricoides, T. trichiura, H. hookeri spp. and T. saginata, T. trichiura) .................................................. 57
4.3.2.1 Prevalence ....................................................................................................... 57
4.3.2.2 Intensity of STH infections ............................................................................ 60
4.4 Multiple helminth infections ..................................................................................... 61
4.4.1 Prevalence ........................................................................................................... 61
4.4.2 Intensity among those with multiple helminth infections .................................. 63
4.5 Factors influencing helminth infection ..................................................................... 65
4.5.1 Reported water contact by pupils ....................................................................... 65
4.5.2 Association between reported water contact and prevalence of S. haematobium infection ........................................................................................................... 66
4.6 Municipal councillors’ reports of water access within 200m from households and the availability of VIP toilets per household in communities surrounding participating schools .................................................. 67
4.6.1 Reported water access by municipal councillors ................................................. 67
List of Tables

Table 1: Access to water services per population in Ugu District……………………………………..32
Table 2: Overall level of sanitation service of the survey of Ugu Household…………………………35
Table 3: Contributions of Environmental Health to the UN MDGs…………………………………...38
Table 4: WHO classification of intensity of infections………………………………………………….49
Table 5: Descriptive information on the pupils aged 10-12 years old (females) from 18 Ugu primary
schools, N=1057…………………………………………………………………………………………………54
Table 6: Prevalence and intensity of *S. haematobium* per amount of urines collected per study
subject………………………………………………………………………………………………………………55
Table 7: Prevalence and intensity (95% CI) of *S. haematobium* amongst pupils attending 18 KwaZulu
Natal primary schools…………………………………………………………………………………………56
Table 8: Prevalence (95% CI) of *A. lumbricoides, T. trichiura* amongst pupils attending KwaZulu Natal
primary schools……………………………………………………………………………………………………58
Table 9: Intensity (eggs/gram) per school of *A. lumbricoides, T. trichiura* amongst pupils attending
KwaZulu Natal primary schools………………………………………………………………………………61
Table 10: Intensity of infection amongst dual and triple infected pupils from 18 Ugu District primary
schools (N=1044)…………………………………………………………………………………………………62
Table 11: Mean intensity (EPG) of *S. haematobium, A. lumbricoides and T. trichiura* amongst pupils at
the 18 Ugu schools………………………………………………………………………………………………63
Table 12: Number of cases of pupils per school with different multiple helminth infections (dual and
triple infections)……………………………………………………………………………………………………64
Table 13: Different forms of water contact reported by pupils…………………………………………66
Table 14: Pupils knowledge and reported history of *S. haematobium*………………………………..70
Table 15: Pupils response to possible indicators associated with *S. haematobium* infection……..71
Table 16: Comparisons of prevalence, intensity and water contact in 1998 and 2010 study…………71
List of Figures

Figure 1: Water supply zones in Ugu district.................................................................30

Figure 2: Ventilated Pit Latrine Toilet.....................................................................34

Figure 3: Map of Ugu District's water and sanitation infrastructural distribution in different community areas of the district........................................................................41

Figure 4: Map of six municipalities in Ugu district, KwaZulu Natal where this study was conducted........................................................................................................44

Figure 5: Comparison of prevalence of *A. lumbricoides*, *T. trichiura* and *S. haematobium* with 95% confident intervals per municipality (Umzumbe and Hibiscus Coast Municipalities) of Ugu District....59

Figure 6: Reported source of drinking water by pupils from 18 investigated Ugu schools (N=1022)…65

Figure 7: Councillors (N=15) reporting change in water access within 200m in communities in areas surrounding schools in the study..............................................................68

Figure 8: Councillors (N=15) reporting percentage change over five years in households with VIP toilets in 18 communities near the eighteen schools in the study............................................69

Figure 9: Pupils’ VIP toilet in one of the schools...............................................................96

Figure 10: Inside the VIP toilet, plastic seat with cap.......................................................96

Figure 11: Closed cemented pits of VIP toilet in one of the schools.................................96

Figure 12: Table top set for examination of helminth eggs under light microscope ............97

Figure 13: Trained research assistants examining urine slide for detection of helminth eggs........97

Figure 14: Study PI, Sipho Zulu preparing test tubed for preservation of urines...............97
Chapter One: Introduction

1.1 BACKGROUND

1.1.1 Introduction to Schistosomiasis (parasitic disease caused by flatworms)

The global prevalence of urogenital schistosomiasis (*Schistosoma haematobium*) is estimated to be about 200 million with 500 – 600 million people from 74 developing tropical countries at risk of being infected due to inadequate sanitary facilities, as a result of poverty, poor housing, and substandard hygiene (World Health Organization, 2005; World Health Organization, 2008). Approximately eighty percent of those infected with schistosome species are in Sub-Saharan Africa (World Health Organization, 2005, World Health Organisation, 2006). The seventy four countries where schistosomiasis is reported include the Middle East, South America, South-East Asia, and particularly Africa, where its impact has negative short and long-term consequences for development (World Health Organization, 2005).

In general, the growth and survival of schistosome parasites and their snail intermediate hosts are sensitive to changes in environmental patterns (World Health Organization, 2009). Previous studies have revealed that there is evidence that *Schistosoma mansoni* in South Africa extends from Messina in the Northern Province down to Port St. Johns and East London in the Eastern Cape, whereas low prevalences were reported in the Rustenburg area of North – West province and in the Bronkhorstspruit region in Gauteng (Vermeulen et al., 1997). High infections (>70%) were reported in Barberton and Nkomazi, Mpumalanga (Vermeulen et al., 1997). In KwaZulu – Natal, only Umbumbulu and Mtunzini had prevalences of 51 –
70% and lower prevalences presented in the coastal regions of Ubombo, Lower Umfolozi, Lower Tugela, Inanda, Umzinto and Port Shepstone (Schutte et al., 1995). The distribution of *S. haematobium* is broader than that observed for *S. mansoni* with the highest prevalences (70.0% – 100%) observed in the Northern Province, Gauteng, North West Province, Mpumalanga and KwaZulu – Natal (Schutte et al., 1981, Moodley et al., 1999).

1.1.2 Epidemiology of schistosomiasis

Schistosomiasis is caused by infection with blood-flukes of the genus *Schistosoma* and is endemic in South Africa. Leder and Weller divide the schistosome species into three major species and two less common species which produce infection in humans: of the major species, *S. mansoni* and *S. japonicum* can provoke intestinal and hepatic complications and *S. haematobium* predominantly leads to renal and bladder sequelae, although occasionally it results in liver disease (Leder and Weller, 2012). The pulmonate snails *Bulinus africanus* and *Bulinus globosus* serve as intermediate hosts for *S. haematobium* which is the most prevalent schistosome in KwaZulu-Natal, although *S. mansoni* has a more focal distribution (Johnson and Appleton, 2005).

Schistosomiasis is also known as a “water-body contact disease” since it is transmitted through skin contact with contaminated water bodies. The lifecycle of schistosomes involve a warm-blooded vertebrate definitive (final) host in which the parasite undergoes sexual reproduction, releasing eggs which are then released from the body with urine and stool, and an aquatic snail (intermediate host of Bulinus spp.), in which the larvae released from the egg undergoes development in the snail in an infective cercariae larvae which enter the snail host from where they are
released into the water as miracidiae, and infect their human host (Center for Disease Control and Prevention, 1994). The adult male and female \textit{S. haematobium} flukes live in the portal veins draining the urinary and female genital tracts, and those of \textit{S. mansoni} live in the veins draining the lower intestine and rectum. About half the \textit{S. haematobium} eggs laid by the female pass into the bladder where they are released to the outside with urine starting the cycle over again, \textit{S. haematobium} eggs are trapped in the vagina, cervix, or uterus. \textit{S. mansoni} live in the lower intestine/rectum and the eggs are released with stool to start the cycle over again.

Approximately one third of the women infected with \textit{Schistosoma spp.} have genital diseases (Poggensee et al., 2001) resulting in a variety of vulvar and perineal diseases including ulcerative, fistulous or wart-like lesions. The genital manifestations resemble cancer-like lesions and the various sexually transmitted diseases (Poggensee et al., 2001; Leutscher et al., 2008).

Several studies have indicated that genital manifestations of schistosomiasis may make women susceptible to human immunodeficiency virus (HIV) infection (Kjetland et al., 2006) and because \textit{S. haematobium} affects the urinary and genital tracts, it has been renamed urogenital schistosomiasis (World Health Organization, 2010). \textit{S. mansoni} eggs are transported by blood to organs such as the lungs and liver (Center for Disease Control and Prevention, 1994).

\textbf{1.1.3 Introduction to Soil Transmitted Helminth (STH)}

Soil transmitted helminths (STHs) are linked to lack of sanitation. They are prevalent in countries where temperatures are conducive to their survival and where there is poverty, hence they are widely distributed in tropical and subtropical areas (World
Health Organisation, 2013). Humid, warm and moist temperatures in these regions (Crompton, 1989) enable fast conversion of freshly deposited infertile eggs into fertile infective eggs (\textit{A. lumbricoides} and \textit{T. trichiura}) or infective larvae (Hookworm spp.).

An estimated 3.8 billion people are infected with STH species (Crompton, 1999; Hotez et al., 2008) with more recent estimations showing that 1,221 million people are infected with \textit{A. lumbricoides}, 795 million with \textit{T. trichiura} and 740 million people with hookworms (de Silva et al., 2003; Hotez et al., 2008). Children are the most affected and live in poor or malnourished populations which result in morbidity and mortality (Hong et al., 2006). Hong et al, further noted that inadequate hygiene, poor health care systems and facilities, social instability, civil war, and natural disasters make the situation worse, and hence poverty and STHs are closely linked (Hong et al., 2006).

\textbf{1.1.4 Epidemiology of STHs}

Soil transmitted helminths are caused by intestinal nematodes, hookworms (\textit{Ancylostoma duodenale} and \textit{Necator americanus}), the roundworm (\textit{Ascaris lumbricoides}), the whipworm (\textit{Trichuris trichiura}), and the threadworm (\textit{Strongyloides stercoralis}) (Olsen et al., 2009, de Silva et al., 2003, Bethony et al., 2006). A more recent estimate from that of de Silva et al above is that 4.5 billion people are at risk of infection with one of the three common STHs (hookworm, roundworm and whipworm) (Utzinger and Keiser, 2004). Tapeworms are cestodes and although they are soil transmitted, they also require an animal intermediate host (Center for Disease Control and Prevention, 1994).
Victims contract infection after ingestion of eggs from contaminated soil or food (*A. lumbricoides* and *T. trichiura*), or through active penetration of the skin by infective larval stages of the parasite present in contaminated soil (Hookworm spp.). STHs do not reproduce inside a person’s body but require the external environment for the full infection cycle to occur. They grow in the host into mature worms and produce eggs, therefore each case of the presence of a helminth in a person’s body is as a result of an infectious event, the consequence of unsanitary defaecation when helminth eggs are released back into the soil. (Ziegelbauer et al., 2012).

### 1.2 Rationale for study

Little is known about the current situation of schistosomiasis and STHs in Ugu District. A similar study in the study area (Ugu District) was last done in 1998 - 2000 by the Parasite Control Programme (PCP) group whose results are compared with those from our study. Since 2005 there has not been a study on the prevalence and intensity of schistosomiasis and STHs on young pupils in KwaZulu-Natal. The province of KwaZulu Natal is the worst affected province in the world by HIV with a prevalence of 50% and it is mostly youth that is infected (Ugu District HIV and AIDS Strategic Plan, 2008, de Klerk et al., 2012) and KwaZulu-Natal also is endemic for schistosomiasis and STHs (Saathoff et al., 2004a, Saathoff et al., 2004b).

Some studies have reported a relationship between schistosomiasis and HIV in adults, and HIV infection has been shown to increase risk of infection by three times amongst schistosomiasis infected women (Kjetland et al., 2006b, Secor, 2006, Chenine et al., 2008). Fighting the spread of HIV requires that other infections that may possibly increase one’s chances of infection be combatted. In order to
recommend mass drug administration for helminth infections and to be able to identify focal areas within the district, a study such as this is needed.

Schistosomiasis is one of the major causes of childhood morbidity in Africa and it negatively affects the nutrition, education, development and productivity of infected children (World Bank, 2003, Hotez, 2008). Helminths parasitizing humans can destroy the organs and tissues in which they live and compete for nutrients with the human host (Muller et al., 2011).

A study in Kenya showed that praziquantel treatment improved mental test scores, attentive test scores and concentration test scores whereas before treatment, all three scores had been worse amongst children with increased egg counts (Kimura et al., 1992).

The current WHO framework for schistosomiasis control recommends school-aged children (including those who are not enrolled at school), adolescents, women of childbearing age and other high-risk groups to be major targets for systematic regular treatment (World Health Organisation, 2002a). Annual mass treatment should be administered to school age children and/or adults if the schistosomiasis prevalence is more than fifty percent but in a case where prevalence is below fifty percent but above ten percent then mass treatment of all community biennially (World Health Organisation, 2002a).
1.3 Aim of research

To determine if improved water and sanitation infrastructure in Ugu District has had an impact on the prevalence and intensity of schistosomiasis and soil transmitted helminths, amongst female pupils aged 10-12 years in primary schools within the Ugu district, KwaZulu-Natal.

1.4 Specific objectives of research

1. To determine the prevalence of schistosomiasis and soil transmitted helminths in pupils 10-12 years age attending schools in Ugu district.

2. To determine the intensity of schistosomiasis and soil transmitted helminths amongst investigated pupils.

3. To investigate factors influencing pupils' river water contact.

4. To review the status of sanitation in communities surrounding schools investigated in the Ugu district.

5. To consider the accessibility of clean water in communities surrounding investigated schools in the area.

6. To describe changes in the prevalence and intensity of helminth infections in Ugu District that has occurred between the years 1998 and 2010.

1.5 Assumptions underlying the study

1. Water accessibility and sanitation infrastructure in communities should have improved in Ugu District since the last time a similar study was undertaken by the Parasite Control Programme (PCP) was between 1998 and 2000. Since then water accessibility and the sanitation infrastructure have been targetted as part of government Reconstruction and Development (RDP) policy (Ugu District
According to the annual reports from the Ugu District Municipality, various projects have sought to ensure that all the communities are able to have these basic services (easily accessible water and proper sanitation) in their homes (de Klerk et al., 2012). This study therefore explored if access to water and sanitation has improved in the study area.

2. Prevalence and intensity levels of helminth infections amongst pupils should have declined when compared to those from the PCP study, 1998-2000. This assumption arises from an understanding that if the living conditions and socio-economic status of the community has improved since then there should be less contact with unclean water and improved sanitation and less unhygienic practices in the area.

3. Although this study only investigates young girls 10-12 years of age, findings from this group can be extrapolated to other primary school pupils in the Ugu District. The prevalence of helminth infection in children of primary school age is similar (Adams et al., 2005) and both boys and girls aged 10-12 years are known to be the group that is at high risk (World Health Organisation, 2002c).

1.6 Operational Definitions Used

1. Schistosomiasis: Schistosomiasis is an infection/disease caused by several species of trematodes. In this report, this term is used to refer to urogenital schistosomiasis infection, which is caused by the fluke, Schistosoma haematobium (World Health Organisation, 2002a). Schistosomiasis is commonly known as bilharzia (Isichenene in Zulu, which is the vernacular language within Ugu district).
2. **Soil Transmitted Helminths (STHs):** STHs are a class of intestinal worms that are transmitted through contaminated soil. In this study the term is used to refer to the two helminths found in the study. That is the roundworm *Ascaris lumbricoides* and the whipworm *Trichuris trichiura*.

3. **Sanitation:** Is generally the proper disposal of sewage wastewater aimed at preventing human contact from any agents that may cause disease (Fincham, 2001a). In this study, the term sanitation is used to refer to safe and clean VIP toilets in households.

4. **Water accessibility:** In this report water accessibility refers to the ability of various communities to access clean safe water from their households usually through standpipes not far from their homes or reticulated water in their homes.

5. **Township:** A densely populated urban area on the periphery of a city

6. **Rural area:** An area located outside the city, mostly underdeveloped that has a low population density.

7. **Urban area:** Areas found within cities characterized by high-density populations.

1.7 **Organisation of the report**

Chapter One provides an introduction to the study as well as the background to the helminth infections that are being investigated in this study (schistosomiasis and STHs), and their epidemiology globally, nationally and also locally i.e. in the study area which is Ugu District Municipality, KwaZulu-Natal, South Africa. In this Chapter, the aims, objectives, and assumptions are also outlined.

Chapter Two is the literature review which reviews the prevalence and intensity of schistosomiasis and STHs from studies on schistosomiasis and STHs and the
epidemiology globally, nationally and also locally i.e. in the study area which is Ugu District Municipality, KZN, South Africa. It includes information from the Parasite Control study in Ugu District from 1998-2000. A further review is of water and sanitation availability and accessibility to water by communities in Ugu. The socio-economic status within this community and the role of the MDGs in respect of the socio-economic status of the community are considered.

Chapter Three describes the type of research undertaken, the study design and the study population. This chapter sets out the sampling framework and issues considered when selecting the schools. It describes the type of samples collected, transportation and management of the samples as well as the computer software and the methods used to analyse the data. It explains the recruitment and training of fieldworkers and describes the data management. Finally the issues of ethical clearance and informed consent from parents and assent from participants are presented.

Chapter Four is a compilation of the results in terms of the objectives of this study.

Chapter Five discusses the results and considers the results of various other similar studies.

Chapter Six contains the conclusions and recommendations of this study.

References and appendices follow.
Chapter Two: Literature Review

2.1 Introducing the Problem

2.1.1 Problem of STHs and Schistosomiasis

Whether the infection with schistosomiasis becomes severe and causes morbidity depends on the fate of the eggs which remain trapped in tissues of various organs in the body. It has been shown repeatedly that treatment with recommended drugs by WHO (anthelminthic drugs) reverses the morbidity resulting from infection with schistosomiasis (World Health Organisation, 2002a). Studies have shown that quantitative improvements in periportal fibrosis, hepatomegaly, and splenomegaly have been observed, as well as improvements in physical fitness, appetite, and school performance (Kimura et al., 1992, Terer et al., 2013). Further deterioration in iron status is prevented by treatment with anthelminthic drugs and iron supplementation improves reduced iron status (World Health Organisation, 2002a). However this reversal of effect of infection is not always guaranteed.

Some studies suggest that schistosomiasis may play a role as a risk factor for HIV infection, and that helminth infections in general, negatively affect the immune system of HIV infected persons (Poggensee and Feldmeier, 2001). Studies done on older women have shown that symptoms similar to those of Sexually Transmitted Diseases (STDs) may actually be caused by schistosomiasis and may possibly be irreversible (Kjetland et al., 2005, Leutscher et al., 2008) since praziquantel only kills the worm but treatment has no effect on the lesions (Kjetland et al., 2008).

Therefore it is assumed that by identifying the problem geographical areas, and treating the infected individuals at an early age, such damage may be prevented. It is
generally thought that the sooner treatment is given, the higher the chances of reversing organ damage (World Health Organisation, 2002b). It has also been estimated that 16 million women will acquire the genital manifestations of *S. haematobium* infection and that, if cured, 120 000 new cases of HIV could be averted through regular praziquantel treatment (tablets used for the treatment of schistosomiasis) in the next decade (Feldmeier et al., 1994, Hotez et al., 2009).

### 2.1.2 Children as Targets

Intensity of infection with schistosomiasis (*S. haematobium* and *S. mansoni*) and Soil Transmitted Helminths (*A. lumbricoides, T. trichuris* and Hookworm spp.) generally peaks in school-going children (World Health Organisation, 2002a). Children have the highest prevalences and intensity of these infections and are also very vulnerable to the effects of worm infections (Saathoff et al., 2004a). Dating as far back as the 1920s it was recognised that high schistosomiasis prevalences and intensities were widespread in South Africa and that the disease produced ill-effects, particularly in children (Leipoldt, 1937). Surveys in the 1970s and 1980s by Schutte in KwaZulu-Natal and by Evans in Mpumalanga, revealed high infection rates for STHs with the result that the extent of the parasite burden on children living in rural areas of the country began to be appreciated (Schutte et al., 1977, Schutte et al., 1981, Evans et al., 1987).

More recent studies by Fincham and team have shown similarly high STH infection rates in the Western Cape (Fincham, 2001b, Fincham et al., 1996). In endemic areas, a pattern has been observed that most children are infected with two or more different species (Kvalsvig, 1994). Children are particularly vulnerable because they play in sand and swim/play in contaminated fresh water. Since these worm infections
cause ascariasis, trichuriasis and hookworm, and are transmitted from person to person through contamination of the soil with human waste/faeces, children are frequently exposed to these infections. Schistosomiasis is transmitted through water contact in rivers and dams that have been polluted with urine and faeces. As a result children who play or wash clothes in rivers or fetch water will be at high risk and since these children are usually over six years of age, the highest intensity level is found between the ages of eight and fourteen years (Kvalsvig, 1994).

At a symposium on schistosomiasis in 1934, Pijper gave an account of the clinical pathology of *S. haematobium* infection in both children and adults and (Kieser, 1934) described the deleterious effects of schistosomiasis on the performance of schoolchildren, and these reports were confirmed by previous findings (Loveridge et al., 1948, Muller et al., 2011). However, although many years have elapsed, the prevalence of schistosomiasis and other helminth problems in South African school children and cost-effective strategies for control and prevention are poorly understood (Voster et al., 1997). Surveys in KwaZulu-Natal by the PCP group revealed that schistosomiasis prevalence peaks at the ages of 10-15 years whereas STH infections peak at a slightly younger age, 5-10 years (Appleton and Kvalsvig, 2006a). Worm burdens (intensities of infection) show a similar age/frequency distribution (Appleton and Kvalsvig, 2006a).

### 2.1.3 Impact of helminth infections on children

The burden of disease resulting from infection with soil transmitted helminths (*A. lumbricoides, T. trichiura* and the hookworms *Necator americanus* and *Ancylostoma duodenale*) has been calculated by classifying the spectrum of possible consequences of infection into defined disease states (World Health Organisation,
According to this WHO document, such classification is based on two worm burden thresholds – lower thresholds above which there are detrimental effects on physical fitness and school performance, which may be temporary or permanent, and a higher threshold above which there is a risk of clinically overt illness (World Health Organisation, 2002a).

STH infections may cause malabsorption of nutrients, loss of appetite, discomfort and intestinal obstruction (ascariasis), and diarrhoea and Hookworm causes anaemia (Kvalsvig, 1994). They hinder physical (ascariasis) and intellectual development (hookworms) and impair school performance (trichuriasis) (Kvalsvig, 1994, World Health Organisation, 2002a).

According to studies on educational performance reported by WHO in Jamaican school children aged 9-12 years, absenteeism was more frequent among infected than uninfected children; the heavier the intensity of infection, the greater the absenteeism, to the extent that some infected children attended school for only half as much time as their uninfected peers (Nokes at al., 1992, World Health Organisation, 2002b). Studies done in Mpumalanga province and at Adams Mission in KwaZulu Natal province have shown that children’s activity levels increased after treatment for schistosomiasis and that the heavily infected children were found to be older for their class than lightly infected or uninfected children (Kvalsvig, 1994). In another study conducted on pre-schoolers in southern KwaZulu Natal, behavioural observations showed that children became more active, sociable and were happier following treatment after being treated twice in one year (Kvalsvig, 1994).
2.2 Comparisons of prevalence of helminth infections (STHs) and schistosomiasis between Boys and Girls

In studies by Adams and colleagues, the results for boys and girls were combined because gender did not influence the prevalence of Ascariasis and Trichuriasis significantly (Adams et al., 2005). In an unpublished study done in northern KwaZulu-Natal by Saarthoff et al., in 1999, males were about 30% less likely to be A. lumbricoides infected than females and they also had lower infection intensities. The prevalence of hookworm infections were slightly (but nevertheless statistically significant) higher in male pupils and the prevalence of T. trichiura was nearly identical in the two groups (Saathoff et al., 2004a).

Although girls swim less frequently after the age of 14 years (Wolmarans et al., 2004), they are more likely than boys to be required to fetch water for household purposes (Hemson, 2007), thus they continue to be exposed to contaminated water even though they no longer swim (Appleton and Maden, 2012).

A study in Kenya found that moderate and heavy intensities of infection with S. haematobium were more common in male pupils than in females; however after treatment the parasitological cure rates were not associated with gender or age (Midzi et al., 2008).

Repeated treatment during childhood reduces the risk of urinary morbidity developing in adulthood, and may have long-term effects on re-infection intensities and the development of severe morbidities even in areas where control has been interrupted for many years (World Health Organisation, 2002b). Hence it is advised
that repeated treatment for high-risk groups including women of child-bearing age be administered to reduce the risk of urogenital morbidity developing at a later stage.

2.3 Findings from 1998 Parasite Control Programme (PCP) in Ugu District, KwaZulu-Natal

Between the years 1998 – 2000, the KwaZulu Natal Department of Health initiated and implemented a Parasite Control Program (PCP) in KwaZulu-Natal and monitored the programme in the southern part of KwaZulu Natal province. Amongst other objectives it aimed at reducing the prevalence and intensity of common parasite infections in the province including soil transmitted helminths and schistosomiasis. The study in Ugu District included forty primary schools and recruited 798 pupils in 1998, (both boys and girls), 677 pupils in 1999, and 591 pupils in the year 2000 (age range 5-17 years). Each pupil was interviewed and one stool sample collected, while two urine samples were collected over two consecutive days. Pupils were treated for STH and schistosomiasis and followed up for two consecutive years.

2.3.1 Prevalence

The PCP study found prevalences of 20.2% *S. haematobium*, 63.0% *Ascaris lumbricoides*, 59.3% *Trichuris trichiura*, and 7.0% hookworm infection at baseline in 1998. These pupils were treated that year and investigated again in 1999 after one round of treatment, when the prevalence was reduced to 12.1% for *S. haematobium*, 33.1% *Ascaris lumbricoides*, 47.4% *Trichuris trichiura* and 2.4% hookworm species.
2.3.2 Intensity

The average mean egg count for *S. haematobium* was high (56.3, SD 270 eggs/10ml urine) and after one round of treatment it improved and dropped to 29.6 eggs/10ml urine. All three soil transmitted helminths that were investigated (*A. lumbricoides*, *T. trichiura* and Hookworm spp.) were light infections though there were few pupils that were moderately infected with all three of these helminths. In total 26.7% pupils had single infections with one of the three STHs found in the study, double infections were 43.1% and only 5.6% cases had triple geohelminth infection.

2.3.3 Water Contact reported by pupils in PCP survey

The majority of the pupils interviewed reported water contact, 70.0% agreed that they fetched water from the river, 51.1% played in the river, half swam in the river, 49.3% washed clothes in the river and 12.6% had other water contact. These findings were also supported by Appleton and Maden when they noted that fetching water and washing clothes were among the most frequent domestic activities whereas playing and swimming in the river were the most frequent recreational activities (Appleton and Maden, 2012).

Most pupils investigated used river water for domestic purposes and another 28.3% used mixed (river and tap) water sources for domestic purposes and only 16.6% used clean tap water.

For the purpose of our study we only analysed the results of female pupils aged 10-12 years to compare with the current prevalence and intensity of helminth infections from this study. All findings presented for comparison with the current study are from
the PCP data that are therefore for female school children of 10-12 years of age (Table 16).

2.4 Water and Sanitation in Ugu District

Ugu district has thirteen water supply zones which supply the various clusters of the community within the district. According to the Ugu District Municipality sector wide infrastructure audit final report these supply zones were not always clearly definable (de Klerk et al., 2012).

Figure 1: Water supply zones in Ugu district (de Klerk et al., 2012)
Sixteen of the total eighteen schools in our study were within the Bhobhoyi water supply zone, and two were within the Umtavuna water supply zone. Current consumption from the Bhobhoyi Water Works varies from 50ML/d to 60ML/d with the plant being rated at 54ML/d. It is predicted that rapid growth of the surrounding rural areas of Bhobhoyi into peri-urban developments will see higher demands in the short term. Population figures for the whole district show that in 2005 the split between the rural and urban water use was in the order of 20% rural and 80% urban (de Klerk et al., 2012).

Despite this, areas supplied by the Bhobhoyi water works normally experience water shortages during the holiday season (December and January) and this is most felt by communities from rural areas. A project has been set up to reduce water shortages through augmenting the Umzimkhulu Water scheme, as failure to do so results in various supply zones experiencing water shortages (Ugu District Municipality, 2011). Despite this, communities around these areas experience water cuts regularly which may last for an hour to several hours. During that time, mobile water tankers are called in to temporarily supply the affected communities.

### 2.5 Accessibility to Water by community of Ugu district

In terms of the National Government’s definition of backlogs, households must have access to a formal water supply within 200 metres walking distance (Department of Water Affairs, 2013). This distance is used as a marker that defines whether the household does have a Reconstruction and Development Programme (RDP) service or not. The RDP is a socio-economic policy framework established by the government of South Africa to assist needy and previously disadvantaged
communities with various basic services with the intention of improving their health, lifestyle and living conditions (Department of Water Affairs, 2013).

Services such as water, sanitation (e.g. toilets), houses, roads, and electricity are some of the services that fall under the RDP. Houses within 200m walking distance from a formal water supply source were viewed as having access to RDP level of service. Houses between 200m and 800m walking distance from the water source were viewed as having reduced access to the required RDP level of service and those that were further away than 800m were considered to be without any water supply (Department of Water Affairs, 2013).

Every household in the urban area of Ugu is fully serviced with water inside the house or in the yard. One in three households in Ugu still have difficulty accessing clean water, having to travel long distances to get water (Table 1).

Table 1: Access to water services per population in Ugu District (Ugu District Municipality, 2011)

<table>
<thead>
<tr>
<th>Settlement Category</th>
<th>Serviced RDP &lt;200m</th>
<th>Serviced RDP 200m - 800m</th>
<th>Not Serviced</th>
<th>Total No of People</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formal Urban</td>
<td>172,114 (100%)</td>
<td>-</td>
<td>-</td>
<td>172,114</td>
</tr>
<tr>
<td>Informal Residential Upgrade</td>
<td>-</td>
<td>2,219 (64%)</td>
<td>1,257 (36%)</td>
<td>3,476</td>
</tr>
<tr>
<td>Linked Rural Upgrade</td>
<td>68,691 (37%)</td>
<td>73,590 (39%)</td>
<td>44,810 (24%)</td>
<td>187,091</td>
</tr>
<tr>
<td>Good Access Rural Upgrade</td>
<td>34,940 (23%)</td>
<td>45,553 (30%)</td>
<td>73,391 (47%)</td>
<td>153,884</td>
</tr>
<tr>
<td>Limited Access Rural Upgrade</td>
<td>26,993 (33%)</td>
<td>24,512 (30%)</td>
<td>31,256 (37%)</td>
<td>82,761</td>
</tr>
<tr>
<td>Scattered</td>
<td>20,482 (19%)</td>
<td>26,692 (24%)</td>
<td>62,695 (57%)</td>
<td>109,870</td>
</tr>
<tr>
<td>Total</td>
<td>323,222</td>
<td>172,566</td>
<td>213,410</td>
<td>709,197</td>
</tr>
<tr>
<td>Percentage</td>
<td>45.58%</td>
<td>24.33%</td>
<td>30.09%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>
2.6 Sanitation status in Ugu

Urban sanitation in Ugu is made up of a combination of waterborne sewerage linked to wastewater treatment works (WWTW) and a system of septic tanks. Waterborne sewerage coverage in formal urban areas has not yet reached 100% but ranges between 40% and 60%, with the exception of Gamalakhe which has sewerage coverage of 80%. Due to various economic activities such as tourism that take place in urban areas and surrounding suburbs, adequate provision of water and sanitation is required. Backlogs in Ugu include maintenance challenges, and ensuring that one hundred percent waterborne sewerage coverage in households is achieved (Ugu District Municipality, 2011).

2.6.1 Rural backlogs in Sanitation in Ugu District

Rural sanitation has many challenges. There is no reliable data spatial or otherwise pertaining to either the location or age of Ventilated Improved Pit Latrine Toilets (VIPs) constructed within Ugu (Ugu District Municipality, 2011). Regardless of this, Ugu District has invested in the improvement of rural sanitation over the past decade through its RDP projects.
Houses in rural areas are supplied with VIP toilets by the municipality. These VIPs are much better than the long drop toilets that people build themselves, which are not safe, smell and are less hygienic. VIP toilets are made of a complete panel kit with a stainless steel or wooden door. They have a ventilated pit that comprises a ventilated pipe with cowl (Figure 2 above). A small survey (0.2% of total rural population) was carried out in 2010 by the municipality of Ugu district to better understand the status of rural sanitation within Ugu.

The scope of the survey is summarised as follows:

The number of households that were surveyed: 241 households (0.2%) of the 106 008 households in the Ugu Water and Sanitation Development Programme. The
average household occupancy (size) was seven people and the survey covered 1674 people in these households. From this survey, all the houses had VIP toilets (76%), only 9% had water on site, and 65% had access to a basic level of water supply within 200m of the household.

Table 2: Overall level of the sanitation service of the survey of Ugu household

<table>
<thead>
<tr>
<th>Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ugu VIP</td>
<td>76%</td>
</tr>
<tr>
<td>Informal Long Drop</td>
<td>22%</td>
</tr>
<tr>
<td>None</td>
<td>2%</td>
</tr>
</tbody>
</table>

The survey conducted shows that the majority of households had VIPs (Table 2). Only about half of these VIPs were in good condition, 29.0% were in a fair condition and 19.0% of them were full. Another striking setback facing the municipality is that toilet paper is supposed to be used in VIP toilets but the survey revealed that only 12.0% used toilet paper and 88.0% used newspaper. As a result of this practice, toilets fill up fast, before the end of their lifespan. According to Ugu, findings from this survey suggested a backlog of 30.0% in the provision of water and proper sanitation in the district (Ugu District Municipality, 2011).

This small survey of rural sanitation indicates that adequate sanitation is not available to many households in the Ugu District.

2.6.2 Sanitation and Water in Schools

Lack of access to clean water and proper sanitation may have an effect on school performance. The Department of Education should ensure that all schools in the
district have access to clean water on the school premises as well as clean toilet facilities, but from visits to the schools the study team discovered that water and sanitation were still a huge challenge in some schools. Schools that are located in the urban areas have better water and sanitation infrastructure than those located in the rural areas, partly due to the well developed facilities found in the coastal urban areas (Figure 3). All the urban schools in Ugu district have water closet toilet facilities. All rural and semi-rural schools in the district have ventilated improved pit latrine toilets but the condition of these toilets varies depending on how well they are maintained by the school authorities.

2.7 Unemployment and income level in Ugu District
Schistosomiasis and STH infections are prevalent especially amongst children from poor communities where employment levels are low (Egwunyenga and Ataikiru, 2005, Hong et al., 2006). South Africa has a high rate of unemployment (Department of Public Works, 2011/2012). As a result, many households survive on a minimal income.

According to verbal interviews conducted in July 2010 in Ugu District, the average household income was between R600 and R1000 per month in the rural and peri urban areas (de Klerk et al., 2012). Many households were supported through social government grants.

2.8 Millenium Development Goals (MDGs)
The Millenium Development Goals are eight goals signed by 189 member states of the United Nations in the year 2000, which range from halving extreme poverty to halting the spread of HIV/AIDS and providing universal primary education, all by
2015 (The General Assembly, 2000). These MDGs aim to advance poverty eradication and sustainable development, by rapidly increasing access to basic requirements such as clean water, energy, health care, food security and the protection of the bio-diversity (World Bank, 2003).

Three of the eight MDGs are directly related to health (reducing child mortality, improving maternal health and combating HIV/AIDS, malaria and other diseases) (Travis et al., 2004). The United Nations Summit on Sustainable Development, held in Johannesburg, South Africa in 2002, revisited the targets set by the Millennium Declararion Goal with regard to safe water supply, and extended it to also include the provision of sanitation, to ensure that the population lacking safe drinking water is cut by half in 2015 (World Summit on Sustainable Development, 2002). The inclusion of safe drinking water and sanitation in the MDGs meant that the world community at large acknowledged the importance of these two health requirements in promoting good health and reducing poverty.
### Table 3: Contributions of Environmental Health to the UN MDGs (Lutchminarayan, 2007)

<table>
<thead>
<tr>
<th>UN MDGs Goals and Targets</th>
<th>Environmental Health Inputs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Goal 1</strong></td>
<td></td>
</tr>
<tr>
<td>Eradicate extreme poverty and hunger</td>
<td>A healthy environment means healthy people, able to secure improved livelihoods and break the cycle of poverty and ill-health</td>
</tr>
<tr>
<td><strong>Goal 2</strong></td>
<td></td>
</tr>
<tr>
<td>Achieve universal primary education</td>
<td>Freedom from diarrhoeal disease and other environmental health hazards will result in increased attendance and participation in school. School sanitation is an important determinant of girls’ attendance.</td>
</tr>
<tr>
<td><strong>Goal 3</strong></td>
<td></td>
</tr>
<tr>
<td>Promote gender equality and empower women.</td>
<td>As the burden of environmental health risks falls disproportionately on women, effective interventions help to improve women’s lives and empower them through increased participation.</td>
</tr>
<tr>
<td><strong>Goal 4</strong></td>
<td></td>
</tr>
<tr>
<td>Reduce child mortality</td>
<td>Appropriate environmental health interventions can significantly reduce the number of children under 5 who die because of unsafe water, inadequate sanitation and poor hygiene.</td>
</tr>
<tr>
<td><strong>Goal 6</strong></td>
<td></td>
</tr>
<tr>
<td>Combat HIV/AIDS, malaria and other diseases</td>
<td>Preventive environmental health measures are as important and at time more cost-effective than health treatments</td>
</tr>
<tr>
<td><strong>Goal 7</strong></td>
<td></td>
</tr>
<tr>
<td>Ensure environmental sustainability. Halve by 2015 the proportion of people without sustainable access to safe drinking water and sustainable sanitation. By 2020, to have achieved a significant improvement in the lives of at least 100 million slum dwellers.</td>
<td>These goals are expressed in terms of environmental health improvements; environmental health measures such as provision of safe water and sanitation contributes to the MDGs.</td>
</tr>
</tbody>
</table>
Chapter Three: Materials and Methods

3.1 Introduction
The aim of the study was to investigate if the improved water and sanitation in Ugu District has reduced the prevalence and intensity of schistosomiasis and STH among pupils of school going age in Ugu district, KwaZulu – Natal, South Africa. The study commenced in February 2010 and was concluded in June 2011.

3.2 Recruitment of Staff
An experienced field supervisor was recruited to train, manage and oversee the collection of information and samples in the field. Due to the nature of the study, only females were recruited as research assistants. The team comprised of ten research assistants, a driver, data capturer and a lab manager (study PI). The whole team attended a two weeks intensive parasitology course which was given by an experienced parasitologist, Mrs Colleen Archer from University of KwaZulu-Natal. Even though all personnel in the team were taught microscopy and the method of identifying and counting parasite eggs and the recording of findings, only five were selected to examine samples at 10x magnification with a light microscope. Mrs Archer then monitored ten percent of the samples which were examined for quality control.

3.3 Type of Research
This is an epidemiological study.
3.4 Study Design

A descriptive cross sectional study design was used.

3.5 Study Area

The study was conducted in the Ugu District on the south coast of the province of KwaZulu-Natal, South Africa. This district has an area of 5866 km² and is one of ten districts and one metropolitan area in the province of KwaZulu-Natal (Department of Provincial and Local Government, 2007; Ugu, 2008). The IsiZulu word “Ugu” means “coast”. The spatial pattern of Ugu District municipality resembles a “T” shape. Areas along the coast (which face the Indian Ocean, with a coastline of 112km) have a well developed infrastructure and thus reasonable economic growth.
Figure 3: Map of Ugu district's water and sanitation infrastructural distribution in different community areas of the district
In contrast, the hinterland is characterized by poor infrastructural provision and high unemployment levels and the area selected was thus appropriate for the study (Ugu, 2008). The biggest towns in the area are Port Shepstone and Margate, the latter being the most popular, which is also the tourism centre of the district (Ugu, 2008). Another town, Scottburgh is the third most popular tourist area in this district (Ugu, 2008). Port Shepstone remains the major employment centre in the district (Ugu, 2008). There are other rural towns which act as administrative centres in the rural areas.

The infrastructural backlogs in the area are very high, and this adversely affects people from the rural communities in the hinterland (Department of Provincial and Local Government, 2007). There is a lack of access to economic opportunities and social services, and the unemployment level within Ugu District municipality is estimated at 30% (Department of Provincial and Local Government, 2007). The majority of the people are employed in the domestic and tourism industry, and on sugar-cane and banana farms in the area (Department of Provincial and Local Government, 2007; Ugu, 2008). The manufacturing sector also contributes a little to the development of the area but this is concentrated around Port Shepstone.

Ugu district has six local municipalities (Figure 3 and 4) and 39 traditional authorities, and is a commercial and subsistence farming area that has seen major developments in the provision of sanitation and running water in recent years. Despite this, water and sanitation services do not reach all households and if they do, at times, are not always reliable, which result in some families resorting to usage of water from the nearby rivers and streams, bore-holes, wells and harvested rain water for their domestic needs (Ugu, 2008).
Faecal disposal facilities for all the communities include water closets (flush) toilets, pit latrines (most common in rural areas as well as in the informal settlements and in townships) and open defecation (Ugu, 2008). The study area is defined as being within a 10-km distance from the sea, an area that is known to be endemic for schistosomiasis and soil-transmitted helminth infections (Saathoff et al., 2004b). With the exception of malaria, there has never been any statutory control programme for any parasitic disease in South Africa (Appleton and Kvalsvig, 2006b). The first pilot programme in the province of KwaZulu-Natal was between 1998-2000, which specifically targeted three common STHs, i.e. *Trichuris trichiura* (whipworm), *Ascaris lumbricoides* (roundworm) and Hookworm species, and *Schistosomiasis haematobium* (urogenital schistosomiasis) and *Schistosoma mansoni* (intestinal schistosomiasis). Schistosomiasis is also known as bilharzia.
Figure 4: Map of six municipalities in Ugu district, KwaZulu-Natal where this study was conducted (Ugu, 2008).
3.6 Study Population

The study is part of a large Female Genital Schistosomiasis (FGS) cohort study taking place in Ugu district. (See also ethical considerations). The study population was females aged 10-12 years old, from primary schools that were randomly selected across Ugu District. These schools were randomised based on the distance from the coast (<10km), using the altitude map to locate potential schools below 300 metres since schistosomiasis prevalence decreases with increase in altitude (Appleton and Kvalsvig, 2006a). In total eighteen primary schools were included in the study. They are scattered in two different municipalities, i.e. Hibiscus and Umzumbe municipality (Figure 4). Seventeen of these schools are classified as rural and only one school, in the Hibiscus municipality, is classified as an urban school.

3.7 Recruitment of Study Subjects

3.7.1 Pupils

All female pupils between 10-12 years at each primary school were invited to participate. Parents were informed about the study and its objectives at the schools’ parents meetings where representatives from the study explained clearly about the study. Parents were encouraged to ask questions and contact details for the study’s Principal Investigators (PI) were given so that they could freely access the study leaders for further information.
3.8 Study Sample

The study sample consisted of 1100 school girls who were tested for the presence of soil transmitted helminths (Ascaris lumbricoides, Trichuris trichiura, Hookworm spp. and Taenia species) and urogenital (Schistosoma haematobium) and intestinal schistosomiasis (Schistosoma mansoni) ova.

3.9 Exclusion Criteria

School girls outside the age range, girls who did not give assent or whose parents did not give consent, or girls who were clinically ill were not included in the study. No pupils were from schools that were above 300 metres altitude.

3.10 Data Collection

Sample collection and analysis were done in two phases. First, the team went to participating schools in the mornings and interviewed selected pupils, and collected stool and urine samples. This took most of the morning. The team then transported the samples to the field laboratory where they were preserved and stored in appropriate conditions before examination under the microscope.

3.10.1 Urine Collection, Packing and Transportation

Three consecutive days’ urine samples were collected per participant though not all were able to give all three required samples. Each school was visited at least thrice. Clean honey jars (350 millilitres) prelabelled with the identity number given to each participant were used to collect urines between 10:00 and 14:00 hours each day. This urine was then packed into cooler boxes that were filled with ice-packs to maintain a cool temperature. From the field it was then transported to the laboratory daily.
3.10.2 Urine Examination Method and Parasitology

At the laboratory, 2 x 10ml urine samples per participant (A and B sample) were preserved with 1ml (10:1) of 2% Merthiolate in 5% formalin solution (formaldehyde solution 38% w/v, Medicolab) and stored in a dark cabinet at cool temperatures. Ten percent of the samples were reserved for external quality control. Preservation of urine samples was done within five hours after collection.

After preparation two different microscopists examined either slide A or B, for internal quality control purposes. The preserved urine sample (11ml) was then centrifuged at 4000 revolutions per minute (RPM) for five minutes to collect the sediment at the bottom of the test tube. All the aliquot in the test tube was discarded. From the sediment, wet-smears were made on two or more slides per 10 ml of urine. The samples were then examined microscopically (10x magnification) for the presence of helminth eggs (Figure 14). If at least one helminth egg was seen under the microscope, that sample was then deemed positive (Berhe et al., 2004, Brouwer et al., 2004, Chiodini, 2005).

All eggs were counted and the egg count was recorded in a record book (Figure 13). Dead or broken eggs were not counted. Intensity of schistosomiasis was expressed as the mean number of eggs per 10 ml of urine. If the slide was flooded with eggs such that it was impossible to do the egg count accurately, ten fields of view were randomly chosen and if all had more than ten eggs, that sample was then recorded as a high intensity sample with more than one thousand eggs (World Health Organisation, 2002a).
All pupils found to be infected with schistosomiasis were offered treatment (praziquantel). We could not get treatment for STHs but advised those infected with STHs to visit their nearest health care centres for STH treatment.

### 3.10.3 Stool Collection, Packing and Transportation

One fresh stool sample was collected from each pupil. Samples were transferred from non-absorbent paper to 40ml specimen jars, which were pre-labelled (with permanent marker). Samples were then packed in cooler boxes with ice-packs and transported to the laboratory.

### 3.10.4 Stool Examination and Parasitology

Stool was preserved within five hours with 10% formalin solution and then stored at four degrees. Ten percent of the stools were chosen randomly and reserved for external quality control. Stool specimens were examined using the Kato-Katz thick smear procedure (Glinz et al., 2010). Briefly, a small amount of stool was collected and sieved through a fine screen to fill a hole of a metal template (25mg). The stool on the slide was covered with cellophane that had been pre-soaked in glycerol and malachite green solution, then pressed against a hard surface so that the stool could spread evenly on the slide, and was left for clarification for 30 minutes.

The slide was then scanned under a light microscope at the low ten times magnification. Two slides (A and B) were made from each stool specimen following the same method as described above. Two different microscopists examined either slide A or B, for internal control purposes, and eggs were counted from the whole slide. If one egg was found then that stool sample was deemed positive for that specific helminth. The stool examination results were recorded in the form of number
of eggs per smear (25mg). Multiplication by a factor of 40 (for 25mg template) then gives the number of eggs per gram of stool (EPG) (Glinz et al., 2010).

Table 4: WHO classification of intensity of helminth infections (Renganathan, 1998)

<table>
<thead>
<tr>
<th>Helminth species</th>
<th>Light Intensity Infections*</th>
<th>Moderate Intensity Infections</th>
<th>High Intensity Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. lumbricoides</td>
<td>1 – 4999 eggs/gram of stool</td>
<td>5000 – 49 000 eggs/gram of stool</td>
<td>≥ 50 000 eggs/gram of stool</td>
</tr>
<tr>
<td>T. trichiura</td>
<td>1 – 999 eggs/gram of stool</td>
<td>1000 – 9 999 eggs/gram of stool</td>
<td>≥ 10 000 eggs/gram of stool</td>
</tr>
<tr>
<td>S. haematobium</td>
<td>&lt; 50 eggs/10 ml of urine</td>
<td>≥ 50 eggs/10 ml of urine</td>
<td></td>
</tr>
</tbody>
</table>

For both urinary and stool analysis, WHO guidelines of rating intensity were used to classify level of intensity of pupils (Table 4).

3.11 Water and Sanitation in the study area

Information related to household coverage, type and availability of water and sanitation infrastructure was sourced from the annual reports and archives in the Ugu District Municipality offices. Data were collected from these archives from the year 2005. A semi-structured questionnaire (Appendix 8) on the water, sanitation and development projects in Ugu District wards was administered to all 15 ward councillors from the areas around the 18 included schools. Three of these ward councillors had two of the included schools in their wards. The information was recorded, transcribed and entered into SPSS Software Package 18.

3.12 Water contact information – Questionnaires

Demographic information was collected through individual interviews with each child at school by trained research assistants. The questionnaire had been translated from English to IsiZulu, back-translated and also piloted in three schools not in the study sample (Appendix 8). Information relating to age, residential area, source of water
used for drinking and other household purposes, type of water used for laundry and how often the child swam in the river and child’s hygiene practices, were collected. A history of symptoms for bilharzia was investigated and whether the child had previously been treated for bilharzia.

3.13 Data Management

Data from the questionnaires (using the study number and not pupils’ names) as well as the laboratory results were captured on Epidata (questionnaires) and Excel (laboratory data) and double entry was done electronically. The questionnaires were entered and hard copies were locked away safely and kept in line with the ethics’ guidelines to ensure the confidentiality of the participants. The electronic data on the computers were password protected and only a few persons (PI and supervisors) had access to this. Names and other information were kept in separate sites (see ethics).

3.14 Comparison of data from 1998 and 2010

A study was carried out in Ugu district from 1998 to 2000 by the Parasite Control Programme (PCP) group. Findings from that study were used to compare with the findings from this child cohort study of 2010. The PCP study included both boys and girls between the ages of five to seventeen years. Forty schools were included in the PCP study as compared to eighteen schools in the 2010 study. Only two of the same schools were investigated in both studies. The amount of urine and stool samples collected from participants who participated in the PCP study (one stool and two urines) and in the child cohort study in 2010 (one stool and three urines) were
analysed similarly. Also similar questions were asked of pupils in both studies concerning water contact and bilharzia symptoms.

The unpublished dataset from PCP was obtained from the study PI and permission was obtained to analyse and use the findings (Appendix 9). Prevalences and intensities of different helminth infections reported by the PCP study were calculated for 10-12 year old female pupils and compared. The responses from the water contact questions from pupils (2010) were also compared with the PCP study data for Ugu District. The similarities and contrasts between the two studies (1998 and 2010) are reported in the results and discussed with reference to the reported changes in water and sanitation service provision in Ugu regarding the prevalence and intensity of schistosomiasis and STHs.

3.15 Data Analysis

The prevalence and intensity of the helminth infections were analysed using univariate analysis. All data were entered and analysed using Statistical Programme for Social Sciences version 18 (SPSS 18) statistical package.

A chi-square ($\chi^2$) test was used to analyze correlations between prevalence of schistosomiasis and factors such as knowledge about schistosomiasis; red urine; dysuria and for analyzing correlations between different helminth infections. A $p < 0.05$ and 95% confidence intervals was used.
3.16 Ethical Considerations

This study drew from a larger project that has been granted permission by four committees: the Biomedical Research Ethics Committee (BREC), University of KwaZulu-Natal KZN on February 20th 2011 (Ref BF029/07) (Appendix 3), the Department of Health, Pietermaritzburg, KZN, February 3rd 2009 (Ref HRKM010-08), the Norwegian ethics committee, Regional Etisk Komité Øst-Norge (REK-Øst), gave ethical clearance September 17th 2007 (Ref 469-07066a1.2007.535) and The European Group on Ethics in Science and New Technologies in 2011 (Ref IRSES-2010:269245). BREC extended permission for this particular study to be conducted, REF: BE005/12 (Appendices 7 and 8). The Department of Health and Education in Ugu district, KwaZulu-Natal and the provincial Department of Education have also given permission (see appendices).

Consent forms were handed directly to parents or children took them home for signatures (Appendices 9 and 10, in English and Zulu respectively). Permission was given by principals and at parents’ meetings. Inclusion in the study took place after individual informed written consent was obtained from a parent or guardian of the child and assent from the child. Therefore all study participants were only recruited into the study once a consent form was received from parents. All subjects found positive for urinary and intestinal schistosomiasis were treated and soil transmitted helminths (STH) were referred for treatment.
Chapter Four: Results

4.1 Introduction

This section provides a demographic profile of the study sample and the prevalence and intensity of schistosomiasis and soil transmitted helminths found, i.e. *S. haematobium, A. lumbricoides and T. trichiura*. This section also reports on different types of water contact reported by pupils from the eighteen different schools in the study sample. The ward councillors’ reports of the current conditions concerning access to water and sanitation in the communities surrounding these schools are also presented.

4.2 Description of Study Sample

Of 1948 eligible girls from 18 randomly selected primary schools, 1241 (63.7%) consented to participate in the study but only 1057 of those were interviewed. Although parental consent was provided, 14.8% (n=184) children did not attend school on the days that the interviews were conducted. Participants (n=1044) were within the age group of 10-12 years, but five were nine years old and six were older than 12 years. In order to categorise the age groups, the five pupils that were 9 years were grouped with the 10 year olds and the six pupils that were 13 years of age were grouped with the 12 year olds for the analysis. The mean age was 11.1 (SD 0.85) years. The age of two pupils was not reported.
Table 5: Descriptive information on the pupils aged 10-12 years old (females) from 18 Ugu primary schools, N=1057

<table>
<thead>
<tr>
<th>Responses by pupils</th>
<th>n</th>
<th>Age (years) 10 to 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>School Grade (n=1010)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 3</td>
<td>67</td>
<td>6.6%</td>
</tr>
<tr>
<td>4 to 7</td>
<td>943</td>
<td>93.4%</td>
</tr>
<tr>
<td><strong>Primary guardian’s relationship to child (n=1023)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>34</td>
<td>3.3%</td>
</tr>
<tr>
<td>Mother</td>
<td>672</td>
<td>65.7%</td>
</tr>
<tr>
<td>Other</td>
<td>317</td>
<td>31.0%</td>
</tr>
<tr>
<td><strong>Sex of primary guardian (n=1023)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42</td>
<td>4.1%</td>
</tr>
<tr>
<td>Female</td>
<td>981</td>
<td>95.9%</td>
</tr>
<tr>
<td><strong>Mother present in household (n=869)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>584</td>
<td>67.2%</td>
</tr>
<tr>
<td><strong>Father present in household (n=869)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>230</td>
<td>26.5%</td>
</tr>
<tr>
<td><strong>Ever lived in city (n=1014)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>938</td>
<td>92.5%</td>
</tr>
<tr>
<td>Yes</td>
<td>69</td>
<td>6.8%</td>
</tr>
<tr>
<td>Do not know</td>
<td>7</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

Even though 1057 pupils were interviewed, some information was missing from the questionnaire and the number of pupils who responded to various questions asked varied as a result. A third of the pupils, 34.6% (349/1010), were doing grade five, with fewer in grades four, 19.5% (199/1009) and six, 25.5% (257/1010). Most of the pupils (95.9%) had females as their first guardian. Of these over half of the pupils, 65.7% (672/1023) had their mothers as their first guardians, and 15.1% (n=155) and 10.7% (n=109) pupils, had grandmothers and aunts as first guardians although a few had stepmothers or their sisters as their first guardians. Few pupils had ever lived in a city, with most exposed to rural disadvantaged conditions.
4.3 Sample Analysis of Schistosomiasis and Soil Transmitted Helminths

4.3.1 Analysis of urine samples for Schistosomiasis

Pupils were requested to provide three consecutive days urine, but not all were able to give all three urines. Some pupils gave one and two urines only. Of the pupils, 970 gave at least one urine sample eligible for analyses. A total of 791 pupils gave all the required three urines (Table 6).

Table 6: Prevalence and intensity of *S. haematobium* per amount of urines collected per study subject

<table>
<thead>
<tr>
<th>Number of urine samples collected</th>
<th>Prevalence % (n) of <em>S. haematobium</em></th>
<th>Mean Intensity of eggs/10ml (SD) of <em>S. haematobium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupils (n=29) providing one urine sample</td>
<td>24.1 (7)</td>
<td>10.6 (43.9)</td>
</tr>
<tr>
<td>Pupils (n=150) providing two urine samples</td>
<td>24.0 (36)</td>
<td>18.3 (60.1)</td>
</tr>
<tr>
<td>Pupils (n=791) providing three urine samples</td>
<td>34.0 (269)</td>
<td>9.6 (32.4)</td>
</tr>
<tr>
<td>Total urine samples (n=970)</td>
<td>32.2 (312)</td>
<td>16.7 (56.7)</td>
</tr>
</tbody>
</table>

The proportion of pupils found to be infected with *S. haematobium* was 32.2% (95% CI 30.1%-37.4%) for three samples, (Table 7). Schools (A-N) were anonymised. Schools are listed in the order that they were visited by our field team from the beginning (February) till the end of the year (October). The distribution of the schools was amongst fifteen different community wards. All investigated schools were in different community wards, except schools A, B and D that were in the same ward 22. Schools H and L also shared the same ward 24. Most schools (16) were in Hibiscus Municipality, except schools N and R that were in Umzumbe Municipality. Schistosomiasis was endemic in all the schools with some schools having a prevalence of up to 66.7% and all schools had mean light intensity of infection.
One third of the investigated schools were found to have schistosomiasis prevalences above 40.0% ranging from 40.0 to 66.7% (Table 7).

Table 7: Prevalence and intensity (95% CI) of *S. haematobium* amongst pupils attending 18 KwaZulu Natal primary schools

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Prevalence (%) of <em>S. haematobium</em> (95% CI)</th>
<th>Intensity of <em>S. haematobium</em> eggs/10ml urine (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>32.2 (29.3 – 35.2)</td>
<td>17 (56.4)</td>
</tr>
<tr>
<td>School A (n = 100)</td>
<td>43.9 (33.0 – 56.0)</td>
<td>19 (47.4)</td>
</tr>
<tr>
<td>School B (n = 71)</td>
<td>40.0 (25.0 – 56.0)</td>
<td>7 (12.57)</td>
</tr>
<tr>
<td>School C (n = 37)</td>
<td>50.0 (27.0 – 73.0)</td>
<td>23 (38.05)</td>
</tr>
<tr>
<td>School D (n = 114)</td>
<td>38.0 (26.0 – 52.0)</td>
<td>31 (81.64)</td>
</tr>
<tr>
<td>School E (n = 11)</td>
<td>20.0 (3.6 – 62.0)</td>
<td>1 (3.71)</td>
</tr>
<tr>
<td>School F (n = 50)</td>
<td>20.5 (11.0 – 35.0)</td>
<td>47 (121.17)</td>
</tr>
<tr>
<td>School G (n = 37)</td>
<td>36.0 (20.0 – 55.0)</td>
<td>11 (46.18)</td>
</tr>
<tr>
<td>School H (n = 101)</td>
<td>46.6 (36.0 – 57.0)</td>
<td>30 (70.52)</td>
</tr>
<tr>
<td>School I (n = 44)</td>
<td>47.5 (33.0 – 62.0)</td>
<td>40 (98.00)</td>
</tr>
<tr>
<td>School J (n = 46)</td>
<td>53.8 (39.0 – 68.0)</td>
<td>32 (66.61)</td>
</tr>
<tr>
<td>School K (n = 172)</td>
<td>10.8 (7.0 – 16.0)</td>
<td>4 (16.86)</td>
</tr>
<tr>
<td>School L (n = 77)</td>
<td>17.0 (9.2 – 29.0)</td>
<td>4 (11.17)</td>
</tr>
<tr>
<td>School M (n = 63)</td>
<td>7.7 (2.6 – 20.0)</td>
<td>3 (12.90)</td>
</tr>
<tr>
<td>School N* (n = 34)</td>
<td>12.9 (5.1 – 29.0)</td>
<td>7 (20.38)</td>
</tr>
<tr>
<td>School O* (n = 22)</td>
<td>10.0 (2.8 – 30.0)</td>
<td>0.44 (1.27)</td>
</tr>
<tr>
<td>School P* (n = 7)</td>
<td>25.0 (4.6 – 70.0)</td>
<td>54 (99.56)</td>
</tr>
<tr>
<td>School Q* (n = 42)</td>
<td>8.8 (3.0 – 23.0)</td>
<td>0.86 (0.30)</td>
</tr>
<tr>
<td>School R* (n = 7)</td>
<td>66.7 (35.9 – 91.8)</td>
<td>14.8 (17.17)</td>
</tr>
</tbody>
</table>

*The last five schools had a low number of pupils as these schools were affected by the teachers’ strike (2010) and were as a result visited during the time teachers were trying to catch up the lost time and also preparing for end of year exams.*
The overall mean intensity (including those not infected) of *S. haematobium* was found to be 17 eggs/10ml of urine (SD 56.4) of which a total of 71.8% (224/312) of infected pupils had low intensity of infection varying from 0.86 to 47 eggs/10 ml urine and 8.9% (87/312) had high intensity of infection. Only one school (n = 7) had high intensity for schistosomiasis (54 eggs/10ml of urine) and the rest of schools had varying intensities of low infection. There was a significant difference (p<0.05) in the intensity of infection with schistosomiasis between schools.

4.3.2 Analysis of stool sample for *S. mansoni* and STHs (*A. lumbricoides, T. trichiura, Hookworm spp.* and *Taenia spp.*)

4.3.2.1 Prevalence
Eighty one percent, (853/1057) pupils in total gave a stool sample. From the single stool sample collected from each participant, only *A. lumbricoides* and *T. trichiura* (Table 7) were found in this population. No Hookworm spp and *Taenia spp.* were found in this sample. For *T. trichiura* and *A. lumbricoides*, n = 221 (25.9%) and n = 209 (24.5%) were infected respectively. None of the schools had STHs prevalence above 50.0%. Although the prevalence levels of *A. lumbricoides* and *T. trichiura* were similar, the difference (1.4%) in the prevalence of infection, was statistically significant (p<0.01). Ten of the schools had *A. lumbricoides* and *T. trichiura* prevalences 20.0% or above (Table 8).
### Table 8: Prevalence (95% CI) of *A. lumbricoides* and *T. trichiura* amongst pupils attending 18 KwaZulu-Natal primary schools.

<table>
<thead>
<tr>
<th>School</th>
<th>Prevalence (%) of <em>A. lumbricoides</em> (95% CI)</th>
<th>Prevalence (%) of <em>T. trichiura</em> (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall schools</td>
<td>24.5 (22.0 – 27.0)</td>
<td>25.9 (23.0 – 29.0)</td>
</tr>
<tr>
<td>School A (n = 100)</td>
<td>34.5 (25.0 – 45.0)</td>
<td>41.7 (32.0 – 52.0)</td>
</tr>
<tr>
<td>School B (n = 71)</td>
<td>32.0 (14.0 – 33.0)</td>
<td>28.0 (1.2 – 30.0)</td>
</tr>
<tr>
<td>School C (n = 37)</td>
<td>40.0 (22.0 – 61.0)</td>
<td>25.0 (5.9 – 28.0)</td>
</tr>
<tr>
<td>School D (n = 114)</td>
<td>25.8 (17.0 – 38.0)</td>
<td>48.4 (36.0 – 61.0)</td>
</tr>
<tr>
<td>School E (n = 11)</td>
<td>11.1 (1.9 – 43.0)</td>
<td>22.2 (6.3 – 55.0)</td>
</tr>
<tr>
<td>School F (n = 50)</td>
<td>25.0 (15.0 – 39.0)</td>
<td>27.1 (17.0 – 41.0)</td>
</tr>
<tr>
<td>School G (n = 37)</td>
<td>18.8 (8.9 – 35.0)</td>
<td>18.8 (8.9 – 39.0)</td>
</tr>
<tr>
<td>School H (n = 101)</td>
<td>24.4 (16.0 – 35.0)</td>
<td>36.6 (27.0 – 47.0)</td>
</tr>
<tr>
<td>School I (n = 44)</td>
<td>36.6 (24.0 – 52.0)</td>
<td>29.3 (18.0 – 44.0)</td>
</tr>
<tr>
<td>School J (n = 46)</td>
<td>37.8 (25.0 – 52.0)</td>
<td>46.7 (33 – 61.0)</td>
</tr>
<tr>
<td>School K (n = 172)</td>
<td>18.6 (14.0 – 26.0)</td>
<td>16.0 (12.0 – 23.0)</td>
</tr>
<tr>
<td>School L (n = 77)</td>
<td>19.1 (11.0 – 30.0)</td>
<td>20.6 (13.0 – 32.0)</td>
</tr>
<tr>
<td>School M (n = 63)</td>
<td>27.1 (17.0 – 40.0)</td>
<td>13.6 (7.0 – 24.0)</td>
</tr>
<tr>
<td>School N* (n = 34)</td>
<td>20.0 (9.5 – 37.0)</td>
<td>16.7 (14.0 – 44.0)</td>
</tr>
<tr>
<td>School O* (n = 22)</td>
<td>15.0 (5.2 – 36.0)</td>
<td>5.0 (0.89 – 24.0)</td>
</tr>
<tr>
<td>School P* (n = 7)</td>
<td>16.7 (3.0 – 56.0)</td>
<td>0.0 (0.0 – 3.9)</td>
</tr>
<tr>
<td>School Q* (n = 42)</td>
<td>0.0 (0.0 – 10.0)</td>
<td>0.0 (0.0 – 10.0)</td>
</tr>
<tr>
<td>School R* (n = 7)</td>
<td>14.3 (2.6 – 51.0)</td>
<td>0.0 (0.0 – 35.0)</td>
</tr>
</tbody>
</table>

*The last five schools had a low number of pupils as these schools were affected by the teachers’ strike (2010) and were as a result visited during the time teachers were trying to catch up the lost time and also preparing for end of year exam.

Fifteen of the schools had pupils with both ascariasis and trichiurasis infections and only two schools had pupils with only one helminth infection. There was significant
(p<0.05) association between the prevalence of *T. trichiura* and *S. haematobium*. There was no significant association between *A. lumbricoides* and the other two helminth infections (*T. trichiura* and *S. haematobium*). One school did not have pupils with any STH infection (Table 8). Differences in infections between schools were significant (p<0.01) for all infections found. Mzumbe municipality had significantly (p<0.05) higher prevalence than Hibiscus municipality for all the infections found (Figure 5).

![Figure 5: Comparison of prevalence of A. lumbricoides, T. trichiura and S. haematobium with 95% confident intervals per municipality (Umzumbe and Hibiscus Coast Municipalities) of Ugu district.](image)
4.3.2.2 Intensity of STH infections

The total number of pupils providing urine samples (n=970) differed from those providing stools (n=853). The overall mean intensity of infection of *A. lumbricoides* was 4110 eggs/gram of stool, n=14 (6.6%) of the cases had a high intensity of infection and n=132 (62.6%) were moderately infected.

An intensity of infection of 146 eggs/gram of stool was found for *T. trichiura*. This helminth species was the least intense infection of the three helminths in this population with only 24 (10.4%) cases found with moderate intensity of infection and just one case (0.4%) that had high intensity and n=205 (89.1%) cases of the n=230 infected were found to have low intensity of infection. The burden of ascariasis and schistosomiasis varied per school from those with a low mean intensity (210 epg) to moderate infection (9689 eggs/gram), whereas for trichuriasis the intensity of infection in all schools was low (Table 9).
Table 9: Intensity (eggs/gram) per school of *A. lumbricoides*, *T. trichiura* amongst pupils attending 18 KwaZulu-Natal primary schools

<table>
<thead>
<tr>
<th>School ID (n)</th>
<th><em>A. lumbricoides</em> eggs/gram (±SD)</th>
<th><em>T. trichiura</em> eggs/gram (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Eighteen Schools</td>
<td>4110 (14841.1)</td>
<td>146 (641.9)</td>
</tr>
<tr>
<td>A (100)</td>
<td>4956 (12 403.22)</td>
<td>188 (411.53)</td>
</tr>
<tr>
<td>B (71)</td>
<td>9640 (26182.26)</td>
<td>139 (350.80)</td>
</tr>
<tr>
<td>C (37)</td>
<td>7091 (17274.20)</td>
<td>49 (88.82)</td>
</tr>
<tr>
<td>D (114)</td>
<td>9689 (37928.90)</td>
<td>141 (449.47)</td>
</tr>
<tr>
<td>E (11)</td>
<td>1951 (5853.33)</td>
<td>102 (246.46)</td>
</tr>
<tr>
<td>F (50)</td>
<td>2762 (6142.97)</td>
<td>140 (554.62)</td>
</tr>
<tr>
<td>G (37)</td>
<td>2380 (5928.55)</td>
<td>231 (1006.76)</td>
</tr>
<tr>
<td>H (101)</td>
<td>2229 (5490.39)</td>
<td>319 (1305.65)</td>
</tr>
<tr>
<td>I (44)</td>
<td>5197 (8408.75)</td>
<td>41 (82.27)</td>
</tr>
<tr>
<td>J (46)</td>
<td>5635 (8569.22)</td>
<td>253 (791.30)</td>
</tr>
<tr>
<td>K (172)</td>
<td>2239 (5684.51)</td>
<td>116 (602.58)</td>
</tr>
<tr>
<td>L (77)</td>
<td>2691 (6386.70)</td>
<td>85 (208.44)</td>
</tr>
<tr>
<td>M (63)</td>
<td>4446 (8127.18)</td>
<td>133 (535.37)</td>
</tr>
<tr>
<td>N* (34)</td>
<td>1726 (4775.06)</td>
<td>197 (967.81)</td>
</tr>
<tr>
<td>O* (22)</td>
<td>2097 (5811.56)</td>
<td>17 (76.03)</td>
</tr>
<tr>
<td>P* (7)</td>
<td>210 (514.39)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Q* (42)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>R* (7)</td>
<td>2857 (7559.29)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*The last five schools had low number of pupils as these schools were affected by the teachers strike (2010) and were as a result visited during the time teachers were trying to catch up the lost time and also preparing for end of year exam.

4.4 Multiple helminth infections

4.4.1 Prevalence

There were n=116 (13.5%) cases of double infection of ascariasis and trichuriasis found, where pupils were infected with both STHs. Another n=85 (10.0%) pupils were found to be infected with both *T. trichiura* and *S. haematobium* (Table 10) and they had moderate to high intensity of infection. There were n=69 (8.0%) pupils reported to be infected with both *S. haematobium* and *A. lumbricoides* and these cases had moderate (ascariasis) to high schistosomiasis infection. Of the pupils,
n=44 (5.2%) pupils were infected with all three helminths, *S. haematobium*, *A. lumbricoides* and *T. trichiura*. None of these individuals were lightly infected. The *T. trichiuris* and *A. lumbricoides* infection intensity and that of *S. haematobium* were high (Table 10).

**Table 10: Intensity of infection amongst dual and triple infected pupils from 18 Ugu district primary schools (n=1044)**

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>n</th>
<th>Mean Intensity</th>
<th>(±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dual infection:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>116</td>
<td>619.35 eggs/gram</td>
<td>1250.31</td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>116</td>
<td>16689.45 eggs/gram</td>
<td>19189.19</td>
</tr>
<tr>
<td><strong>Dual infection:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. haematobium</em></td>
<td>85</td>
<td>440.80 eggs/gram</td>
<td>843.68</td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>69</td>
<td>15783.31 eggs/gram</td>
<td>16731.51</td>
</tr>
<tr>
<td><strong>Dual infection:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. haematobium</em></td>
<td>69</td>
<td>70.14 eggs/10 ml</td>
<td>70.13</td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>69</td>
<td>15783.31 eggs/gram</td>
<td>16731.51</td>
</tr>
<tr>
<td><strong>Triple infection:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. haematobium</em></td>
<td>44</td>
<td>90 eggs/10 ml</td>
<td>116.55</td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>44</td>
<td>17819 eggs/gram</td>
<td>18456.25</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>44</td>
<td>552 eggs/gram</td>
<td>905.14</td>
</tr>
</tbody>
</table>
4.4.2 Intensity among those with multiple helminth infections

*S. haematobium* was found to be at high levels of intensity (60 eggs/10ml urine) amongst 27.9% of infected pupils. Overall moderate intensity levels of infection of *T. trichiura* and *A. lumbricoides* were found amongst those infected.

Table 11: Mean intensity (epg) of *S. haematobium*, *A. lumbricoides* and *T. trichiura* amongst pupils at the 18 Ugu schools

<table>
<thead>
<tr>
<th>Helminth</th>
<th>n</th>
<th>Mean Intensity</th>
<th>(±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. haematobium</em></td>
<td>312</td>
<td>52 eggs/10ml urine</td>
<td>89.77</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>230</td>
<td>561 eggs/gram</td>
<td>1163.94</td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>211</td>
<td>17006 eggs/gram</td>
<td>26348.94</td>
</tr>
</tbody>
</table>

Investigating whether particular schools were at high risk, it was found that schools A, H, D, I and K had the most pupils with dual infection and then schools I (Table 12). In total, 14 schools (77.8%) had multiple infections. These schools were located within the Hibiscus Coast Municipality (Table 12).
Table 12: Number of cases of pupils per school with different multiple helminth infections (dual and triple infections)

<table>
<thead>
<tr>
<th>School name (ID) and n</th>
<th>Double Infections</th>
<th>Triple Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{A. lumbricoides and T. trichuria}</td>
<td>\textit{T. trichiura and S. haematobium}</td>
</tr>
<tr>
<td>A (100)</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>B (71)</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>C (37)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>D (114)</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>E (11)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>F (50)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>G (37)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>H (101)</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>I (44)</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>J (46)</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>K (172)</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>L (77)</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>M (63)</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>N* (34)</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>O* (22)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P* (7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q* (42)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R* (7)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
4.5 Factors influencing helminth infection

4.5.1 Reported water contact by pupils
The majority of the pupils reported that at home they use a standpipe or communal standpipe that the municipality provided (Figure 6). A very small fraction (<2%) reported that they rely on the river as a source of drinking water. *S. haematobium* infection was significantly associated with the source of drinking water (p<0.05). However, 12.4% (n=26) and 83.7% (n=175) of infected pupils who drank water from an indoor tap, or a standpipe/communal standpipe, were respectively found to be positive for *S. haematobium*. This indicates that pupils with indoor tap are less at risk of getting infected than those who rely on communal standpipes.

![Figure 6: Reported source of drinking water by pupils from 18 investigated Ugu schools (n=1022)](image-url)
4.5.2 Association between reported water contact and prevalence of S. haematobium infection

Most pupils reported that they neither swim (n=770) nor play in the river (n=691), for the latter see (Table 13). There was also little laundry activity in the rivers reported by these pupils. The majority of pupils reported that they did not bath (n=681), wash clothes (n=600) or wash blankets (n=744) in the river. All these activities were however significantly associated with S. haematobium infection (p<0.05). Few pupils collected water from the river and a small number reported that they cross a river regularly. None of the pupils (n=1010) reported fishing in rivers. However, 18.3% (55 of 307) of the pupils who denied water contact were found to have bilharzia.

**Table 13: Different forms of water contact reported by Ugu pupils, n = 1057**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Never % (n)</th>
<th>Rarely % (n)</th>
<th>Sometimes % (n)</th>
<th>Often % (n)</th>
<th>Daily % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Play (n=1024)</td>
<td>67.5 (691)</td>
<td>12.5 (128)</td>
<td>13.6 (139)</td>
<td>6.1 (62)</td>
<td>0.4 (4)</td>
</tr>
<tr>
<td>*Wash/bath (n=1023)</td>
<td>66.6 (681)</td>
<td>8.0 (82)</td>
<td>9.3 (95)</td>
<td>4.1 (42)</td>
<td>12.0 (123)</td>
</tr>
<tr>
<td>*Laundry (n=1023)</td>
<td>58.7 (601)</td>
<td>9.6 (98)</td>
<td>14.6 (148)</td>
<td>14.1 (144)</td>
<td>3.1 (32)</td>
</tr>
<tr>
<td>*Wash blankets (n=1022)</td>
<td>72.8 (744)</td>
<td>10.1 (103)</td>
<td>11.4 (116)</td>
<td>5.8 (59)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Collect Water (n=1023)</td>
<td>73.4 (751)</td>
<td>7.5 (77)</td>
<td>7.1 (73)</td>
<td>4.0 (41)</td>
<td>7.9 (81)</td>
</tr>
</tbody>
</table>

*Significant association with S. haematobium infection (p<0.05)*
4.6 Municipal councillors’ reports of water access within 200m from households and the availability of VIP toilets per household in communities surrounding participating schools

The 18 schools were represented by 15 ward councillors, three of whom were from the Umzumbe Municipality and the rest from the Hibiscus Coast Municipality. Two councillors each had two participating schools in their ward while the rest had one participating school. All the interviewed councillors (n=15) reported that there has been an improvement over the past five years regarding the infrastructural facilities in their communities. Installation of Ventilation Improved Pit (VIP) toilets was reported to be a major challenge when compared to water installation projects in their communities. None of the councillors said they had 100% household VIP coverage in their communities.

4.6.1 Reported water access by municipal councillors

All municipal ward councillors (n=15) reported that there had been a positive change in the provision of water to communities surrounding the 18 Ugu schools compared to five years previously. More than half of the councillors reported that everyone in their communities had water access within 200m from their households (Figure 7) and that most pupils had the use of clean water at home. It was also established through interviews with the community ward councillors that some people within their communities still do use rivers and streams for bathing/laundry, or washing blankets as it is believed that by using rivers ensures that the above-mentioned activities are done thoroughly. This still happens despite people having access to water within 200m and according to ward councillors, it was elderly people who still held firmly to this practice so the number of people using rivers had declined.
4.6.2 *Reported improved sanitation by municipal councillors*

Although pupils were not asked about sanitation conditions in their households, all fifteen ward councillors reported that toilet installation in households in their respective communities was a challenge. One councillor reported that every household in his community had a Ventilated Improved Pit (VIP) toilet five years ago but this has since changed because the population has increased. Two-thirds of the interviewed councillors reported that about 75.0% of households in their communities had VIP toilets (Figure 8). All ward councillors reported that there has been an increase in the number of households with VIP toilets now compared to five years ago despite the various challenges.
Figure 8: Councillors (n=15) reporting percentage change over five years in households with VIP toilets in 18 communities near the eighteen schools in the study.
4.7 Association between knowledge of bilharzia and prevalence of *S. haematobium* infection

The majority of the pupils (57.6%) responded that they knew what bilharzia is (Isichenene in the Zulu language), and described bilharzia as red urine or when someone’s urine has blood in it. More than half of the pupils reporting previous schistosomiasis infection had been treated for bilharzia before and as can be seen in Table 13; both these factors were significantly associated with currently having *S. haematobium* infection, (p<0.05).

<table>
<thead>
<tr>
<th>Question (N)</th>
<th>Response</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Know what bilharzia is (1020)</em></td>
<td>No</td>
<td>393</td>
<td>38.6</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>587</td>
<td>57.6</td>
</tr>
<tr>
<td></td>
<td>Unsure</td>
<td>39</td>
<td>3.8</td>
</tr>
<tr>
<td><em>History of bilharzia within family (1022)</em></td>
<td>No</td>
<td>796</td>
<td>77.9</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>149</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>Don’t Know</td>
<td>77</td>
<td>7.5</td>
</tr>
<tr>
<td><em>Ever Had Bilharzia (1020)</em></td>
<td>No</td>
<td>773</td>
<td>75.8</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>226</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>Don’t Know</td>
<td>21</td>
<td>2.1</td>
</tr>
<tr>
<td><em>Ever treated for bilharzia (237)</em></td>
<td>No</td>
<td>102</td>
<td>45.1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>129</td>
<td>54.4</td>
</tr>
</tbody>
</table>

*Variable significant for *S. haematobium* (p<0.05)

A significant association (p<0.05) was found between those who reported knowing what bilharzia is and having *S. haematobium* infection and 63.5% (195/307) of these pupils tested positive for *S. haematobium* infection. Of pupils who had been treated for bilharzia before, 44.9% (40/89) were found to have been re-infected. A statistically significant relationship was observed between infection with schistosomiasis and both red urine and dysuria (p<0.05 for both).
Table 15: Pupils’ response to possible indicators associated with \textit{S. haematobium} infection

<table>
<thead>
<tr>
<th>Question (N)</th>
<th>Percentage Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Dysuria ever (1020)</td>
<td>22.2</td>
</tr>
<tr>
<td>*Red urine ever (1023)</td>
<td>17.8</td>
</tr>
<tr>
<td>*Untreated bilharzia (222)</td>
<td>44.6</td>
</tr>
<tr>
<td>*Any water contact (1057)</td>
<td>63.1</td>
</tr>
</tbody>
</table>

*Variable significantly associated with \textit{S. haematobium} infection (p<0.05)

Of the pupils who were previously infected with bilharzia 62.9\% (132/306) tested positive for bilharzia in our study. About one fifth of the interviewed pupils reported a history of bilharzia symptoms and almost half reporting untreated bilharzia (Table 14).

Table 16: Comparisons of Prevalence and Intensity of Helminth Infections and Water Contact in 1998 and 2010 studies in Ugu District

<table>
<thead>
<tr>
<th></th>
<th>1998; ( n = 398 )</th>
<th>2010; ( n = 1057 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prevalence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{S. haematobium} &amp; 14.9 (11.7-18.7) &amp; 32.2 (29.3-35.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{A. lumbricoides} &amp; 62.4 (57.7-67.1) &amp; 24.5 (22.0-27.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{T. trichiura} &amp; 58.6 (53.7-63.4) &amp; 25.9 (23.0-29.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intensity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{S. haematobium} (eggs/10ml urine) &amp; 22.53 (SD57.21) &amp; 16.7 (SD56.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{A. lumbricoides} &amp; 72.84 (SD63.80) &amp; 4110.2 (SD14841.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{T. trichiura} &amp; 64.97 (SD59.6) &amp; 146.0 (SD642.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Water Contact</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetch water from river &amp; 71.6 (66.9-75.9) &amp; 7.9 (6.4-9.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash clothes in the river &amp; 44.0 (39.1-49.0) &amp; 12.0 (10.2-14.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swim in the river &amp; 47.8 (42.7-52.7) &amp; 0.47 (0.15-1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Play in the river &amp; 47.4 (42.5-52.5) &amp; 0.47 (0.15-1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other water contact &amp; 10.9 (8.2-14.6) &amp; 63.1 (60.2-66.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Unlisted fresh water contact
A total of 398 female pupils aged 10-12 were investigated in 1998. Prevalence of *S. haematobium* was high in 2010 compared to 1998 but prevalences of *A. lumbricoides* and *T. trichiura* were lower in 2010 compared to 1998. However, the intensity of *S. haematobium* was lower in 2010 and the intensity of the STHs was higher. Water related risk behaviours were lower in 2010 in comparison to 1998.
5.1 Prevalence of helminth infections

A third of pupils investigated tested positive for *S. haematobium* infection. A quarter also tested positive for the STHs, *T. trichiura* and almost a quarter were infected with *A. lumbricoides*. Ten percent were infected with both STHs. No other STHs were found in the study. Few pupils had high intensity of infection for the three helminths found with the majority found to have light and moderate intensity of infection. Soil transmitted helminths (two) found in our study were less prevalent than those found in the PCP study (three), since no Hookworm *ssp.* was found in our study. The prevalence of *A. lumbricoides* and *T. trichiura* prevalences has decreased by more than half but for *S. haematobium* the prevalence was found to have increased when compared with the 1998 PCP study. Water and sanitation status in the surrounding communities had improved as more households had easy access to safe water and VIP toilets. These helminth infections can cause short and long term health problems and with the current available treatment options, this needs to be addressed.

An insignificant variation in helminth infections between the schools investigated was seen. A total of 970 pupils provided urines and three urine samples were collected from each of 791 pupils. The high variability of multiple *S. haematobium* counts makes single egg counts a less than optimal tool for estimating total prevalence and for identifying the infection status of individuals (Savioli et al., 1990, Saathoff et al., 2004b). The prevalence amongst pupils who gave all three urines (32.2%) for schistosomiasis was similar to the overall prevalence for all urines collected (n=970). Only a proportion of 81.0% (n=853) of the total participants in the study gave stool


samples and about a quarter of these were infected with each of the STHs (*T. trichiura* and *A. lumbricoides* respectively). No other helminths were found in the study.

There has been a decrease in the prevalence of STH infections since the 1998 Parasite Control Programme (PCP) study where *A. lumbricoides* and *T. trichiura* were reported to be 62.4% and 58.6% respectively whereas *S. haematobium* had increased from 14.9% in 1998 to 32.2% in 2010 (Kvalsvig et al., 2001). Temperature, dryness and UltraViolet (UV) light are the main factors that can influence death of helminth eggs (Maya et al., 2012). Ugu district has not been faced with drought in recent years but there has been an attempt to improve water and sanitation facilities at household level which may have contributed towards a reduction in the prevalence of helminth infections (Ugu District Municipality, 2011).

Cases of high STH prevalence have been reported in South Africa for decades. An *A. lumbricoides* prevalence of 70.0% was noted among children in Cape Town attending the outpatients department of the Red Cross War Memorial Children’s Hospital (Louw, 1966), while a survey of inpatients at King Edward VIII Hospital in Durban yielded 80.0% positive for helminths (Adeloye, 1987), and a survey among Cape school children in the Tygerberg area showed a prevalence of 96.0% (Burger, 1968).

Also studies conducted in other parts of the province of KwaZulu-Natal in the past have reported higher prevalences than those the current study found. A study done in northern KwaZulu-Natal school pupils found a prevalence of *S. haematobium*
infection of 68.0% (Saathoff et al., 2004b), and 59.8% pupils were infected with *T. trichiura* and a lower 22.0% were found to be infected with *A. lumbricoides* (Saathoff et al., 2004a). In another study that investigated the prevalence of ascaris and other helminths in children attending a rural Natal hospital and its referring clinic, 38.0% and 22.0% were found to be positive for *A. lumbricoides* and *T. trichiura* respectively (Bradley and Buch, 1994). Whilst these findings from the past studies show us that the problem of helminths has been present for some time in the province of KwaZulu Natal, they also show us that *S. haematobium*, *A. lumbricoides* and *T. trichiura* are co-endemic in many regions of the province.

### 5.2 Effects of Helminths

Haematuria (blood in urine) was the most popularly known symptom of *S. haematobium* infection as where ever we visited to raise awareness, pupils’ exclusively associated red urine with *S. haematobium* infection. Some pupils also reported dysuria (pain when urinating) as one other common symptom of bilharzia. Few pupils were aware of other symptoms associated with bilharzia or STHs in general. Schistosomiasis in children can result in anaemia, stunting and reduced ability to learn (World Health Organisation, 2002a) hence our study focussed on school aged pupils to identify and assist in solving the problem.

These problems can be reversed by administering recommended treatment (Praziquantel) in correct dose (40mg/kg), however if untreated, the damages caused cannot be reversed even though egg deposition can be reduced significantly. Urogenital schistosomiasis can result in bladder and ureter, and kidney damages which are often diagnosed at later stages of infection. Bladder cancer is another possible late-stage complication. In woman, urogenital schistosomiasis may lead to
genital lesions, vaginal bleeding and pain during sexual intercourse (Wright et al., 1982, Kjetland et al., 2006a).

Males also do suffer immensely from infection if they are not treated. Urogenital schistosomiasis infection can induce pathology of seminal vesicles, prostate and of other organs and may have long-term irreversible effects consequences, including infertility (Center for Disease Control and Prevention, 1994).

5.3 Infection Reduction

In coastal Kenya, age stratified analysis (less than 12 years) showed that overall children in the older (12-20 years) age groups had less infection than those in the younger (5-11 years) age groups, although at the outset of the program in 1984, the older children had the higher prevalence (71.0%) compared with the younger (63.0%) (Satayathum et al., 2006). Older children may have a lower risk for infection because of an acquired immunity that is not found in younger children, as was suggested by earlier studies on immune response and reinfection (Sturrock et al., 1983, Hagan et al., 1987, Etard et al., 1995, Wolmarans et al., 2004, Hemson, 2007, Appleton and Maden, 2012). Satayathum et al, in the findings published in 2006 showed that sex-specific prevalence of infection pattern over years was not constant as they found over period of nine years of study, indicating that a number of factors may influence sex-specific prevalence (Satayathum et al., 2006).

Our study found a reduction of more than half for both prevalences of A. lumbricoides and T. trichiura in pupils aged 10-12 years since 1998. Also the overall prevalences for these two helminths, for all pupils investigated (ages 5-14 years) in
1998 was more than twice what we found in our study. Only prevalence of *S. haematobium* was found to have increased in comparison to the PCP findings from 1998 for both group of pupils aged 10-12 years and for all pupils aged 5-17 years. However, the differences in *S. haematobium* prevalences were not as high as those found in STHs. Such changes in infection prevalence may not be attributable only to improvements in water and sanitation but to the combinations of improvements in factors that spur increase of these infections such as health education and public awareness of importance of hygienic behaviour.

A study that examined the combinations of water, sanitation and chemotherapy in Iran reported a decrease in Ascaris infections by 79% and egg counts by 88% and these were greater than the reductions for the group with only water and sanitation which had a 28% and 60% reduction respectively (Arfaa et al., 1977). Both investigated groups in our study and PCP study only received treatment the following year and the results post chemotherapy are not documented in this report. No mass treatment for schistosomiasis or STHs took place in Ugu during the period of between the PCP study in 1998 and our study in 2010.

Other studies have found that improved water supplies and chemotherapy produced a greater reduction in the prevalence of schistosomiasis than that resulting from provision of water supplies alone (Pitchford, 1970, Negron-Aponte and Jobin, 1979, Jordan et al., 1982, Mason et al., 1986). Strategies that adopt combinations of various approaches have repeatedly been successful (Pitchford, 1970, Negron-Aponte and Jobin, 1979, Tameim et al., 1985) and the provision of water supplies to
those who have been treated with drugs can prevent reinfection (Jordan et al., 1982).

### 5.4 Intensity of helminth infections
The majority of cases found in our study were light infections. These findings are in agreement with the findings from the PCP study which reported overall light and moderate intensity of infections (Kvalsvig et al., 2001). The PCP study was done in the same area but reported a mean egg count of (22.5, SD 57.2 EPG) eggs/10ml urine for *S. haematobium*, which was similar to that found in our study (16.7, SD 56.4 EPG).

Pupils with helminth dual co-infections comprised almost the third of the pupils in our study and had moderate to heavy infections. Those with *S. haematobium* had heavy intensity of infections in both the PCP and our study. This indicates that pupils with co-infections of either *T. trichiura* or *A. lumbricoides* are suffering from heavy worm burdens which may, if unattended, lead to morbidity. Co-infections are common in endemic areas and Bradley and Buch also reported cases of *Ascaris*, *Trichuris* and *Taenia* co-infections in earlier studies conducted in the province (Bradley and Buch, 1994).

Helminth eggs can survive 10–12 months in the soil upon excretion in tropical climate conditions (Larsen, 1999, Sanguinetti et al., 2005, World Health Organisation, 2006) with *Ascaris* eggs being the most resistant (World Health Organization, 2006). This characteristic of helminths ova may enable rise of co-
endemicity through introduction of new infections whilst old infections have not yet been eradicated.

Amongst all the 18 schools included in the study, at least two of the three targeted helminths were found in pupils attending each school. These findings confirm that these areas are endemic for all three targeted helminths.

5.5 Schools Included in the Study

The national teachers unions’ strike affected the field work of our study in the last five schools resulting in a low participation of pupils from those schools. Thus this is the reason all the last five schools had overall low sample size except for one school (Q) as our team got disrupted in these schools. There is a possibility that the findings from these schools may be an under-representation of the true extent of their pupils’ helminth infection prevalence.

All randomly selected schools were located at an altitude of below 300m and were near rivers, the furthest being 1.7km. The altitude was selected based on the studies by Appleton and colleagues who showed that in KwaZulu-Natal, the prevalence of helminth infections decreased with increasing altitude (Appleton et al., 1999, Moodley et al., 1999). In our study, a pattern was observed which showed that schools furthest from rivers had lower prevalences for all three heminth infections and that those that were closer to rivers had higher prevalences.

Few pupils, 5.8% (59/1023), admitted that they cross the river daily as opposed to 68.8% (704/1023) that never crossed rivers but this was significantly associated with
S. haematobium infection. However, a group working in Kenya did not find frequent water contact to be a significant indicator of schistosomiasis infection amongst pupils aged less than 12 years (Satayathum et al., 2006). Factors that increase risk of infection may vary and be complex and these include poor living conditions such as a lack of clean water, inadequate sanitation and resource allocation and external environmental factors such as suitable climatic conditions like temperature extremes, droughts, and flood events (Moodley et al., 1999). It is commonly known that constant contact with fresh water bodies increases chances of infection by S. heamatobium hence schistosomiasis is often referred to as a water body contact disease. Whether schools that are closest to rivers in our study are at an increased risk of infection with schistosomiasis is an issue that will have to be critically explored in future surveys.

Our teams had to visit participating schools on at least five consecutive days in an effort to get all three required urines and one stool sample from all the participants. School visits were not easy as most of the schools are located in rural areas where there is poor infrastructure, especially roads, and some pupils have to cross shallow rivers to get to school. When raining these rivers overflow and become risky to cross and as a result many of the schools had a very low turn out during rainy days and also the reason we were unable to collect the required number of urine samples from the participants. Absenteeism in schools was high during rainy days and also on Fridays.

Fridays in South Africa are short school days. Schools close at 13:00 instead of the normal 14:30 making it impossible for teams to reach as many pupils on Fridays. We
had four full days (Monday to Thursday) and one half day (Friday) to collect samples in the schools. Prevalence rates for all three helminth infections between schools varied but not significantly. *S. haematobium* transmission occurs mainly during the hot and humid summer (Saathoff et al., 2004b) and has a pre-patent period, infection to egg-excretion by host, of about eight to ten weeks (Sturrock, 1986). Urine samples were collected in summer but this is also the time of the year of increased rainfall in KwaZulu-Natal (Appleton et al., 1999).

Findings from a study done in northern KwaZulu-Natal reported that *S. haematobium* prevalence from boys slowly increased from 60.0% in the youngest to 79.0% in the oldest age group whereas the youngest girls had a much lower prevalence (37.0%) but the increase with age was steeper (Saathoff et al., 2004b). However, another study in Ugu District found no statistically significant gender differences (Taylor et al., 2001). Also a Kenyan study on pupils less than 12 years did not find any significant difference in schistosomiasis infection between boys and girls (Satayathum et al., 2006). Our findings however may be an underestimation of the real prevalence of helminth infections in school age pupils in general (both boys and girls) as our focus was on females from a narrow age group but it presents a reflection of the helminth infection status in Ugu district.

Whilst other studies have found that school children present the highest prevalence and intensity of *S. haematobium* infection (Saathoff et al., 2004b), Schutte et al reported differences in prevalences of between 55% and 92% in the same area (Schutte et al., 1981), but this was many years ago and since 1994 efforts have been made to improve the local living conditions.
5.6 Water and Sanitation

The phrase “improved access to water and sanitation” refers to water sources that are protected, safe and clean and sanitation that is safe, healthy and clean, and both services should be provided by the municipality (Department of Water Affairs, 2013). Such water sources include a household water connection, borehole, protected well, protected spring, or rainwater collection. Improved sanitation includes public sewer or septic system or the use of ventilated pit latrines (Department of Water Affairs, 2013). More than two thirds of the population in Ugu District have access to safe piped water, communal stand pipe or mobile tankers (mainly when there are water shortages).

As of 2006, about 42.0% of the population in Sub-Saharan Africa was without improved water, and about 64.0% is without improved sanitation in the region (Montgomery and Elimelech, 2007).

In South Africa, the population without access to improved water has decreased from 19.7% in 1996 to 8.8% in 2011, but in KwaZulu-Natal alone, 14.1% of population lacks access to improved water (Statistics South Africa, 2011). In order to assess the development that has taken place over the years in Ugu District we were able to interview the community ward councillors from communities surrounding the investigated schools and they provided information from their perspective as community leaders, and reported that the overwhelming majority in their communities had access to improved water.
Water and sanitation information from the community ward councillors and that collected from our study also concurred with the 2012 annual report from the survey conducted by the Ugu municipality which showed that 76.0% and 65.0% of the households within the community respectively have VIPs and access water within 200m (Ugu District Municipality, 2011). In most countries the proportion of population with access to improved water increased from 1990 to 2002 (World Health Organisation and UNICEF, 2004). WHO has declared 2005-2015 the decade of water, with the goal of establishing a framework to eventually provide full access to improved water supply and sanitation for all people (Montgomery and Elimelech, 2007) in an effort to bring global attention and resources to the problem.

Studies on improvements in water and sanitation done in other countries in previous years have showed that water and sanitation does impact prevalences in helminth infections (Mason et al., 1986, Lima e Costa et al., 1987). School children who lived in communal lands without piped water supply had prevalences of *S. mansoni* and *S. haematobium* of 4.8% and 4.4% whereas pupils who lived on the same lands but with piped water had prevalence 0.8% and 0.4% for both *S. mansoni* and *S. haematobium*, respectively (Mason et al., 1986).

Another study in Brazil found that children aged 5-14 years were 7.3 times more likely to have splenomegaly (an indication of severe schistosomiasis) if they had no piped water in their home (Lima e Costa et al., 1987). Over a seven year period, children under 14 years of age in small North East community in Brazil showed a reduction in the prevalence of schistosomiasis, and this was 77% greater in the treatment than in the control villages (Barbosa et al., 1971).
Statistics of the South African population without improved sanitation access have declined from 13.6% in 2001 to 5.2% in 2011 and the number of the population with flush toilets connected to the sewerage system increased from 49.1% to 57.0%, flushing toilets with septic tanks increasing from 2.8% to 3.1%, and pit toilets with ventilation (VIP) from 5.7% to 8.8% from 2001 to 2011 (Statistics South Africa, 2011). These overall reports indicate that there has been an improvement in the areas of water (as discussed previously) and sanitation throughout the country even though such success for the latter is much less compared to that of water provision.

Sanitation remains a challenge for some communities in Ugu, and according to the councillors’ reports this is due to improper maintenance of VIPs by households, for example, that cannot afford to buy toilet paper due to the cost. The use of newspaper instead of toilet paper then leads to pits filling up fast as they do not readily decompose fast but take time to decompose. This may also be the reason fourteen of the fifteen councillors reported a backlog in the provision of VIPs as some households pits are already full/almost full and will require new VIPs whilst there are some households that are still waiting to get their first VIPs and have been waiting for years.

According to the national South African policies, citizens in rural areas must have access to clean water no more than 200m away from where they live (Department of Water Affairs, 2013). Ugu District Municipality provides its population with communal standpipes for water, in order to improve access but there is not always sufficient water to meet the community’s requirements (Ugu District Municipality, 2011).
5.7 Water Contact (Risk Behaviour)

Knowledge of the prevalence of infection as well as age groups at risk and areas that are worst affected within the population, is key for adequate patient management and for guiding the design, implementation and monitoring of community-based infectious disease control programs (Katz et al., 1972, Peeling et al., 2006). Our study found that children from households using standpipes or communal standpipes had a higher risk of contacting *S. haematobium*, (83.7% were positive), than children from households with indoor tap water (12.4%). These findings were supported by what other studies have found, there was a reduction of 37% Ascaris prevalence for a group that had lavatories and indoor plumbing and 12% for those with lavatatories and a yard well (Henry, 1981). Another study found that the presence of piped-water in the home (Jordan et al., 1982, Lima e Costa et al., 1987) was associated with larger reductions in the prevalence of schistosomiasis (12.4% in Zimbabwe) than that produced by community water supplies (Mason et al., 1986).

Valuable time has to be reserved for collection of water from nearby communal standpipes due to the demand. In areas not well serviced with improved water, collection of water may represent an additional burden as up to six hours each day may be spent in search of water to meet household needs (World Health Organisation and UNICEF, 2005). Due to the time spent fetching water for all household activities, some families end up not collecting enough. In south-east Brazil, children aged 5-14 years were 2.3 times more likely to be infected if they had no piped water in their home (Lima e Costa et al., 1987). Children may be inclined to use unsafe water rather than queue.
Our study found that few (less than a quarter) of the pupils admitted that they practise water contact risk behaviours regarding unsafe water contact. Risk behaviours are water contact related activities that may increase the chances of getting infected with *S. haematobium*. Activities such as swimming in the river/dam, playing/swimming in the river, washing/bathing in the river, laundry, washing blankets and collecting water were all listed since they are still being commonly practised in these communities. Earlier studies in the same area found that recreational activities accounted for most of the water contact and unlike household related water contacts, showed strong seasonal variation (Kvalsvig and Schutte, 1986). There are no water related recreational facilities such as swimming pools in rural areas in Ugu; there is only one in Gamalakhe, an urban township.

It has been found in other studies in Saint Lucia and south-east Brazil that access to improved water supplies that included laundry and shower facilities was associated with reduced contact with infected waters and thus reduced infection (Jordan et al., 1982, Lima e Costa et al., 1987). From the pupils’ interviews it appeared that the problem of bilharzia was not new in the area (Table 14). Pupils’ knowledge of bilharzia was suprisingly high compared to their age which may indicate that they may have learnt about bilharzia through cases of friends or relatives who have had bilharzia. However, a number of pupils themselves had been infected including those who reported bilharzia before. The majority of pupils do not have both their parents living with them and adequate supervision and proper guidance may be lacking at home as indicated by almost half reported untreated bilharzia cases.
The findings from our study show that only prevalence of *S. haematobium* had increased but the STHs' prevalence had decreased since the last time a similar study was done in Ugu district. This reduction in STHs' prevalence may be due to improved water and sanitation infrastructure conditions which have occurred over the years. Also a change of lifestyle within communities from subsistence farming to relying on processed goods for consumption as seen in communities may have contributed to this reduction over the years. Other water contact from that reported in this report, even though infrequent, may still increase chances of *S. haematobium* infection.

5.8 Limitations of the Study

1. Access to participating schools after a heavy rainfall was very difficult. Roads were slippery and vehicle access was difficult. Due to the cost factor, the vehicles hired for use in this study were not four wheel drive vehicles. We had to postpone visiting some schools during rainy days as it would have been almost impossible for us to reach them since roads leading to them were in such a bad state. Fortunately, there were not so many schools that had impossible roads to drive on when wet, so we were able to still work in most schools during wet days, but children often did not come because of the weather and this affected the collection of three consecutive days urine samples.

2. There was a noticeably high absenteeism rate in schools during wet and in some instances, cold days. A number of reasons were cited for this high rate of absenteeism, one being that some pupils have to cross slow moving
shallow waters to get to school and when it rains such water bodies fill up with water and overflow making it impossible to cross.

Some pupils have only one school shirt which has to be washed daily after school and when it is raining, the shirt cannot dry in time for the following school day making it difficult for pupils to attend school. This made it difficult for our team to collect all the information and samples needed in the three days planned, resulting in our team having to visit schools for more than one week, to collect as many samples as possible.

3. Risk behaviour activities that increase pupils’ chances of getting infected with STHs as well as a history of STHs’ infection in pupils were not collected in this study as much as those for S. haematobium. Risk behavioural information from the previous PCP study was useful in predicting the events that may be considered as risk for getting infected with STHs.

4. Towards the end of 2010 there was a teachers’ strike that lasted for a couple of weeks towards the end of the academic year. This strike meant that the interaction we had with schools was very limited and when the strike was over, teachers were making up for the lost time and also preparing for exams. This led to the last five schools we visited having a low number of participants, as noted in our findings.

5. It was later realised that the questionnaire should have included a questions relating to the sanitation/toilet present at home, the type of toilet and also if
there is tap water at home. This kind of information would have been helpful in comparing our findings to findings from the PCP study and being able to clearly delineate if there has been a change in the provision of such infrastructure.

6. Community ward councillors were interviewed about the development in the communities around the schools included in the study, especially with regards to water and sanitation. However since they are in political structures, their replies may have been biased. We were however able to compare their responses with reports from the annual documents released by Ugu municipality and these supported their responses.

7. We were not able to assess the extent of development in the area due to lack of documents in the Ugu District detailing the precise number of VIPs and standpipes installed per annum. From documents viewed at the archive room of Ugu Water and Sanitation department, most were financial documents detailing the financial breakdown, and none had detailed figures of the number of VIPs and standpipes erected per household in a month or year. This then made it difficult for us to be able to say how much has been done in the area.

8. Our study focussed on eighteen randomly selected schools that were clustered in the Hibiscus Coast Municipality, few were in UMzumbe and no schools were included from the other four municipalities (Mdoni, Vulamehlo, Ezingolweni and Mziwabantu) within Ugu were included. This then made it
difficult to compare accurately the findings from our study with those from the PCP study (1998) which sampled forty schools from across all six municipalities in Ugu. Only two schools from the PCP study were also included in our study, the rest were different schools selected using random numbers.

9. Only female pupils aged 10-12 years were included in our study as compared to both boys and girls from ages 5 to 17 years in the PCP study. This study was nested within a bigger study on Female Genital Schistosomiasis (FGS) and female pupils were the only participants in the whole study. Previous studies have reported that ages 9-13 are the most vulnerable and most likely to contract helminth infections. Our study focused on this group, the most vulnerable age group making it possible to determine the prevalence of children in Ugu schools situated at less than 300m altitude. Although only females were included in the study, we are able to draw conclusions for both boys and girls using evidence from findings from previous studies that have found that both boys and girls are infected similarly with helminths (Taylor et al., 2001).

10. Water and sanitation conditions were not investigated formally at the participating schools but we were able to observe the type of sanitation and water facilities at each school and were able to incorporate such information in our findings.
11. Pupils were neither asked about their knowledge of soil transmitted helminths nor about a history of soil transmitted helminths or whether they had been previously treated for it, which prevented us from establishing if there had been any reinfection.
Chapter Six: Conclusion and Recommendations

6.1 Conclusion

After describing demographic, socio-economic indicators, the current situation and conditions of water and sanitation supplies in the area, as well as the prevalence and intensity of schistosomiasis and soil transmitted helminths amongst pupils in schools investigated, we were able to assess the extent of the problem of helminths in the area and also the helminth types present in the area:

- This study enabled us to determine the helminth species (*Ascaris lumbricoides, Trichiurus trichiura* and *Schistosomiasis haematobium*) present in areas investigated and we were able to note a change in helminth species present in Ugu district, in comparison to the species present in the district the last time a similar study (PCP study conducted between 1998-2000) was conducted.

- Through interviews with community ward councillors from communities surrounding the investigated schools, we were able to learn of the developments that have happened over the past five years and such developments have been happening since the early 1990s to varying degrees. Despite the increase in prevalence of *S. haematobium*, it was also noted that the number of households and families with improved access to water and sanitation has increased. We were also able to determine that fewer pupils now practise water risk behaviour such as playing, doing laundry and washing in rivers, as compared to the past. This may indicate that there
are other factors that may be attributed to this increase other than inadequate safe water infrastructure.

- The level of knowledge of bilharzia amongst pupils was assessed and we discovered that more than half knew what bilharzia is and 18.3% that denied having bilharzia tested positive for bilharzia. Despite these findings, the *S. haematobium* (32.2%) was higher then that the PCP study found (14.9%).

- We were able to conclude that access to water and sanitation has improved in comparison to the past. The prevalence and intensity of all three helminths has decreased. We were unable to attribute such a reduction in the problem of helminths solely to improved access to water and sanitation as there was general improvement in infrastructure despite an increase in population sizes. However it was noted that in UMzumbe Municipality, which has some challenges of water and sanitation had high prevalence and intensity of STHs in comparison to Hibiscus Coast Municipality which had less challenges, hence water and sanitation may be cited as important factors in explaining this trend. Therefore it is possible that a combination of factors may have contributed to this reduction in the problem of helminths in Ugu when compared to findings from the PCP study.

- Although there has been a reduction in the prevalence of helminth infections, nearly a third of the samples were infected with schistosomiasis and a quarter with soil transmitted infections emphasising the need for further measures to reduce these infections.
6.2 Recommendations

Improvements in water and sanitation can be expected to also improve other aspects of health other than reducing the prevalence or incidence of helminth infections. When infection rates are reduced by anthelminthic treatment, water and sanitation facilities prevent infection rates from increasing again to pretreatment levels.

The following recommendations are made:

- To achieve maximum health impact, greater focus is needed in ensuring installation of safe hygienic toilets and safe water for personal and domestic hygiene in communities;

- Proper sanitation facilities should be installed at the same time as water facilities to significantly effect optimum reduction of water-faecal related infections;

- Access to water supply should be as close as possible to each house in order to reduce the temptation of using unsafe water sources and maximise hygiene practices;

- School and health programmes should emphasize hygiene education to encourage personal hygiene. Pupils should also be taught early at schools about infections/diseases that are endemic in their communities and how such diseases can be prevented from spreading;
• Programmes from the DoH, DoE and Environmental Affairs should work together to raise awareness about parasitic infections and practices that minimise the risks of infection; thereby reducing new cases of infection.

• Mass treatment programmes to control helminth infections (soil transmitted helminths and schistosomiasis) are required based on WHO guidelines which advocate targetting school-age pupils. Regular mass treatment using single dose praziquantel for schistosomiasis and albendazole for STHs have few side-effects and reduce the prevalence and intensity of helminth infections (WHO, 1998).
Appendix 1: Pictures of water and toilet facilities in schools from the 2010 study

Figure 9: Pupils VIP toilet in one of the schools

Figure 10: Inside the VIP toilet, plastic seat with cap

Figure 11: Closed cemented pits of VIP toilet in one of the schools
Appendix 2: Photos of field laboratory used to analyse samples in the 2010 study

Figure 12: Table top set for examination of helminth eggs under light microscope

Figure 13: Trained research assistants examining urine slide for detection of helminth eggs

Figure 14: Study PI, Sipho Zulu preparing test tubes for preservation of urines
Appendix 3: Ethics’ Approval Letter for FGS Study

01 March 2011

Prof. M Taylor
Department of Public Health Medicine
Nelson R Mandela School of Medicine
University of KwaZulu-Natal


RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 20 February 2011
Expiration of Ethical Approval: 19 February 2012

I wish to advise you that your application for Recertification dated 01 December 2010 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The approval will be ratified by a full sitting of the Committee at a meeting to be held on 12 April 2011.

Yours sincerely

[Signature]
Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics
Appendix 4: Ethics’ Provisional Approval Letter for Masters Study

21 June 2012

Mr. SG Zulu
Department of Public Health,
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Mr Zulu

PROTOCOL: Has improved water and sanitation changed the prevalence of schistosomiasis and soil transmitted helminths (STH) amongst female primary school aged children in Ugu District of KwaZulu-Natal, South Africa. REF: BE005/12

PROVISIONAL APPROVAL

A sub-committee of the Biomedical Research Ethics Committee has considered your application received on 12 January 2012.

The study is given PROVISIONAL APPROVAL pending a response to the following:

1. Permissions from school authorities are required.
2. Informed Consent: Most components have been included but the submitted Informed Consent Forms refer to the umbrella study Ref: BF029/07, not specifically to the current proposed study.
3. How is the researcher going to separate Demographic Data from the rest of the Questionnaire because it looks like one document.
4. Principal investigator’s details are not on the information document. Please include your details.
5. There were concerns about the researchers Parent study which were published in the local newspaper. How will these be addressed with the community?

Only when full ethical approval is given, may the study begin. Full ethics approval has not been given at this stage.

PLEASE NOTE: Provisional approval is valid for 6 months only - should we not hear from you during this time - the study will be closed and reapplication will need to be made.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHPR) Federal-wide Assurance (FWA 678).

Yours sincerely,

Mrs A Marinuthu
Senior Administrator: Biomedical Research Ethics
Appendix 5: Ethics’ Approval Letter for Masters Study

29 November 2012

Mr. SG Zulu
Department of Public Health,
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Mr Zulu

PROTOCOL: Has improved water and sanitation changed the prevalence of schistosomiasis and soil transmitted helminths (STH) amongst female primary school aged children in Ugu District of KwaZulu- Natal, South Africa. REF: BED05/12

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 12 January 2012.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 17 July 2012 to queries raised on 12th June 2012 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 29 November 2012.

This approval is valid for one year from 29 November 2012. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.


BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee’s decision will be RATIFIED by a full Committee at its next meeting taking place on 11 December 2012.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

[Signature]

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

[Address]

[Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx]
Appendix 6: Informed Consent - English

REDUCING ISICHENENE – FEMALE BILHARZIA PROJECT
Information and request for participation in a research project - guardian

Dear parents and guardians
Thank you very much for your cooperation last time when we were working with your child. We would like to follow her up.

The University of KwaZulu-Natal and the University of Oslo in Norway are doing research on Bilharzia to find out how Bilharzia affects girls
- to investigate if treatment works better in the young
- to investigate if treatment of Bilharzia can protect girls from other diseases

Why your girl child is being asked to participate
The treatment has been tried in adult women, but it did not work properly. We know that treatment kills the parasite Bilharzia. We know that it works best for urinary diseases in the young. Now we wish to test it in children.
We ask your girl
- because she may have had contact with the Bilharzia water
- because she is still young
- because we hope to protect her from the disease
Approximately 4000 girls and young women are invited

Her rights
If you agree to participate you have a right to access all personal information we have registered about your daughter. She can correct all faulty information. She may at any time withdraw and then she may request material/information to be destroyed.
Consequences for your daughter

Participation in this study will mean that
- she will give urine, stool and have blood tests, and an interview will be done
- she will receive the best known treatment
- she will be invited annually, maybe for 10 years
- If we discover special problems, she will be treated or referred as necessary

Benefits

We strongly recommend that all girls who have ever been in contact with water should be treated every year even if their urine is clear. This is because damages can be unnoticed or may occur later. Participants will be checked and treated every year as recommended by the World Health Organization. Her Bilharzia will die and she will feel healthier. She will receive treatment for other diseases we may discover.

Risks

She may feel sick but not serious, vomit, get rash and have diarrhea when she takes the tablets for Bilharzia. This can last for a couple of days. If she gets many dead worms she may feel very sick.

How her tests and personal information are taken care of

The information that we collect in this research will give new information about Bilharzia in girls and we will share this information with doctors and nurses who need this. However no one will ever know about your daughter’s personal information. Tests and interview will be analyzed and stored without your daughters name on it. Anything with your daughter’s name is stored in another place. The samples will be tested in the best laboratories in the world without your daughter’s name. If you agree to let your daughter participate in the study, you also give permission for this. The information will only be used to study Bilharzia together with girls and women’s diseases and risk for infection for up to 20 years. It will not be used for other things. The principal researchers are formally responsible that everything is kept safely. They have access to the address file.
Who gave permission to the project
The project has been given permission from the KwaZulu-Natal Department of Health and ethical committees in both South Africa and Norway.

Economy
The study is financed by research money from overseas and from South Africa. There are no plans for working together with industry or earning money. The researchers involved in the study have no personal financial gain in this project.

You decide
Participation in the study is entirely voluntary. You may ask any questions and we will try to answer. You do not have to give a reason if you do not wish your girl to participate. Her treatment now or in the future will not be affected by the decision. She may also interrupt the participation if you or if she wishes.

More information
Please feel free to contact the project manager: Prof M. Taylor 031 260 4499 or 031 266 1592. Other responsible contacts: Ms Pumla Mkhiva: 0765508220 Dr J. D. Kvalsvig 031 209 3735, The Department of Public Health medicine at the University of KwaZulu–Natal, Dr Eyrun Kjetland, Department of Infectious Diseases, University of Oslo, Norway. The Medical Research Administration: phone 031 260 4405; fax number: 031 260 4410; email address: ethicsmed@nu.ac.za

Yours sincerely
Myra Taylor PhD and Dr E. Kjetland
REDUCING ISICHENENE – FEMALE BILHARZIA PROJECT

Request for participation in a research project – parent/guardian

Participation in the study is based on voluntary, informed consent. You are free to ask any additional information. If you, after having received all the information you deem necessary wish for your daughter to participate in the study, you must sign this consent form.

I, ________________________________, the guardian/parent (name of parent/guardian in capital letters)

agree □ don’t agree □

If you agree please furnish with

Your child’s date of birth ________________________________

National ID number: ________________________________ (if you have)

Contact number: ________________________________

School: ________________________________ Grade: _____ Section: _____

of ________________________________, confirm that I have received written information about the study and have had the opportunity to ask additional information, and that I will let my daughter / protégée participate in the project.

Signature ________________________________ Date ________________________________

(Signed by guardian/parent) (Dated by the guardian/parent)

If it happens that you move away from this area please give us the name and the contact number of a person you’ll inform so that we’ll also know: ________________________________

__________________________________________

__________________________________________
Appendix 7: Informed Consent - Zulu

UKUNCIPHISA ISICHENENE – UHLELO LWABESIFAZANE LWESICHENENE
Ulwazi nesi cele sokuba ubambe iqhaza ocwaningweni – mzali/mbheki.

 Bazali nababheki

Isikungo sezempundo esiphakene saseUniversity yakwaZulu, Natal ne-University yaseNorway i-Oslo benza ucwaningo ngischenene
- ukuthola ukuthi isicheneni siwaphatha kanjani amantombazane
- ukuphanya ukuthi ukwelashwa kusebenza kangocon kwabancane
- ukuphanya ukuthi ukwelashwa kwesichenene kungabavikela yini kwenzine izifo

Kungani intombazane yakho icelwa ukuba ibambe iqhaza
Sicela intombazane yakho
- ngoba kungase kube ithintene namanzi anesichenene.
- ngoba isencane
- ngoba sinethemba lokuyikela esifani

Cishe amantombazane angu 4000 kanye nabesifazane abasebancane bayamenya

Amalungelo akho
Uma uVuva ukubamba iqhaza ucalo lujungle lokuba ubone lonke ulwazi esilibhalile ngendodakazi yakho. Angalungisa nomia yiluphi ulwazi oluyiputhu ngaye. Angaboza nomia yingasiphi isikhathi futhi angacela lonke ulwazi lushatshaliyawo
Okulandelayo ngendodakazi yakho
Ukubamba iqhaza ocwaningweni kusho ukuthi
- uzosisika umchamo, indle kanye negazi
- uzothola ukwelashwa okungcono kakhulu
- uzomenywa njalo ngonyaka, mblawumbe iminyaka ewu 10

Izinzuza:

Ingcuphe:
Angase azizwe egula kodwa okungatheni, aphalaze abenkugubuka abanje sise kusinde, uma ephuza amaphilisi esichenene. Lokhu kungaba okwezinskwana, Uma ethola izikelemu eziningi azifile angaziwazi egula kakhulu.

Nguwe onqumayo
Ukubamba iqhaza kungkuzinkelka okupheleleyo. Awunweke wani sakutha uma ungafisi intombazane yakho ukuthi ibambe iqhaza. Ukuphathwa kwakhe manje, nasesikhathini osizayo angeke kuthikamezwe yisingumo sakho, Uma ethanda, angakungamula/misa ukubamba iqhaza kwakhe.

Ulwazi olungaphelivu
Siza uzizwe ukuhulatele ukuthinti umphathi wocwaningi uDr M Taylor kulenombolo 031 2604499, nomz 2661592. Omunye ongathinta: Dr JD Kvalsvig kulenombolo 031 2604499, The Department of Community Health at the University of Kwa-Zulu Natal. Dr Eyrun Kjetland, Department of Infectious diseases, University of Oslo, Norway. The Medical Research Administration: phone 031 2604769, fax: 031 2604609; email: BREC@ukzn.ac.za
UKUNCIPHISA ISICHENENE – UHLELO LWABESIFAZANE LWESICHENENE

Isicelo sokuba ubambe iqhaza ocwanningweni – mzali/mlheki

Waziswe ngocwanningo u ________________________________

Ukubamba iqhaza ocwanningweni kungukuzinikela ngomyume. Ukhululekile ukuba ucela,
ofunye ulwazi olungezeleleleki. Uma emva kokuthola lolwazi ucalanga ukuthi
kunesidilile ukuba indodakazi yakho ibambe iqhaza ocwanningweni ungasayina lelifum
lemyume.

Mina, __________________________________________________________

Nama in capital letters)

Ngiyavuma  □ Angivumi  □

Umheki/mzali ka ____________________________________________________

Uma ukuva scele usiphe

Usuku lokuzalwa lwangane yakho:

Inombolo yocingo __________________________ Grade: □ Section: □

Ngiyisisekisa ukuthi ngilubhile ulwazi ngocwanningo nguba nthuba lokubuza ngolwazi
olungezeleleleki.

Ukusayina ____________________________ Usuku ____________________

(Signed by guardian/parent) (Dated by guardian/parent)

Uma kwenzeke nisuka endaweni scele igama nenombolo yocingo yalowo,
eniyokumazisa ukuze sazi. ____________________________________________

______________________________________________________________
Appendix 8: Questionnaire

REDDUCING BILHARZIA PROJECT

NB: Please use a tick where there are pre-coded responses
Name of Interviewer: ______________________ Date: ________

A. Personal data page
1. Isibongo / Surname(s) ____________________________
   Nickname/first name(s) ____________________________
2. Um Ptya / Age (years) ________
3. Wazalwa nini? / When were you born? _________
4. WaZalwa mphaphi? / Where were you born? ________
   School: ____________________________ Area: ________
   Grade: ________ Section: ________
5. Ubani igama likathisha wakho kulonyaka? / What is the name of your class teacher this year?

6. Uhlala kuphi isikhathi esiningi? (Ikhelela isicatho) / Where do you live most of the time? (Physical address)

7. Ujwayele ukulala kanganakanani lapha? sonke isikhathi / How often do you sleep here? (All the time/Most of the time)
   □ inxenye yesikhathi □ /
   Landline number ____________________________
8. Ikhele leposi / Postal address
   a. Inombolo kamakhalekhwini / Cell phone number
      ____________________________
   b. Inombolo yocingo lwasekhaya / Landline number ____________________________
9. Kungani ubelapha isikhathi esiningi? / Why are you here most of the time?
   a. Ubani okunakekelayo lapha? (Igama) / Who is looking after you here? (Name)
      b. Igama lombhekxi / Name of a guardian ________ sex M/F □ □
      c. Ubuhlolo umama / Umalume/umngani / Omunye (chaza) / Relation (mother
         /uncle/friend/other (specify) ____________________________
      d. Ngubani oyinhiyo yekhaya? / Who is the head of the household? ________
      e. Ngubani ongumninimizu? / Who owns the house? ____________________________

10. Ingabe ikhona enye indawo ohlala kuyo ngezimpelasonto, amaholide nomz
    ezinye izinsuku? yebo cha / Uma kungu cha 11/ /Is there another place where you
    stay on weekends, holidays or other days? (Yes, no) (IF NO = 11)
    a. Ujwayele ukulala kanganakanani lapha? ngezinye zeziinsuku zesikole
       □ ngezimpelasonto □ /amaholide □ /Okunye (chaza) /How often do you sleep there?
       (some school days, weekends, holidays, other-explain) ____________________________

Page 1 of 7
b. *Ikheli lapho ublala khona* / Physical address

|__________________________________________|

|__________________________________________|

c. *Ikhelileposi* / Postal address

|__________________________________________|

|__________________________________________|

d. *Inombolo kamakhalekhukhwinini* / Cell phone number

|__________________________________________|

|__________________________________________|

e. *Inombolo yocingo lwasekhaya* / Landline number

|__________________________________________|

|__________________________________________|

f. *Kungani ublala lapha ngesiniye isikhathi?* / Why do you stay here sometimes?

|__________________________________________|

|__________________________________________|

g. *Ubani okunakekelayo lapha? (igama)* / Who is looking after you here? (name)

|__________________________________________|

|__________________________________________|

h. *Ubuhlobo umama* / umalume / umngani / etc. / Relation (mother/uncle/friend/etc.

|__________________________________________|

|__________________________________________|

i. *Ngubani oyinhloko yekhaya? (ubuhlobo)* / Who is the head of the household? (Relationship)

|__________________________________________|

|__________________________________________|

j. *Ngubani ongumninimuzi?* / Who owns the house?

|__________________________________________|

|__________________________________________|

**11. Unaso esinye isihlobo esisondele kuwe esihlala kwenergy indlu? yebo □ cha □**

|__________________________________________|

|__________________________________________|

a. *Ubani igama?* / What is the name

|__________________________________________|

|__________________________________________|

b. *Ubuhlobo umama* / umalume / etc / Relation (mother/uncle/etc)

|__________________________________________|

|__________________________________________|

c. *Ingabe ublala endaweni efanayo njengayakho yebo □ cha □* / Does he/she live in the same area as you? (Yes, no)

|__________________________________________|

|__________________________________________|

d. *Ikheli lapho ublala khona* / Physical address

|__________________________________________|

|__________________________________________|

e. *Ikhelileposi* / Postal address

|__________________________________________|

|__________________________________________|

f. *Inombolo kamakhalekhukhwinini* / Cell phone number

|__________________________________________|

|__________________________________________|

g. *Inombolo yocingo lwasekhaya* / Landline number

|__________________________________________|

|__________________________________________|

**12. Uma ungase ube nohambo nomal usuke kulendawo, ubani ongaba,nemininingwane yakho, yokukuthinta (ngaphandle kwalena engenhla)? If you were to travel or move away, who would have your contact details (other than the above)?**

|__________________________________________|

|__________________________________________|

a. *Igama / Name*

|__________________________________________|

|__________________________________________|

b. *Ubuhlobo umama* / umalume / etc / Relation (mother/uncle/etc)

|__________________________________________|

|__________________________________________|

c. *Ikheli lapho abhala khona* / Physical address

|__________________________________________|

|__________________________________________|

d. *Ikhelileposi* / Postal address

|__________________________________________|

|__________________________________________|

e. *Inombolo kamakhalekhukhwinini* / Cell phone number

|__________________________________________|

|__________________________________________|

f. *Inombolo yocingo lwasekhaya* / Landline number

|__________________________________________|

|__________________________________________|

Page 2 of 7
13a. Ubani osayine ifomu lakho lemvume? (ubuhlobo, igama) / Who signed your consent form? (relation, name) 

b. Kungani kunguyena? / Why this person? __________________________________________

/You haven’t yet mentioned your biological mother and/or biological father.

a. Ingabe umama wakho usaphila? Yeho [ ] cha [ ] angazi [ ] / Is your mother still alive? (Yes, no, DK)

b. Ingabe ubaba wakho usaphila? Yeho [ ] cha [ ] angazi [ ] / Is your father still alive? (Yes, no, DK)

Uma kungu CHA: Ngiyadabuka ukuzwa ukuthi umama noma ubaba wakho usaphila. Ngizokubuza ngomzali usaphila. I’m sorry to hear that your mother or father has passed away. I am going to ask you about the parent who is alive.

c. Uma kungu CHA kubonobhili abazali yiya kufif/ If No for both parents go to 15.

<table>
<thead>
<tr>
<th>14d.</th>
<th>Igama likamama okuzalayo / Biological mother</th>
<th>Igama likababa okuzalayo / Biological father</th>
</tr>
</thead>
<tbody>
<tr>
<td>i)</td>
<td>Unako ukuthintana nomama/ubaba wakho?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Do you have contact with your mother/father?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>yebo [ ] cha [ ] akwenzeki [ ] yes [ ] no [ ] NA</td>
<td>yebo [ ] cha [ ] akwenzeki [ ] yes [ ] no [ ] NA</td>
</tr>
<tr>
<td>ii)</td>
<td>Ingabe umama/ubaba wakho ubhala eduza kwala ubhala khona?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Does your mother/father live near you?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>yebo [ ] cha [ ] akwenzeki [ ] NA</td>
<td>yebo [ ] cha [ ] akwenzeki [ ] NA</td>
</tr>
<tr>
<td>iii)</td>
<td>Ingabe yilaphi zinga lefundo esemthethweni likamama/baba wakho?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>What is your mother/father’s top education?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ayikho imfundo esemthethweni [ ]</td>
<td>Ayikho imfundo esemthethweni [ ]</td>
</tr>
<tr>
<td></td>
<td>No formal education [ ]</td>
<td>No formal education [ ]</td>
</tr>
<tr>
<td></td>
<td>Primary school [ ]</td>
<td>Primary school [ ]</td>
</tr>
<tr>
<td></td>
<td>Impundo ephezulu [ ]</td>
<td>Impundo ephezulu [ ]</td>
</tr>
<tr>
<td></td>
<td>High school [ ]</td>
<td>High school [ ]</td>
</tr>
<tr>
<td></td>
<td>Impundo ephekeme [ ]</td>
<td>Impundo ephekeme [ ]</td>
</tr>
<tr>
<td></td>
<td>Tertiary [ ]</td>
<td>Tertiary [ ]</td>
</tr>
<tr>
<td></td>
<td>Angazi Don’t know [ ]</td>
<td>Angazi Don’t know [ ]</td>
</tr>
<tr>
<td>iv)</td>
<td>Ubani igama likamama/baba wakho?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>What is your mother/father’s name?</td>
<td></td>
</tr>
<tr>
<td>v)</td>
<td>Lithini ikheli likamama/baba wakho?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>What is your mother/father’s address?</td>
<td></td>
</tr>
</tbody>
</table>

| e. | Ingabe ubaba wakho uhlayla nawe ekhaya? Yeho [ ] cha [ ] Uma kungu CHA → 15a | Does your father live with you at home? (Yes, no) (If no → 15a) |
| f. | Ingabe ubaba wakho uyakusiza ngomsebenzi wakho wesikole uma udinga usizo? Yeho [ ] cha [ ] | Does your father help you with schoolwork if you need help? (Yes, no) |
| g. | Ingabe ubaba wakho uke akujazeni ngokwenza okungalungile? Yeho [ ] cha [ ] | Does your father ever punish you for doing wrong? (Yes, no) |
| h. | Ingabe lokho kuyakuvimba/gwema ukuthi ungakwenzi futhi? Yeho [ ] cha [ ] | Does that stop you from doing it again? (Yes, no) |
| i. | Ungasho uthi ubaba wakho unomthetho oqinile? Yeho [ ] cha [ ] |                                            |
Questionnaire

Reducing Bilharzia Project

1. Would you say that your father is too strict? (Yes, no)
2. Is he stricter than your friends' fathers? (Yes, no, DK)
3. Does your mother live with you at home? (Yes, no) (If no → 16a)
4. Does your mother help you with schoolwork if you need help? (Yes, no)
5. Does your mother ever punish you for doing wrong? (Yes, no)
6. Does she stop you from doing it again? (Yes, no)
7. Would you say that your mother is too strict? (Yes, no)
8. Is she stricter than your friends' caregivers? (Yes, no, DK)

16a. Who is your main caregiver?

M = mother  F = father  B = brother  S = sister
Gm = grandmother  Gf = grandfather  U = uncle  A = aunt
C = cousin  Sf = step-father  Sm = step-mother  Sb = step-brother
N = none  O = other (please list)

16b. Does she/he help you with schoolwork if you need help? (Yes, no)
16c. Does she/he ever punish you for doing wrong? (Yes, no)
16d. Does she/he stop you from doing it again? (Yes, no)
16e. Would you say she/he is too strict? (Yes, no)
16f. Is she stricter than your friends' caregivers? (Yes, no, DK)

B. Family and living

1. How long have you lived here? (years)
2. Have you ever lived in a city? (Yes, no, DK)
3. How old were you when you lived in the city? (DK)
4. For how long did you live there? (Less than 1 year, 1-5 years, more than 5 years)
5. Qala ngomdala kunaboboke endini? [Start with the oldest in the household.]

<table>
<thead>
<tr>
<th>Ubani obhla kulendu yakini? Who lives in your house?</th>
<th>Isilungisa seminyaka Approximate age</th>
<th>Umsebenzi Yebo, cha, Work (Yes/No)</th>
<th>Umfundzi (Yes/No)</th>
<th>Izinga lemfundo eliphazelulo (Ayiako/Ephansi/Ephenu/Ephakeme) Top education (0=None/1=Primary/2=High/3=Tertiary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>M = mother</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>F = father</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>B = brother</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>S = sister</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Gm = grandmother</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Gf = grandfather</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>U = uncle</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>A = aunt</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>C = cousin</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Sf = Step-father</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Sm = Step-mother</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Ss = Step-sister</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Sb = Step-brother</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>O = other (friend, housekeeper etc)</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>CC = Our Child</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No = nephew</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>N = niece</td>
</tr>
</tbody>
</table>

6. Ngubani omandla okunuhlizenkeleniphani ekhaya? (igama nobunyolo) /Who is the main provider in the household? (name, and relation)

---

C. Ukuthinta amanzi / Water contact

Manja ngizokubaza imibuzo ngakuthinta amanzi.
Now I will ask you some questions about water contact.

1. Ilwatholani amanzi okuphuzza? emfuleni ☐ empopnpi/whomphakathi ☐ esiphethwini esivikelelele ☐ esiphethwini esingakilekile ☐ kuzubena empopnpi ongaphakathi ☐ Where do you get drinking water? (taps, standpipe, communal standpipe, protected spring, unprotected spring, river, indoor tap)

2. Ingabe amantombazane ekilasini lakho ayabhukuda emfuleni noma edamini ezinsukwini ezishisayo? yebo ☐ cha ☐ angazi ☐ / Do girls in your class swim in the river or camp on hot days? (Yes, no, D/K)

3. Ingabe umngane wakhlo omkhulu uyakwenza lokho? yebo ☐ cha ☐ angazi ☐ / Does your best friend do this? (Yes, no, D/K)

4. Uyakwenza wena? yebo ☐ cha ☐ / Do you do this? (Yes, no)
5. **Manje ngizokubuza ngezinhlobo zezinto ozenzayo ngamanzi, nizenza kanganakanani, isikhathi eside kanganakanani osihlala emanzini nokuthi uuthintana kanganakanani umzimba wako namanzi?** /Now I will ask you what kind of water activity you do, how often you do them, for how long you stay in the water and how much of your body that is in contact with the water.

<table>
<thead>
<tr>
<th>Umfula/river</th>
<th>Kangaki? How often?</th>
<th>Umosa kanganakanani emanzini?</th>
<th>Umzimba uwathinta kanganakanani emanzini agakakhathi weza leziinto?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amazi amile</td>
<td>Kuvucvela (3)</td>
<td>Kuvucvela (3)</td>
<td>Nsongenzi (3)</td>
</tr>
<tr>
<td>Amazi avela</td>
<td>Kwesinye isikhathi (2)</td>
<td>Kwesinye isikhathi (2)</td>
<td>Kwesinye isikhathi (2)</td>
</tr>
<tr>
<td>Kulukuqebuka</td>
<td>Kuthukelaqabuka (1)</td>
<td>Kuthukelaqabuka (1)</td>
<td>Kuthukelaqabuka (1)</td>
</tr>
<tr>
<td>Ngabe (0)</td>
<td>Rarely (1)</td>
<td>Rarely (1)</td>
<td>Rarely (1)</td>
</tr>
<tr>
<td>Never (0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Uyayala /</th>
<th>Uyabukuda? Do you play / swim?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uyakthaza /</td>
<td>Uyakeza Do you wash / bathe?</td>
</tr>
<tr>
<td>Uyakthaza</td>
<td></td>
</tr>
<tr>
<td>Uyakthaza</td>
<td>Uyakthaza zokulala? Do you wash blankets?</td>
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<tr>
<td>Uyakthaza</td>
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<td>Uyakthaza</td>
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<td>Uyakthaza</td>
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Page 6 of 7
**F. Ezempilo / Health**

Imibuzo elandelayo ingezempilo yakho. The next questions are about your health.

1. Ingabe uyazi siyini isichenenene? yebo [ ] cha [ ] anginasiquniseko [ ] Do you know what Bilharzia is? (Yes, no, unsure)

   *Isichenene yisifo ongasithola ngokuthina amanzi angcicolile.* Bilharzia is an infection you can get through contact with infected water.

2. Ingabe kukhona emndenini onaso nom a owake wabanesichenene yebo [ ] cha [ ] Angazi [ ] I has anyone in your family ever had Bilharzia?

3. Wake waba naso isichenenene? yebo [ ] cha [ ] angazi [ ] / Have you ever had Bilharzia? (Yes, no, D/K)

   *Uma kungu yebo:* If yes:

   4a. Wake walsheba isichenene phambilini? yebo [ ] cha [ ] / Have you ever been treated for Bilharzia before? (Yes, no)

   *Uma kungu CHA yiya ku Q5* / If No go to Q5

   *Uma kungu YEBo yiya ku 4b* / If Yes go to 4b.

4b. Walsheba nini isichenene (iminyaka) [ ] [ ] [ ] [ ] [ ] [ ] 1st time 2nd time 3rd time / When were you treated for Bilharzia (age)...

**Emantombazaneni isitho sangasase sinezimbobo/gudu ezintathu. Imibuzo elandelayo igxile ikakhulu embotsheni yesibili ebizwa ngokuthi yinkomo (isitho sangasase sowenisifazan).** In girls the private parts consist of three openings. The next questions focus mostly on the second opening, called the vagina.

<p>| | | |</p>
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<tbody>
<tr>
<td>6</td>
<td>Wake waba nayo inkinga noma yiphi ngokuchama njenge: Have you ever had any problems with urination like:</td>
<td>Esontweni eselule: This last week</td>
</tr>
<tr>
<td>6a</td>
<td>Zinhlengu uchama / Pain when you urinate</td>
<td></td>
</tr>
<tr>
<td>6c</td>
<td>Iconisi lomchamo uma ukguma, ukhohlela nom a uhleka / Drop of urine if you jump, cough or laugh</td>
<td></td>
</tr>
<tr>
<td>6d</td>
<td>Umchamo obomvu / Red urine</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 9: Permission Letter to Use PCP dataset

To Whom it May Concern,

I hereby confirm that the student Mr Siphozenkosig Gift Zulu, student number 204511397 who is registered for a Masters in Medical Sciences with School of Public Health Medicine at University of KwaZulu-Natal had the permission to use the data and information collected from the Parasite Control Program (PCP) study which was done in Ugu District, KwaZulu-Natal between 1998 and 2000.

Kin Regards,

[Signature]

Dr. Jane Kvalsvig (Principal Investigator for PCP Study)
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