UNIVERSITY OF THE WITWATERSRAND

FACULTY OF HEALTH SCIENCES

SCHOOL OF PUBLIC HEALTH

RESEARCH REPORT

TITLE:

SPATIAL ANALYSIS OF *SCHISTOSOMA HAEMATOBIUM* INFECTION AMONG SCHOOL CHILDREN IN A RURAL SUB-DISTRICT OF SOUTH AFRICA: AN APPLICATION OF GEOGRAPHICAL INFORMATION SYSTEMS (2009)

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Research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg in partial fulfillment of the requirements for the Degree of Master of Science in Medicine in the field of Population Based Field Epidemiology

Johannesburg, South Africa 2009.

DECLARATION

I, Azongo K. Daniel declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in the field of Population Based Field Epidemiology in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature:

Full Name: Azongo Kwaku Daniel

1st day of May 2009

Dedication:

I dedicate this work to my Dad Mr Frank Azongo Atugba for his steadfast love and care.

Abstracts

Background

Assessing risk of schistosomiasis requires knowledge of the spatial distribution of the disease and its association with demographic, socioeconomic, behavioural, and environmental factors over time and space. The objective of this study was to advance such knowledge by analyzing the spatial distribution of *schistosoma haematobium* infections in relation to the demographic attributes and environmental covariates of the Africa centre Demographic Surveillance Areas (DSA) in rural KwaZulu-Natal. The study also examined the association between household socio-economic conditions and rates of *S. haematobium* infection with particular emphasis on the impact of pipe water on rates of infection.

Methods

The study is a crosses sectional study, involving all 33 primary schools in Africa Centre DSA. 2110 grade five and six children took part in the study. Statistical analysis was done using chi square tests to compare statistical significant differences between sex and age groups. Bivariate and multivariate logistic regression models were used to explore factors that are significantly associated with infection. Spatial analysis was done to examine the spatial distribution of the disease using geographical information systems techniques. Microscopic analysis of the urine samples was done using the filtration technique.

Results

Of the 2110 school children who were screened for infection, 347 tested positive for the presence of

S. haematobium, representing an overall prevalence of 16.6%. Prevalence levels were higher in boys (20.8%) than females (8.5%) (P<0.001). 57.6% were heavily infected (eggs ≥50 eggs per 10ml urine) as compared to 42.5% who had light infection (eggs<50 eggs/10ml of urine). Whereas, prevalence was significantly age-dependent (Pearson chi2 (3) = 28.4184, P< 0.001), intensity of infection was not significantly age dependent (Pearson chi2 (3) = 3.2579, P<0.354). Altitudinal variation, access to portable water, toilet, and distance to water bodies were significantly associated with infection. Prevalence of infection was clustered around the Eastern part of the study area.

Conclusion

While there may be several factors associated with schistosoma infection in the study area's school children; age, sex, water contact behaviour, homestead altitude and distance to permanent water bodies, were the most significant risk factors explaining the spatial distribution of S. haematobium infection in the Africa Centre DSA. Selective Mass treatment of *S. haematobium* infection in 7 clustered areas is recommended for the control of the disease.

Acknowledgements

This research work was carried out in one of the well established Health and demographic research centres in the world, the Africa Centre for Health and Population Studies in KWaZulu-Natal, South Africa, in partnership with the University of Witwatersrand Johannesburg, South Africa. Many people have contributed in diver's ways to ensure the success of this work and I sincerely acknowledge their precious contributions.

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I would in particular like to thanks the Director of the Africa Centre, Professors Marie-Louise Newell for proposing the research topic and giving me access to data to carry out this project. I am highly indebted to my two supervisors, namely Professor Frank Tanser, of the Africa Centre who is the principal investigator of the schistosomiasis study and Edmore Marinda of the University of Witwatersrand Johannesburg. Both have contributed in designing this work and guiding me through the scientific thinking and writing, for which I have very much appreciated. Particularly, I wish to express my sincere gratitude to Prof. Tanser who in addition, has taught me basic GIS and taken me through the difficult but fascinating spatial analysis processes in this work. He has also given me the drive and the needed encouragement to finish this work. I very much appreciate his financial support during the later part of my stay on campus.

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ABREVATIONS AND ACRONYMS

ACIDS - Africa Centre Demographic and Information System

BS - Bounded structure

BSID - Bounded Structure Identification Number

CI - Confidence Interval

DALY - Disability-adjusted life year

DSA - Demographic Surveillance Area

DSS - Demographic Surveillance System

GBD - Global Burden of Disease

GIS - Geographic Information Systems

GPS - Geographic Position System

INDEPTH - International Network for Continuous Demographic Evaluation of Populations and Their impact on Health in Developing Countries

MASL- meters above sea level

NDVI - Normalized Difference Vegetation Index

PTA - Parent Teachers Association

PZQ - Praziquantel

RS - Remote Sensing

SQL- Structured Query Language

MDG - Millennium Development Goals

UNICEF – United Nation International Children and Educational Fund

WHO -World Health Organization

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CHAPTER ONE

INTRODUCTION

1.0 Background Information

Schistosomiasis causes widespread infection in Africa. A careful review of the epidemiological literature shows that the prevalence of schistosomiasis infections is still high in areas of seasonal transmission. In many areas, a higher proportion of school-age children bear the largest disease burden and share both the highest prevalence and intensity of infection [1]. Schistosomiasis is a disease of poverty that causes much suffering and death; in addition, the disease also has a debilitating effects on the cognitive performance and growth of children, and reducing the work capacity and productivity of adults, thereby perpetuating the cycle of poverty and suffering in poor populations across the globe [2]. The WHO report on the global burden of diseases (GBD) in 1990 indicates that Africa has the highest estimated disability adjusted life years (DALY) associated with Schistosomiasis in the World.

Schistosomiasis is also a rural disease mostly affecting people working in agriculture and freshwater activities [3]. High rates of morbidity and mortality are found in resources poor countries including Sub-Saharan Africa, Asia and Southern Americas, especially in Brazil. In Asia, China is the most affected country and in 2002, an estimated 90 million people were at risk of acquiring the disease and 820,000 were infected with the disease [4]. Brazil is the most endemic country in the Americas with 3 million estimated morbidity cases and over 25 million people at risk of infection.

Efforts to reduce the burden of the disease rely to a great extent on a clear understanding of its determinants and spatial distribution in the population. Findings from other epidemiologic investigations have showed that demographic, environmental and behavioural factors are associated with schistosomiasis infection. Human factors responsible for transmission include water contact activities and the uses of sanitation facilities [5, 6] Environmental determinants of infection include temperature, rainfall and altitude [7, 8] (indirectly through its relationship with temperature and rainfall).

Control and surveillance programmes aimed at controlling Schistosomiasis and other helminthes infections are difficult to implement in resources constrained settings partly due to lack of information on the clustering of the diseases in affected populations [9]. This problem poses serious challenges in meeting the World Health Assembly Resolution WHA54.19 adopted in 2001, aimed at attaining a minimum target of regular administration of chemotherapy to at least 75% of all school-age children who are at risk of morbidity by 2010 [1, 2].

The World Summit on Sustainable Development, held in Johannesburg in 2000, advocated governmental support for the provision of clean water and sanitation facilities. The aim was to halve, by 2015, the number of people without access to clean water and sanitation facilities in order to reduce the disease burden caused by Schistosomiasis and other helminthes infections [10]. This study intends to explore the spatial distribution, determinants and prevalence of *S. haematobium* infection in the Africa Centre Demographic Surveillance Area (DSA) and delineate areas of significant clustering of infections using Geographical Information Systems (GIS). Geographic information systems (GIS) and remote sensing (RS) provide useful tools for determining environmental factors related to the distribution of intermediate host snails of schistosomiasis, and thus mapping and prediction of disease transmission in endemic communities [11]. The application of such new technology in disease surveillance has contributed greatly in the planning of national control programmes and making effective utilization of health resources [12].

1.1 Statement of the problem

There is absence of a national research and control programme for human schistosomiasis in South Africa [13] resulting in a significant gap in our knowledge of the spatial distribution and focal clustering of the disease states of *S. haematobium*, especially among school-age school children. The lack of accurate data on the burden of the disease is hampering the scientific assessment and quantification of its impact of on public health. However, estimation of the prevalence of *S. haematobium* and the spatial distribution of populations infected with the disease are important components of the management and control of infectious diseases. A map of populations at risk of infection would guide health planning and at the same time help maximise the use of resources to control the disease. The government of South Africa is currently rolling out pipe water programmes to make portable water accessible to disadvantaged communities through out the country. It is important for such policies to be guided by the health states of communities.

1.2 Rationale of the study

Although, there is a broad understanding of the distribution of *Schistosoma haematobium* infection in KwaZulu-Natal province, much of the data is out of date and insufficient for greater understanding of the disease clusters. Investigating factors behind the distribution of the disease, may guide the Department of Health and the Department of Education in planning and implementing surveillance and other intervention programmes to control the disease.

Limited resources constitute a major problem facing most health care systems in sub-Saharan Africa. The allocation of these scarce resources is normally dependent on two important factors, the number of people living in a particular catchments' area and the disease state of the area. Therefore a clear delimitation of populations at greater risk of infection will help focus local resources on priority areas where control programmes should be targeted. The application of GIS plays an important role in formulating schistosomiasis control programmes, evaluating the impact of interventions in transmission over space and time and allocating resources for sustainable control [14].

Sustainable and effective control of the disease also requires a clear and precise understanding of the distribution of the disease according to environmental, geographical and epidemiological data. The application of GIS and spatial analysis present a powerful methodology for integrating these different data sets, suitable for mapping populations at risk of Schistosomiasis infection, and finding factors associated with transmission [15]. The design of Schistosomiasis control programmes in sub-Saharan Africa is often constrained by the lack of comprehensive survey data. According to Moodley et al. [13], methodological constraints and limited disease data make reaching an accurate estimation of the prevalence of Schistosomiasis infection in South Africa difficult.

Combining GIS and spatial analysis, this study examines the spatial distribution of *S. haematobium* infection, among grade five and six school children and characterized the prevalence and intensity of infections within the Africa Centre DSA of KwaZulu-Natal/South Africa. The results presented in this work would enable the Department of Health (DOH) and the Department of Education (DOE) to indentify Schistosomiasis high prevalence areas, thus utilizing fewer resources than they would have done using mass screening. By examining the impact of access to pipe water on rates of infection, the study would also guide the government in rolling out pipe water supply to deprive and vulnerable populations. The study also forms part of a wider research agenda at the Africa Centre that is examining rates of *S. haematobium* re-infection in the DSA with particular emphasis on the impact of access to pipe water on rates of infections.

1.3 LITERATURE REVIEW

1.3.1 *Schistosomiasis haematobium*

Schistosomiasis (also known as Bilharzia) is a disease caused by a blood born fluke (trematode) of the genus *Schistosoma* that live in human blood vessels around the bladder or intestine. There are two main types of schistosomiasis commonly found in Africa; these are the urinary type, caused by *Schistosoma haematobium*, and the intestinal type, caused by *Schistosoma mansoni*. *S. haematobium* is transmitted by snails of the species *Bulinus* and during their life cycle prefers to inhabit in permanent water bodies and wet lands.

The clinical effects of urinary schistosomiasis (caused by *Schistosoma haematobium*) “leads to blood in urine (haematuria) and painful urination (dysuria) as early symptoms whilst sever complications leads to calcification of the bladder wall, bladder stones, bladder carcinoma, hydronephrosis and kidney failure [16]. Studies have shown that genital schistosomiasis affects the reproductive health of men and women and are suggestive of it being a risk factor for the spread of HIV infections [17].

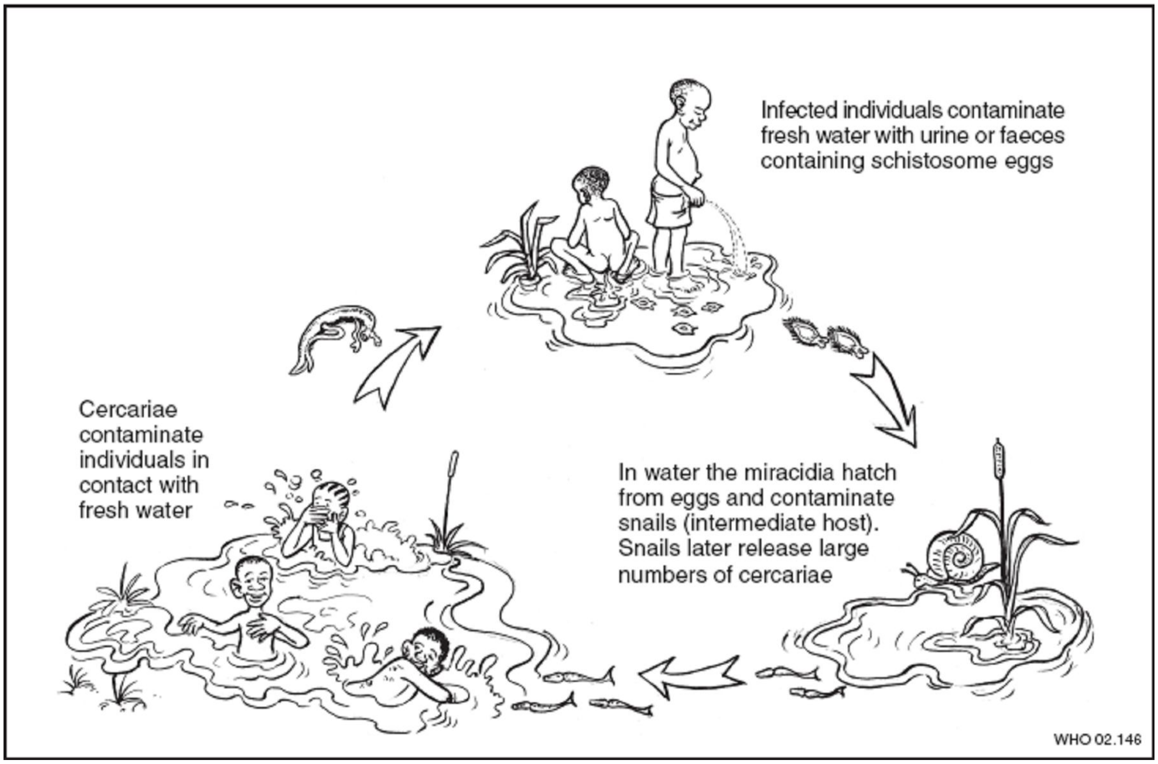

Figure 1.1 Life circle of schistosomiasis: Adopted from the WHO 2002 report, helminth control in school-age Children: A guide for managers of control programmes

A review of the relevant literature indicates that the disease constitutes a major public health problem in sub-Saharan African. The WHO report on Schistosomiasis [1] indicated that an estimated 200 million people are symptomatic and more than 600 million people were living in areas where Schistosomiasis is endemic. The burden of the disease is high in sub-Saharan Africa, where an estimated 85% of all cases are concentrated. For instance, recent estimates suggest that, about 436 million people live at risk of infection with *S. haematobium*, and 393 million people are at risk of *S. mansoni*. It has also been estimated that, the annual mortality rate due to schistosoma might well exceed 200, 000 deaths [1, 18]. The global (DALYs) caused by Helminth infections including schistosomiasis are large, ranking first among school-age school children. It is estimated that, the morbidity burden due to schistosomiasis in sub-Saharan Africa is 3.5 million DALYs [2]

Schistosomiasis affects mostly school-age children who have daily contact with (contaminated) water for recreational and domestic activities. Children between the ages of 5 and 14 years are mostly at risk of infection and predominantly bear the morbidity burden. [19]. Studies show that, schistosomiasis and other helminth infections pose both physical and intellectual challenges to infected individuals. In school children heavy infections could result in anemia due to nutritional deficiency, absenteeism and higher dropout rates in schools and generally retard child growth and development [19]. Neglecting the disease could retards achievements made in child survival programmes and will continue to yield a generation of school-age children incapacitated by this debilitating disease that compromises economic growth and development [20].

A study conducted in Mapumtaland in Northern KWaZulu-Natal to examine the impact of praziquantel in a cohort of school children indicted that baseline prevalence was 65.9% and 70.3% in males and females respectively with a mean prevalence of 68.3% [21]. The study further indicates that, praziquantel was highly efficacious and resulted in a drastic reduction of the prevalence and intensity of infection after three weeks of treatment.

Identifying and treating pockets of individuals and communities at greater risk of infection will contribute greatly to the achievements of some of the Millennium Development Goals (MDG), including achieving universal primary education, reducing child mortality and in the long term help in eradicating hunger and poverty [1].

Historical studies in South Africa using climatic data and population census have attempted to estimate the prevalence of schistosomiasis infection in school-age children and the number living at risk of infection. In 2000, it was estimated that, between three to four million school children were at risk of infection [13]. Some climatic and environmental factors have been reported to favour snail breeding, and the transmission of schistosomiasis. Most of the determinants are heterogeneously distributed, while others change with time and space. Temperature and habitat stability [22] are found to be the most predominant factors responsible for the distribution of host snails in sub-tropical South Africa. Temperature and rainfall are the most significant climatic factors that contribute to the occurrence of urinary schistosomiasis. For instance an increase in the summer and winter minimum and maximum temperatures predicted a decrease in the probability of *S. haematobium* occurring in a particular geographic area [22]. However, there still exist wide variations in the prevalence of infections in zones with similar climatic conditions, because of differences in seasonal transmissions and other socio-economic factors, especially in rural and agricultural areas [15, 23, 24]. Migrants infected with schistosoma can also introduce the parasite to uninfected communities. For instance a study conducted in north-eastern KwaZulu-Natal reported that, infected migrants with intestinal Schistosomiasis were potential carriers and agents of infection and therefore pose a serious threat to public health in the province [25].

The distribution of *S. haematobium* in South Africa (based on temperature suitability) stretches from the north-western provinces, through Gauteng, Limpopo and Mpumalanga and southward to KwaZulu-Natal and the East Cape [5] Bilharzia Atlas of South Africa. However, the distribution of *S. haematobium* is more pronounced than that of *S. mansoni,* with a recorded prevalence of above 70%, especially through Limpopo, Mpumalanga and KwaZulu-Natal [22]. It has been observed that, while such ecological studies, integrating climatic and demographic data are sufficient to explain factor suitable for infection and to estimate populations at risk of infection, they are subjective [11], and do not present detailed information on the spatial distribution of infection and the worm burden of the disease for a particular action to be taken. It is important that, the mapping of high-risk areas based on climatic conditions be supported by epidemiological evidence in the form of disease data. This project work seek to fill this void, by integrating different data sets from environmental, demographic, behavioural, socio-economic and disease characteristics, to explore the spatial distribution of schistosomiasis infection in a rural South Africa population. This study is unique in the sense that, it is one of its kinds evaluating the impact of access to piped water on rates *of S. haematobium* infections in the region. The study also presents a detailed examination of environmental and household socio-economic factors that could influence the risk of schistosoma infection. For instance, household socio-economic status, and school children water contact pattern has been reported to correlate with schistosoma infection [15, 19, 24].

1.3.2 Geographical Information Systems (GIS) and Schistosomiasis

There have been recent advances in the application of spatial analysis and mapping in epidemiological studies by public health administrators to enhance their planning, analysis and monitoring of disease [26]. GIS and spatial analysis are valuable research tools used in strengthening the whole process of epidemiological research information management and planning. Functionally, GIS is applicable in "data management (acquisition, storage and maintenance), analysis (statistical and spatial modelling) and display (graphics, and mapping) of geographic data" [27]. GIS is therefore useful in integrating different data sets (environmental, parasitological and socio-demographic) to present a complex inter-relationship of health information. In Africa, GIS technology has considerable application for public health research and planning and plays a pivotal role in explaining the micro-geographical distribution of diseases [28]. Writing on the application of geographic information systems for risk screening and epidemiology, Wartenberg, D [29] reported that, the application of GIS could make screening programmes more efficient and cost-effective, by mapping high-incidence neighbourhoods that require screening and treatment.

GIS is becoming more relevant in Demographic Surveillance System (DSS) across the developing world, and have been extensively used to plan and execute fieldwork [30]. It is expected that, this new trend will support the overwhelming interest and significant commitment by international organization to control both soil-transmitted helminths and schistosomiasis in Africa. For instance the WHO/UNICEF joint programme on Health Mapping (HealthMap) has developed a user-friendly mapping interface and database management system that is currently being applied in the West Africa helminths control programme [31]. GIS has been used in complex analyses of satellite images to determine the association between parasitic and vector-borne diseases to environmental factors (temperature, climate, altitude, rivers etc) [15, 32, 33]. It has also been widely applied in understanding the spatial distribution and as a tool to enhance planning and implantation of schistosomiasis control programmes [34, 35]. In Uganda the application of GIS in public health research has resulted in the selective mapping of focal clustering of schistosoma prevalence exceeding 50% (WHO recommended threshold for mass treatment), in areas within 5km of Lakes Victoria and Albert, making control programmes highly successful and cost-effective [36].

In China GIS has been successfully applied in the planning and control of schistosomiasis, which was highly endemic in 12 provinces, and of public health importance [37]. The government of China, with technical and financial support from the World Bank lunched a national control
Programme to control both transmission site and individuals infected with the disease. Using GIS and remote sensing technologies to map exact transmission sites have resulted in a substantial reduction in the disease burden, in 5 of the 12 endemic provinces.

Historical studies in South Africa, using GIS have been descriptive in nature. However, from the epidemiological point of view, conducting an analytical study using location specific risk factors would probably hold more promise in quantifying the association between exposures and outcome. The application of GIS to identify high-risk communities for treatment is a vital component in the management and implementation of cost-effective schistosomiasis control programmes. It has a huge potential to provide evidence-based research, for estimating and predicting disease outcome and its association with socio-demographic factors. With the application of GIS, together with statistical techniques, this study has presented an explicit *S. haematobium* risks map and further identify environmental factors associated with the disease in rural KwaZulu-Natal.

1.4 Research question

What is the distribution *of S. haematobium* in grades five and six school children in the Africa Centre DSA, and what factors are associated with this distribution?

1.5 Hypotheses

1. The prevalence of *S. haematobium* infections is not homogenously distributed across the Africa Centre DSA.
2. Lack of access to pipe water is associated with high levels of *S. haematobium* infections among primary school children in the Africa Centre DSA.

1.6 **Aim of study**

The overall aim of this study was to explore the spatial distribution of *S. haematobium* infection and to investigate the demographic, environmental, behavioural, and socio-economic factors (particularly access to pipe water) associated with infection among grade five and six school children.

1.6.1 Specific Objectives:

1. To quantify the prevalence and intensity of *S. haematobium* infection among grade five and six

school children in the Africa Centre DSA 2007.

2. To investigate the determinants of *S. haematobium* infections and examine the impact of access to piped water on rates of infection.
3. To explore the spatial distribution of *S. haematobium* prevalence among grade five and six school children in the Africa Centre DSA.

CHAPTER TWO

MATERIALS AND METHODS

2.0 Demographic characteristics of the study area

The Africa Centre Demographic Surveillance Area (Figure 2.1) is situated in the Hlabisa district of Umkhanyakude in northern KwaZulu-Natal, about 250km north of the city of Durban. The district extends between latitudes 28.18° and 28.47°S and longitudes 31.97° and 32.38°E and covers an area of about 434 km^2. The land is flat to undulating, with altitudes ranging from 20-350m above sea level. The vegetation is typical of semi-arid savannah with vegetation ranging from sparse grassland to thick vegetation, natural reserves, and commercial farmlands. Hydrological features such as perennial rivers, streams, dams and ponds are spatially scattered across the landscape.

2.1 Africa Centre Demographic information systems

The Africa Centre Demographic Information System (ACDIS) collects demographic, socio-economic and behavioural data on the 85,000 individual's resident at one of the 11,000 homesteads every six months. Residential status of both resident and non-resident members of households are collected which makes it possible to distinguish between household members (self-defined on the basis of links to other household members) and a resident household member (residing at a physical structure within the surveillance area at a particular point in time).

The study area falls well within the endemic schistosomiasis belt and is designated as coastal lowlands (<300 MASL) (Figure 2.2) within which high prevalence of schistosomiasis occur [22]. The study area has been classified in to three distinct locations, with the rural areas occupying about 94.5%, peri-urban 5.2% and urban 0.2%. Provision of piped water and sanitation is increasing rapidly in the area (Figure 2.3) with 55% and 75% of households having access to piped water and toilets facilities respectively, in 2003/2004 [38] (about 15% increase in both amenities from 2001). By 2007, 90% of households ware projected to have access to piped water.

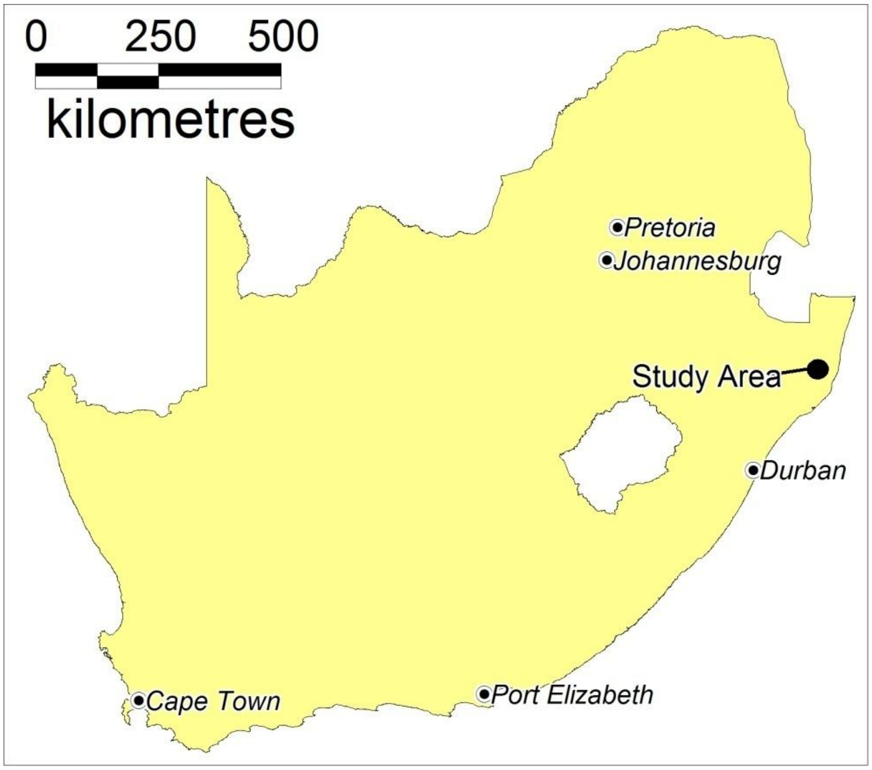

Figure 2.1 Map of South Africa showing the study area. (*The shaded area depicted the African Centre DSA*

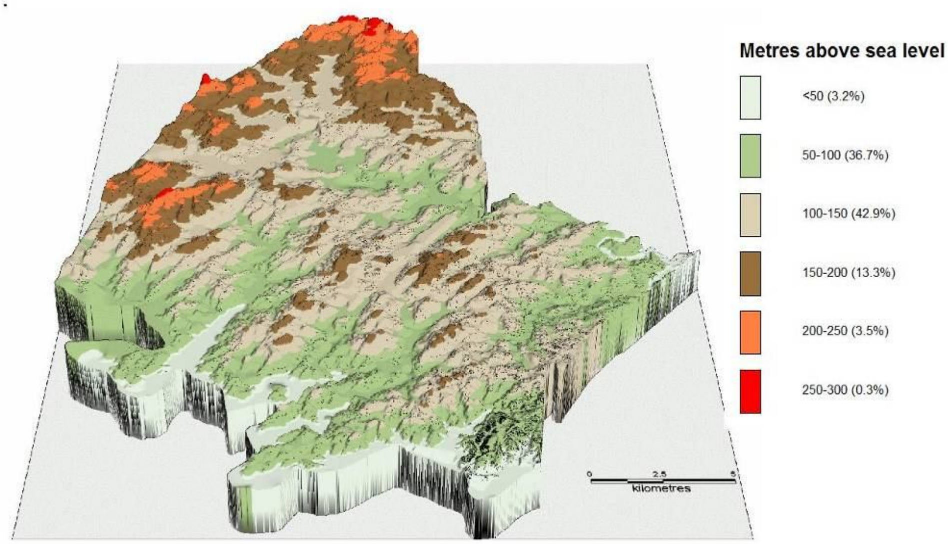

Figure 2.2 Altitudinal zones (and proportion of homesteads in each zone) in the Africa Centre DSA with all homesteads superimposed

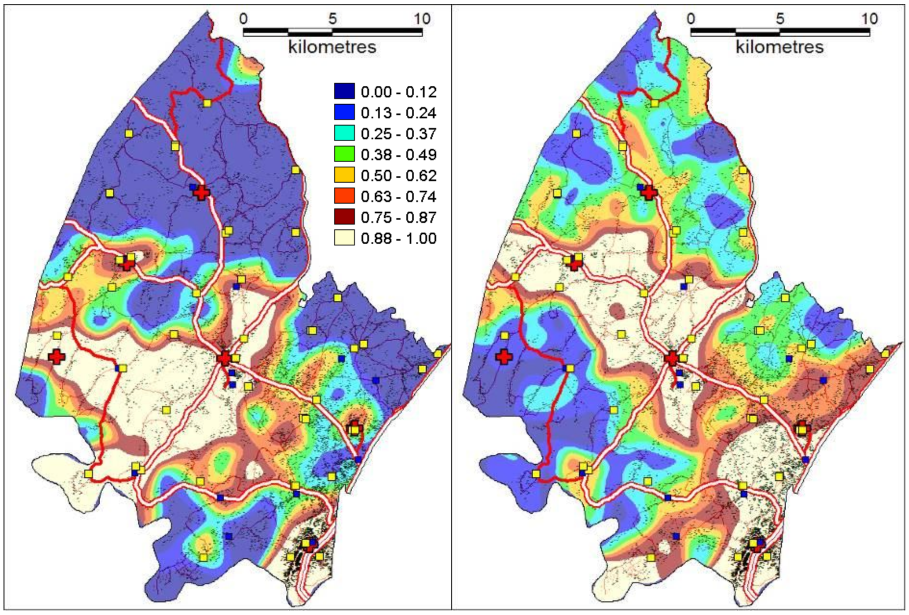

Figure 2.3 Proportion of homesteads having access to piped water (left) and toilets (right) with roads and homesteads superimposed. Yellow squares = primary schools, blue squares = secondary schools and red crosses = clinics. A 4km distance-weighted standard Gaussian filter to perform the analysis (F. Tanser, unpublished work)

2.2 Africa Centre Geographic information systems

Since its inception ACDIS has developed and maintained GIS capacity that allows the spatial analysis of any of the variables collected by the surveillance system. All 11,000 homesteads, schools and all health facilities in the study area have been geo-referenced using differential global positioning systems (Trimble Geoexplorer, Sunnyvale, CA) and a differential correction is applied using Pathfinder™ Office software, version (Trimble) to ensure a location error less than two meters (to an accuracy of < 2m). The ACDIS can provide important geo-referenced explanatory and confounding variables associated with schistosomiasis outcomes including basic demographics, household education levels, source of household water, presence of latrine and household expenditure [30]

All households, healthcare facilities, schools and streets, have been geo-referenced using hand-held GPS receivers (Trimble Geoexplorer, Sunnyvale, CA). A differential correction is applied using Pathfinder™ Office software, version 1.10 (Trimble) to ensure a location error of less than two meters. Each household's location is linked to the ACDIS data using MapInfo GIS software [30]. Homesteads are assigned unique bounded structured identification numbers (BSID) and given a bar-coded label for the purpose of the longitudinal survey. The exact locations of water bodies (including rivers, ponds, and pipes) and other natural reserves are digitized from 1:50 000 topo-cadastral maps.

2.3 Study population

The study is a cross-sectional study involving all grades five and six school children at all the 33 primary schools in the demographic surveillance area. All schoolchildren who were present on the day of testing were eligible for participation in the study. To be recruited into the study all the school children must consent and submit singed consent and assent forms.

2.4 Field investigations and laboratory procedures

The fieldwork started in the 2007 academic year. Screening and testing started in April 2007 and was postpone in May due to the nation wide strike action by teachers. The study resumes again in October 2007 and ended in November 2007. The study started with consultations with heads of participating schools and the parents and teachers association (PTA) of all the schools participating in the study. The purpose and methods of the survey was well explained and understood by both teachers and parents. Consent and assent forms ware distributed to schoolchildren to send to their parents for endorsement prior to the day of testing. All grade 5 and 6 pupils present on the day of testing were eligible for inclusion. Pupils who did not have both consent and assent forms were not eligible for testing. After receiving the written consent and assent from the study participants, they were given glasses of juice to drink and given a 500ml honey jar to provide urine samples between the hours of 10h00 and 14h00. The urine samples were labelled with unique bar code to correspond to the subject data sheet to link the results to individual data and improve on the handling of the samples. Two nurses did a rapid specimen test using urinalysis reagent strips (Bayer Uristix®) to test for the presence of micro haematuria and proteinuria in other to identify infected schoolchildren for treatment. Since haematuria is the most common visible symptom of urinary bilharzias, a positive reading for blood in the urine (haematuria) was an indication of infections. Testing for haematuria using the dipsticks take 1 minute and all pupils who tested positive ware treated with praziquantel. After the testing and treatment the specimens were then transported to the Africa Centre Laboratory for processing. Two fieldworkers were responsible for processing the samples under the direct supervision of a lab technician. The process involves mixing the urine well by inversion and then using a 10ml. syringe to draw up 10ml. of the specimen from the 500 honey jay into a 15 ml. conical test tube [39]. This was repeated in another conical test tube which was then label subscript A and B with the corresponding child identification number (ID). In other to prevent the schistosomiasis oval from hatching the two urine specimens were diluted (1 ml. to each 10 ml. urine sample) with a preservation solution, formalin Merhoilate concentration. All samples (irrespective of infection status) were transferred on a weekly basis from the Africa Centre laboratory to an experienced lab technician at the Biological sciences laboratory of the University of KwaZullu-Natal in Durban for microscopic analysis.

The microscopic analysis was done using the filtration technique as described by the world Health Organization [39]. To analyze the intensity of infection or egg count per specimens, two filtrations (of 10ml specimen each) were examined through paper filters with a pore size of one micrometer with all the filters stained with ninhydrin. All eggs on the slides were counted using microscopes with electric light illumination and the results were recorded as egg counts/10 ml of urine.

2.5 Treatment of study participants

After the testing and questionnaire interview (see section 2.6) all pupils who tested positive with haematuria were treated with a single oral dose of praziquantel (Biltricide®, Bayer) with reference to their weight (40mg per kg weight) and in according with current national Public health guidelines for treating schistosomiasis in South Africa and WHO standard protocols [2]. Treatment was administered and strictly supervised by the nurses. Weights of the children were also recorded to determine the drug dosage to administer to pupil who tested positive. False negative children were treated in a subsequent follow-up visit if microscopic analysis detected the presence of *S. haematobium* ova.

2.6 Questionnaire survey

A simple questionnaire was also administered to the school children after the testing and treatment. Two locally trained fieldworkers were recruited to administer the baseline questionnaire to the study participants. Items included on the questionnaire include school children baseline characteristics (i.e. names, age, sex, and homestead identification number). The school children were also asked about their water contact behaviour, main source of drinking water and type of toilet facilities used in households. In order to identify school children who have been treated for schistosomiasis, they were asked to recall if they have received any treated for schistosomiasis during the past six month preceding the current study.

2.7 Data management

The disease data and the baseline questionnaire were entered at the schools via a web-based interface linked to Structured Query Language (Microsoft SQL Server 2000). Data was thoroughly validation for inconsistencies on the field and uploaded to a central database upon return to the Africa Centre. An electronic copy of the microscopic data was sent to the Africa Centre and backed-up on the server. Altitude and permanent water bodies' variables were obtained from the Surveyor General at 1:50,000 scale and extracted using MapInfo. Distance to permanent water bodies was calculated using the distance tool in Mapinfo, which measured the Euclidean, distance between homestead and the nearest permanent water body in kilometres. Stat transfer version 8.0 was used to convert the GIS data to stata for the statistical analysis.

2.8 Socio-economic data

The Africa Centre collected information on household possession, expenditure and income annually as part of the demographic survey. Household assets were obtained for each individual by linking the baseline information (Bounded structure ID) to the ACDIS socio-economic database using Bonded Structure Identification Number (BSID). In a small number of instances (<10%), there was more than one household resident at a single Bounded structure (BS). In such circumstances, a household was selected at random and a number of assets from this household used in the calculation. This is unlikely to affect any result as multiple households at a single bounded structure share many assets including water and toilet, and thus wealth measures are likely to be similar. The household assets variables were weighed using principal component analysis [41]. In rural Africa, household assets indices are valid proxies for assessing wealth in health research [42]. Assets-based approach was used to stratify schoolchildren into five wealth quintiles as either belonging to the most poor, very poor, poor, and less poor or least poor on the assets index scale. These five categories of relative wealth were chosen because they have been found to capture wealth effects well in a number of previous studies in poor setting of South Africa [43, 44, 45]. The first principal component obtained in a principal component analysis on 27 household assets was used to form an assets index. The assets include items used for both consumption and production, such as beds, bicycles, tables, telephones, television sets, sewing machines, block makers, wheelbarrows, tractors, cattle, and livestock.

2.9 Statistical analysis

Data analysis was done in stata IC version 10.0 [46]. The outcome variable was infection status and is categorical (discrete) and binary. An infection with *S. haematobium* was defined as the presence of at least one egg in 10ml urine sample using the filtration method and the number of eggs counted in a

10ml sample of urine specimen was used to determine the intensity of infection. Samples with less than <50 egg per 10ml of urine specimen were categorized as light infected whiles those with more then ≥50 eggs per 10ml of urine specimen were categorized as heavy infection [2]. Participants were categorised into two groups; those who tested positive for *S. haematobium* as the cases and those who tested negative *S. haematobium* as the comparison group. A comparison of prevalence differences between sexes as well as between age groups was carried out using chi-square test.

Graphical representation of the distribution of the prevalence of infection among the age group were constructed using Microsoft excel, 2007. There were few missing data (9.81%) in the socio-economic data, this was because some households either migrated or were formed after the assets data were taken. Therefore a dummy variable (missing) was generated and included in the model. However, the results were not reported.

2.9.1 Test of Association

Study participants with complete parasitological data (i.e. two 10ml of urine samples) and questionnaire data were included in the final analysis. The school children ages ranged from 8 years to 19 years, consisting of the following age categories; 8-10 years, 11 yeas, 12 years, and 13-19 years. These age categories where considered based on the median age in the study population and for the purpose of quantifying the age specific morbidity in this study.

In this report, water collection pattern was used to analyze the frequency of water contact. The aim was to explore patterns of water contact to detect individuals at risk, and to set an indicator to identify children at risk of infection as a target group for treatment. I also explored the relationships between distance from homestead to permanent water bodies and altitude to construct a model that predicts higher risk transmission areas. The mean altitude is 115.99 and Std. Dev. 42.1842 meters above sea level (masl). Altitude was categorized into three levels, altitude <100 meters, altitude 100 – 150 meters and altitude >150 meters. This categorisation was found suitable for the analysis in this study. Distance from homestead to permanent water bodies rages from a minimum of 0.01 kilometres to a maximum of 9.93 kilometres. The distance variable was categorized into four groups; distance within 1 kilometre, within 2 kilometres, within 3 kilometres and above 3 kilometres. Similarly, there was no scientific base for choosing these categories.

School children who have access to piped water were coded as yes (1) and those without access to pipe water coded as no (0). Similarly, those with access to toilet facilities were coded as yes (1) and those without access to toilet facilities also were coded as no (0). These classifications were chosen to simplify the analysis in this report. To explore possible factors associated with the outcome variable (infection status) and the explanatory variables, a chi- square test at (0.05) statistical level of significance was chosen. The proportions of prevalence among the age groups and sex was tabulated and graphed.

2.9.2 Bivariate Logistic Regression Analysis

Bivariate logistic regression analysis were first fitted to examine the association between infection status and the explanatory variables; sex, age group, access to piped water, toilet, location of homestead, altitude, household socio-economic status, distance from homestead to permanent water bodies (categorized as 1 kilometres, 2 kilometres, 3 kilometres and above ≥3 kilometres were estimated).

Before fitting the bivariate models, the outcome variable was regenerated as a binary variable with 1 for a positive outcome (infected) and 0 for a negative outcome (not infected). Odds Ratios, together with 95% confidence intervals were calculated for all the bivariate logistic regression estimates.

2.9.3 Multivariate analysis

Multivariate logistic regression analysis was also fitted to determine factors associated with *S. haematobium* infection. Again the statistical significance of the covariates were assessed using the likelihood ratio test. Various multivariate models were first explored before arriving at a parsimonious model. Tests of interactions between sex and age group were done, in addition, correlation analysis were done for distance to permanent water bodies and water contact behaviour. The final model was assessed for a model fit for multicollinearity and the assumption of constant error variance. Again a P-value of 0.05 or less was considered statistically significant. The results were presented in a tabular form with the adjusted odd ratios, the p-values and the 95% confidence interval. Linktest and lfit was used to assess model fit.

2.10 Spatial analysis

Spatial analysis was explored to produce disease maps showing areas with higher infection levels. The spatial analysis was carried out in MapInfo 8.5 software [47] I analysed the prevalence of infections by school catchments area. School catchments areas were created using Thiessen (Voronio) polygons [48] in MapInfo. Thiessen polygons are polygons whose boundaries define the area that is closest to each point relative to other points. Mathematically, there are defined by the perpendicular bisectors of lines between all points. This method has the advantage of being relatively quick and easy to perform. Previous analysis at the Africa Centre has shown that pupils usually attend the nearest school based on Euclidean distance and thus supports the use of this method (personal communication with Frank Tanser of the Africa Centre for Health and Population Studies). I used school level prevalence to construct disease maps by school catchments area. The prevalence categories of 0-10%, 10-20, 20-30, 30-40 and >40 was used to construct the map.

2.11 Ethical considerations

Ethical clearance for the primary study was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (with reference number E165/05) Appendix 2. Ethical approval was also obtained from the Committee for Research on Human Subjects of the University of Witwatersrand with Protocol Number (M061141) Appendix 3. Meetings were held with parents and teachers from the participating schools to explain the purpose of the study and to discuss issues related to the study participants' privacy and confidentially. Prior to data collection, consent and assent forms ware issues to all children at the school for their parent to consent before they were enrolled in to the study. In order to ensure confidentiality in the map, all other important geographic features were suppressed in the map.

CHAPTER THREE

RESULTS

3.0 Descriptive summaries of major findings

The study covered all the 33 primary schools in the Africa Centre demographic surveillance area in the 2007 school academic year, which began in April and completed in November. There were 3049 school children present on the day of testing at the 33 schools surveyed. The mean number of school children in each school were 95 (range: 13–231) However, a total number of 2,110 (67%) schools children, consented, and provided sufficient urine samples to be included in the parasitological data for the analysis.

There were slightly more females (51.4%; *n*=1,084) than males (48.4%; *n*=1,026). Age of the school children in the sample ranged from 8 years to 19 years, with a median age of (11, SD 1.57). About 34.1% of the study participants live at altitude less than 100 meters above sea level (masl), 41.6% at altitude between 100 and 150 meters and 24.3% lived at altitude above 150 meters. The proportion of school children reported having access to piped water is 88.1% compared to 11.9% who did not have access to piped water. Similarly, 83.8% of the school children reported using toilet facilities in their households as against 16.2 who did not have any toilet facilities. A detailed description of the frequencies of risk factors amongst study participants is shown in table (3.1) below. For multivariate risk factor analysis, 1,982 (93.6%) school children with valid BSID were included. The rest of the school children 128 (6.4%) were either resident outside the DSA or could not provide adequate/valid information for them to be linked to their demographic and geo-reference data in the Africa Centre Demographic information system database (ACDIS).

Table 3.1: Socio-demographic characteristics of study participants in the Africa Centre DSA

Factor	Number	Proportion (%)
Sex		
Male	1026	48.6
Female	1084	51.4
Age in Years		
≤10	512	24.3
11	625	29.7
12	515	24.4
≥13	457	24.6
Altitude		
<100masl	720	34.1
100-150masl	877	41.6
>150masl	378	24.3
Drinking water		
No Piped	250	11.9
Piped	1,851	88.1
Toilet		
No Toilet	340	16.2
Toilet	1,758	83.8
Water collection pattern		
None	1,492	71.1
At least once	606	28.2
Location		
Urban	42	2.1
Peri-urban	1,462	73.8
Rural	478	24.1
Distance to permanent water bodies		
Within 1km	610	28.9
Within 2km	620	29.4
Within 3km	374	17.7
Over ≥3km	378	17.9

3.1 Prevalence of *S. haematobium* infection.

Examination of two 10ml urine aliquots per individual revealed that about 347 of the school children had *S. haematobium* eggs in their urine. Table (3.2) below shows the sex distribution of the prevalence level of infections among the various risk factors. Figure (3.1) also depicts sex and age specific prevalence of infection. The prevalence level of infection was 16.5% for all the individual age groups considered. However, there was a progressive increase in prevalence levels with age. Prevalence was 11.9 in the 8-10 year's age groups, 13.3%, 19.2%, and 22.8% in the 11, 12 and ≥13 year groups respectively. Statistically, males were more likely to be infected with the parasite than females (22.2% vs. 11.1%, Pearson chi2 (3) = 28.4184, $P < 0.001$).

The prevalent level of infection was lowest (15.7%) among school children who reported the use of piped water as their main sources of drinking water, as compared to those without access to piped water (19.8%). However, there was no statistical significance difference between the two groups (Pearson chi2 (1) = 2.6133   P < 0.106).

3.2 Intensity of S. *haematobium* infection.

Out of the 347 school children infected with *S. haematobium*, 57.6% had heavy infections (>50 eggs/10mls urine) while 42.4% (<50 eggs/10mls urine) had light infection (refer: table 3.3). Generally, sex was not found to be significantly associated with infection intensity, (Pearson chi2 (1) = 2.36   P < 0.125). The proportion of girls and boys who had heavy infection was more than those who had light infection. On the other hand, older age groups showed consistently higher infection intensities than their younger counterparts even though this was also not statistically significant (Pearson chi2 (3) = 3.26   P < 0.354).

Table 3.2: Infection prevalence (%) among school children stratified by sex and the overall in the Africa Centre DSA

Factor (%)	Males	Female	Overall
Age			
≤10	34 (18.5)	27 (8.2)	61 (11.9)
11	49 (16.8)	34 (10.2)	83 (13.3)
12	62 (23.3)	37 (14.9)	99 (19.2)
≥13	82 (28.8)	22 (12.8)	104 (22.8)
Drinking water			
No Pipe	193 (23.5)	98 (16.3)	291 (19.8)
Pipe	28 (21.5)	21 (10.3)	49 (15.7)
Toilet			
No toilet	174 (19.1)	100 (8.1)	274 (13.7)
Toilet	47 (22.5)	19 (11.9)	66 (17.0)
Distance to permanent water bodies			
Within 1 km	71 (23.9)	42 (13.4)	113 (18.5)
Within 2 km	83 (28.8)	37 (11.1)	120 (19.4)
Within 3 km	36 (19.9)	29 (15.0)	65 (17.4)
Over ≥3 km	24 (12.2)	7 (3.9)	31 (8.2)
Altitude			
<100	123 (54.2)	74 (61.7)	197 (56.8)
100-150	71 (31.3)	32 (26.7)	103 (29.7)
>150	33 (14.5)	14 (11.7)	47 (13.5)
Water collection pattern			
None	139 (19.2)	73 (9.6)	212 (14.2)
At least once	82 (28.2)	46 (14.6)	128 (21.1)
Wealth quintiles			
Most poor	45 (21.8)	34 (30.6)	79 (24.9)
More Poor	43 (20.9)	21 (18.9)	53 (20.2)
Poor	45 (24.8)	17 (15.3)	62 (19.6)
Less Poor	39 (18.9)	34 (14.4)	55 (17.4)
Least poor	34 (16.5)	23 (20.7)	57 (18.0)

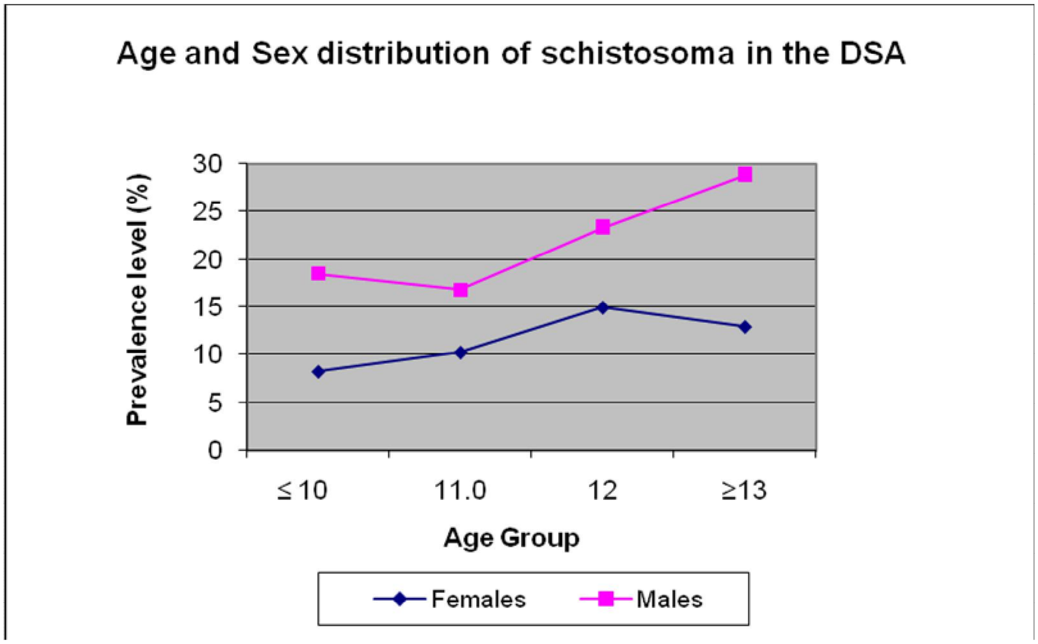

Figure 3.1: Prevalence of *S. haematobium* among grade 5 and 6 school children by sex and age group.

Table 3.3: Proportion of school children (%) with different infection intensities with *S. haematobium* infection, stratified by age and sex (n = 347) in the Africa Centre DSA.

Variable Sex	Light infection (1-50 eggs)	Heavy infection (>50eggs)	χ^2	p-value
Females	48.0	52.0	2.36	0.125
Males	39.5	60.5		
Age years				
≤ 10	46.8	53.2	3.26	0.354
11	48.8	51.2		
12	38.4	61.6		
≥13	38.5	61.5		
Geometric mean EPC	0.58, Std. Err. 0.49			

3.3 Spatial distribution of S. haematobium infection among school children in the DSA

A map showing the distributions of *S. haematobium* prevalence is presented in Figure (3.2). Areas of high prevalence (<30%) were apparent toward the South-eastern part of the DSA. These areas are highlighted in red colour in the map below. Prevalence appeared to be lowest in the North-western parts of the DSA as compared to the rest of the DSA. Areas of zero or low prevalence are found in the central and western parts of the DSA. There is also one isolated high prevalence area in the Northern part of the DAS.

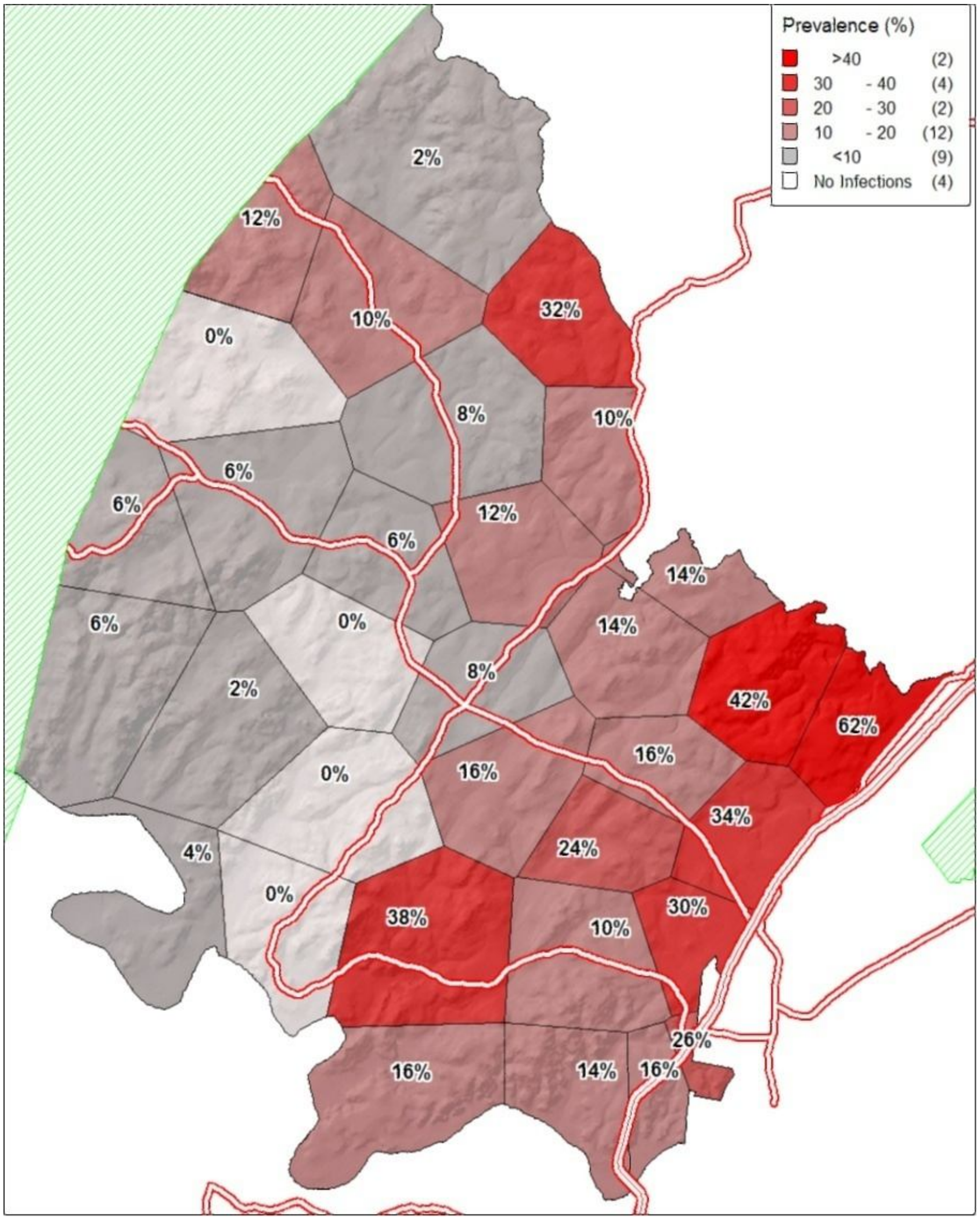

Figure 3.2: the spatial distribution of *S. haematobium* prevalence in the Africa Centre DSA; Thesian polygons represent school catchments area.

3.4 Bivariate logistic regression analysis of the associations between *S. haematobium* infection and demographic, environmental, and socio-economic covariates

Bivariate logistic analyses were carried to assess the association between infection and potential risk factors for *S. haematobium* infection. The results of the bivariate analysis are shown in Table 3.4 below. Boys were about two times more likely to be infected than girls (odds ratio (OR) = 2.28, *P* <0.001, 95% CI: 1.79, 2.90). In terms of infection intensity, there were no statistical differences between boys and girls, (odds ratio (OR) = 1.58, *P* <0.126, 95% CI: 0.88, 2.84). However, Age was found to be significantly associated with infection. The older age groups (13-19years) were at greater odds of being infected than the under 10 year's age group (odds ratio (OR) = 2.18, *P* <0.001, 95% CI: 1.55, 3.09).

All the environmental covariates were significantly associated with *S. haematobium* infection using the bivariate models. School children living at higher altitude were less likely to be infected than those living at lower altitude. For example, school children living above 100-150masl were at least 65% less likely to be infected compared with school children who live below 100masl (odds ratio (OR) = 0.35, *P*<0.001 CI: 0.27, 0.46). Similarly school children who live at altitude above 150 meters were 73% less likely to be infected compared with school children who live below 100masl (odds ratio (OR) = 0.27, *P*<0.001, 95% CI: 0.19, 0.38).

Decreased risk of infection was also associated with increased distance from homestead to permanent water bodies. Even though the odd ratios shows that school children living at locations 2 kilometres away from permanent water bodies have slightly increased odds of infection (odds ratio (OR)=1.05, *P*< 0.710, 95% CI: 0.79, 1.40), school children from homestead that are located further away from permanent water bodies had decreased odds of infection. School children living at 3 kilometres away from water bodies were not statistically different in terms of infection to those living at a distance of about 1 kilometres or less, (odds ratio (OR)=0.92, *P*< 0.65, 95% CI: 0.66, 1.29). For school children living at a distance of over 3 kilometres from permanent water bodies, they were about 61% less likely to be infected than those living at 1 kilometres or less to permanent water bodies (odds ratio (OR)=0.39, *P*< 0.001 95% CI: 0.29, 0.59). However, females school children living at distance of over 3 kilometres from permanent water bodies had a reduce odds of getting infected (odds ratio (OR) = 0.26, *P*< 0.001, 95% CI: 0.11, 0.59) compared to males (odds ratio (OR) = 0.44, *P* < 0.002, 95% CI: 0.27 0.73). The odds of *S. haematobium* infections where high for school children living in peri-urban and rural areas as compared to those living in the urban area (odds ratio (OR) = 5.22, *P*< 0.024, 95% CI: 1.24, 21.98) for per-urban and (odds ratio (OR) = 3.69, *P*< 0.073, 95% CI: 0.88, 15.40) for rural area.

Schoolchildren who reported to have had any contact with permanent water source were 62% more likely to be infected than those who did not report of collecting water from permanent water bodies (odds ratio (OR) = 1.62, *P*<0.001, 95% CI: 1.26, 2.06). With regards to access to piped water, schoolchildren who reported lack of access to piped water as their main sources of drinking water were not statistically different in terms of infection from those who reported the use of piped water as their main source of drinking water (odds ratio (OR) = 1.31, *P* <0.107 95% CI: 0.94, 1.84). However, after stratifying by sex, the odds of infection became statistical significant for girl who reported lack of access to piped water compared to those with access to piped water (odds ratio (OR) = 1.69, *P* <0.04, 95% CI: 1.01, 2.82).

Table 3.4: Bivariate logistic regression of the associations between *S. haematobium* infection and demographic, environmental and socio-economic risk factors from the Africa Centre DSA adjusting for sex. Odds ratios (OR) are displayed with their 95% confidence intervals (CI)

Variable	Unadjusted Both	Adjusted Males	Adjusted Females
Age group	Odds Ratio 95% (CI)	Odds Ratio 95% (CI)	Odds Ratio 95% (CI)
≤10	1.00	1.00	1.00
11	1.13 (0.79, 1.61)	0.89 (0.55, 1.45)	1.26 (0.74, 2.14)
12	1.76 (1.25, 2.49)*	1.34 (0.83, 2.14)	1.94 (1.15, 3.29)*
≥13	2.18 (1.55, 3.09)*	1.78 (1.13, 2.80)*	1.63 (0.90, 2.97)
Sex			
Females	1.00		
Males	2.28 (1.79, 2.90)	-	-
Drinking water			
Piped	1.00	1.00	1.00
No pipe	1.32 (0.94, 1.85)	1.23 (0.72, 1.77)	1.69 (1.01, 2.82)*
Toilet			
Toilet	1.00	1.00	1.00
No toilet	0.78 (0.58, 1.04)	0.81 (0.57, 1.16)	0.65 (0.39, 1.09)
Location			
Urban	1.00		
Peri-unban	5.22(1.24, 21.98)*	3.65(0.82, 16.32)	1.14(---)
Rural	3.69(0.88, 15.40)	1.98(0.45, 8.69)	1.09(0. 75, 1.69)
Altitude			
< 100 masl	1.00	1.00	1.00
100-150 masl	0.35 (0.27, 0.46)*	0.33 (0.23, 0.46)*	33 (0.21, 0.52)*
> 150 masl	0.27 (0.19, 0.38)*	0.24 (0.15, 0.36)*	26 (0.14, 0.47)*
Distance to permanent water bodies			
Within 1km	1.00	1.00	1.00
Within 2km	1.05 (0.79, 1.40)	1.29 (0.89, 1.86)	0.81 (0.50, 1.29)
Within 3km	0.92 (0.66, 1.29)	0.79 (0.50, 1.24)	1.14 (0.68, 1.90)
Over ≥3km	0.39 (0.29, 0.59)*	0.44 (0.27, 073)*	0.26 (0.11, 0.59)*
Water collection			
None	1.00	1.00	1.00
At least once	1.62 (1.26, 2.06)*	1.66 (1.21, 2.27)*	1.62 (1.09, 2.41)*
Wealth quintiles			
Most poor	1.00	1.00	1.00
Very poor	0.79 (0.55, 1.14)	1.07 (0.66, 1.73)	0.53 (0.29, 0.96)*
Poor	0.76 (0.53, 1.10)	1.15 (0.72, 1.85)	0.41 (0.23, 0.77)*
Less poor	0.72 (0.49, 1.04)	1.05 (0.64, 1.70)	0.42 (0.22, 0.79)*
Least poor	0.76 (0.53, 1.11)	0.82 (0.50, 1.36)	0.69 (0.39, 1.23)

* Significant Odds Ratios at 95% significant level.

Socio-economic status was not statistically associated with infection at the bivariate level. However, when the analysis was stratified by sex, Socio-economic status was significantly associated with infection for girls but not boys (odds ratio (ORs) = 0.53, $P < 0.037$, 95% CI: 0.29, 0.96) for the very poor, (odds ratio (ORs) 0.41, $P < 0.006$, 95% CI: (0.23, 0.77) for the poor, (odds ratio (ORs) 0.42, $P < 0.008$, 95% CI: (0.22, 0.79) for the less poor and (odds ratio (ORs) 0.69, $P < 0.22$, 95% CI: (0.39, 1.24) for the least poor as compared to the most poor female wealth quintile.

3.5 Multivariate logistic regression analysis of the associations between *S. haematobium* infection and demographic, environmental, and socio-economic covariates.

A multivariate-logistic regression model was formulated to determine the association between infection and demographic and socio-economic variables. Socio-economic status and toilet facilities, even though not statistically significant in the bivariate model were retained in the final model because they have been shown to be associated with schistosomiasis infection in previous studies and to account for any confounding effects. The results are presented in Table 5 below. In the adjusted multivariate logistic regression model, sex, age group, toilet facilities, household location, distance from homestead to permanent water bodies and water collection pattern were significant predictors of infection.

The findings indicated that boys were 2½ times more likely to be infected with *S. haematobium* compared to girls (adjusted odds ratio (AOR) = 2.49, $P < 0.001$, 95% CI: 1.90, 3.24).

The final model also showed that school children who were 12 years old were nearly 50% more likely to be infected than those aged 8 to 10 years, (adjusted odds ratio (AOR) = 1.46, $P < 0.050$, 95% CI: 1.00, 2.13) while the older age group (13 to 19 years) were about 80% more likely to be infected with *S. haematobium* compared to those between 8 and 10 years (adjusted odds ratio (AOR) = 1.79, P< 0.003, 95% CI: 1.22, 2.64) after adjusting for possible confounders. The 11 years old groups were not statistically different from the 8 to 10 years age bracket in terms of infection, (adjusted odds (AOR) =1.00, P< 0.971, 95% CI: 0.65, 1.47).

The model also shows that all the environmental covariates were statistically associated with *S. haematobium* infection in the study area. Increased distance from homestead to a permanent water body was negatively associated with infection. School children living in homestead over 3 kilometres were 66% less likely to be infected compared to school children living in homesteads less than or equal to 1 kilometre from a permanent water body (adjusted odds (AOR) = 0.34, P< 0.001, 95% CI: 0.20, 0.56). However, school children living within 1 to 2 kilometres were not statistically different from those living at 1 kilometres or less from a permanent water body in terms of infection (adjusted odds (AOR) = 0.96, P< 0.788, 95% CI: 0.70, 1.30) for those living within 2 to 3 kilometres (adjusted odds (AOR) = 0.94, P < 0.742, 95% CI: 0.64, 1.37) and for those living within 3 kilometres from a permanent water body).

Homestead altitude was also negatively associated with infection. School children living in homesteads at an altitude above 150 meters below sea level were 0.16 or 84% less likely to be infected as compared to children living in homesteads 100 meters below sea level (adjusted odds (AOR) = 0.16, P < 0.001, 95% CI:0. 09, 0.25). Similarly, school children living at altitude between 100 and 150 meters were 0.29 times less likely to be infected compared to those living below 100 meters (adjusted odds (AOR) = 0.29, P < 0.001, 95% CI: 0.220, 0.39).

With regards to access to pipe water, school children who reported lack of pipe water as their main source of drinking water were 1.92 times more likely to acquire infections compared to school children who have access to pipe water (adjusted odds (AOR) = 1.92, $P < 0.007$, 95% CI: 1.20, 3.07). With respect to access to toilet facilities, school children who do not have access to toilet facilities where 35% less likely to be infected compared to those with access to toilet facilities (adjusted odds (AOR) = 0.65, $P < 0.021$, 95% CI: 0.46, 0.94).

The multivariate analysis also revealed that school children who were living in the peri-urban and rural areas were at a significantly higher risk of infection than those living in the urban areas (adjusted odds (AOR) = 5.82, $P < 0.018$, 95% CI: 1.34, 25.11) for peri-urban and (AOR) = 7.29, $P < 0.008$, 95% CI: 1.69, 31.75 for rural). The wide confidence interval shows a small number of people in urban areas compared to those in rural areas.

Fetching water from a permanent water body was significantly associated with infection, adjusted odds (AOR) = 1.84, $P < 0.001$, 95% CI: 0.32, 0.83). The use of toilet facilities by household members was significantly associated with infection in the adjusted model though not significant in the bivariate model. Socio-economic status was the only variable not associated with infection. The Odds Ratio, p-values together with 95% confidence interval is reported in table (3.5).

Table 3.5: Multivariate logistic regression of the associations between *S. haematobium* infection and demographic, environmental and socio-economic risk factors in the Africa Centre DSA, Adjusted Odds ratios (AOR) are displayed together with p-values and 95% confidence intervals

Variable Sex	Odds Ratio Adjusted	P-value	95% Confidence interval
Females	1.00		
Males	2.49	0.001*	1.90, 3.24
Age group (yrs)			
8-10	1.00		
11	1.00	0.971	0.65, 1.47
12	1.46	0.050*	1.00, 2.13
13-19	1.79	0.003*	0.22, 0.39
Drinking water			
Pipe	1.00		
No Pipe	1.92	0.007	1.20, 3.07
Toilet			
Toilet	1.00		
No toilet	0.65	0.021*	0.46, 0.94
Altitude			
<100 masl	1.00		
100-150 masl	0.29	0.001*	0.22, 1.39
>150 masl	0.16	0.001*	0.09, 0.25
Distance to permanent water bodies			
Within 1 km	1.00		
Within 2 km	0.96	0.788	0.70, 1.30
Within 3 km	0.94	0.742	0.64, 1.37
Over ≥3 km	0.34	0.001*	0.20, 0.56
Water collection pattern			
None	1.00		
At least once	1.84	0.001*	0.32, 0.83
Location			
Urban	1.00		
Peri-urban	5.82	0.018*	1.34, 25.11
Rural	7.29	0.008*	1.69, 31.75
Wealth quintiles			
Most poor	1.00		
Very poor	1.02	0.890	0.68, 1.53
Poor	0.89	0.559	0.58, 1.33
Les poor	0.79	0.313	0.51, 1.23
Least poor	0.79	0.286	0.51, 1.21

* Significant Odds Ratios at 95% significant level

CHAPTER FOUR

DISCUSSION

This study investigates the spatial distribution of *S. haematobium* infections in relation to the demographic attributes and environmental covariates of the Africa centre Demographic surveillance area in the Hlabisa district of KwaZulu-Natal, South Africa. The study examines the determinants of *S. haematobium* infection including; household socio-economic status, access to piped water, age, sex, distance to permanent water bodies, altitude of homestead and location of homestead. While there may be several factors associated with schistosoma infection in the study area, the age of the school children, sex, access to piped water, homestead altitude, location, and distance from homestead to permanent water bodies, were the most significant factors associated with the spatial distribution of *S. haematobium* infection among school-age children in the study area.

The overall infection prevalence of 16.5% in this study is quite low compared to results from historical studies conducted 150 kilometres north of the study area, where 68% of school children were reported to be infected with *S. haematobium* [19]. The possible explanation for this low prevalence may be due to improved access to piped water and sanitation in the DSA. Household access to piped water and toilet facilities have increased rapidly from 55% and 75% respectively in 2003 to 78% and 77% in 2006 [49]. Improvement in social service has a positive effect on the standard of living and the health status of populations and could account for the declining rate of transmission in the area. However, the pattern of age specific prevalence of *S. haematobium* infection among boys and girls reported in this study are consistent with results of the earlier study [19].

The higher proportion of boys reported to have water-contact activities than girls strongly supports the hypothesis that prevalence and intensity of infections in school-age children is typically male-biased as a result of higher cercarial exposure in contaminated waters. In previous studies, heterogeneity in water contact behaviour among males and females has been reported as a major risk factor for the differential levels of infection between males and females [50, 51]. This is probably the main reason accounting for the high infection prevalence among boys than girls, but not due to any kind of sex-difference in the immunological mechanisms of resistance to infection [52]. It is also logical to suggest that, in rural Africa males move more freely and are more adventurous than girls and are therefore, more likely to come into contact with water bodies including contaminated ones. This assertion is supported by a study conducted in Maputaland (150 kilometres North of this study area), which demonstrated a significant gender differences in levels of infection, suggesting that girls are being mainly infected through their domestic water collection activities [19].

Proximity to permanent water bodies is a major pathway of infection for most at risk populations including school-age children. School children living in homesteads located at close proximity to permanent water bodies were at significantly higher risk of *S. haematobium* infection than children living in homesteads situated further away from a permanent water body. This finding is supported by early studies conducted in Tanzania, Coˆte d'Ivoire and Kenya [53, 11, 54], where the prevalence and intensity of *S. mansoni* and other soil-transmitted helminths infections were distance-dependent from the homestead to a permanent water body. According to Clennon et al. [54], higher infection levels were found clustered around ponds known to contain *Bulinus nasutus* snails that shed *S. haematobium* cercariae and low transmission found in rivers where intermediate snails were rarely found.

Contacts with permanent water body were significantly negatively correlated with the distance variables, indicating that school children living closer to permanent water bodies had more frequent contact with contaminated water than those living far from permanent water bodies. The policy implication of this finding is that, if control and intervention programmes are targeted at communities' closer to permanent water bodies, it is possible to have a substantial reduction in the levels of *S. haematobium* infection in the DSA.

The results reported in this study on the intensities of *S. haematobium* infection are not consistent with other studies. The results show that a substantial number of infected individuals have heavy infections compared to those who had light infection. The 2000 WHO report indicates that most infected individuals have light intensity of infections while a few are heavily infected [2]. In endemic communities it is the heavily infected individuals who suffer from the clinical effects of the disease and pose serious source of infection for other individuals in the community. Severe schistosoma infection can cause high rates of absenteeism and drop out among school children because of seqular, thereby lowering educational achievement and reversing progress made towards meeting the MDG's on achieving universal primary education by 2015 and the elimination of neglected diseases.

Altitudinal variation is associated with the distribution of *S. haematobium* infection, with very little transmission occurring in areas above 150 meters. Climatic conditions such as temperature and rainfall [13, 55] have an indirect influence on the development, distribution and density of the snails host and therefore rate of schistosomiasis transmission [56]. Previous studies have examined the relationship between altitude and exposure to schistosomiasis infection and clearly predicted the altitudinal limits for the development of schistosomiasis transmission. For instance, in I'vory coast, Brooker et al. [14] reported that school children living at locations below 400 meters were at a 5-fold higher risk of *S. mansoni* infection when compared to those living at higher altitudes.

The design and implementation of any schistosomiasis control programme requires knowledge of both the epidemiology and spatial distribution of populations at risk of transmission for effective and efficient intervention. For instance defining environmental limits of schistosomiasis transmissions could help health workers to clearly delineate geographic areas where control activities should be given priority. In the present study, the prevalence levels were high in areas where altitude is below 150 meters and could be earmark for immediate control activities. Other potential benefit of these environmental covariates is that, when the population of the area is overlay on these environmental variables, it is possible to adequately estimate the at-risk population at the sub-district level for effective planning and control. As indicated in table 3.1 about 68.4% of the study participants live in areas where altitude is below 150 meters above sea level. An earlier study [13] indicated that between 3.9 million to 4.4 million school age school children in South Africa live in areas where temperature and other climatic conditions are suitable for schistosome transmission.

This study has shown a marked difference in the prevalence of infection between urban, peri-urban and rural areas. Children who reported residing in peri-urban and rural areas were significantly more likely to be infected with *S. haematobium* than those living in the urban areas. The reasons for this is are twofold. The first relates to better access to water and sanitation over a longer period. The results show that more households in the urban areas have better access to pipe water (100% vrs 85%) than those in the rural areas. However, I control for this in the multivariate model and there was an independent protective effect of living in an urban area. One reason for this may be that many children in the rural areas have only recently had access to piped water and therefore could have been infected before the introduction of piped water in their community. I was unable to control for historical access to safe water supplies in the model. The second reason concerns the increased opportunity for children in rural communities coming into contact with infected water bodies. Most rural areas in sub-

Saharan Africa are characterized by poor hygienic conditions resulting in an intense transmission of schistosomiasis and other soil helminth infection. Contrary to this finding, Phiri K et al. [57] reported that living in an urban community was associated with significantly higher risk of helminthes infection than those living in rural areas. However, the authors attributed this unusual finding to the differences in the prevalence of *Ascaris lumbricoides* infection reported in their study.

The study also shows that access to piped water has a protective effect against *S. haematobium* infection. This is in agreement with earlier studies investigating the impact of access to portable water and sanitation on risk of schistosomiasis infection [58, 59]. The results indicate that risks of exposure were highest among school children with no access to piped water as compared to those without access to piped water (AOR 1.92, P<0.007, 95% CI 1.20, 3.07). The proportion of individuals who might not have access to piped water are likely to be school children living in deprived communities, and are thus more exposed to the risk of infection because of their socioeconomic status including access to pipe water and poor sanitary facilities. An earlier study conducted in the area in 2001 shows that about 27% of households obtain water for cooking and drinking from rivers or ponds, while 39% of households had no toilet facilities [38]. Access to toilet facilities has improved in the area. However, it is possible that the quality of toilet facilities provided at the schools and homes are not hygienic enough to prevent transmission of *S. haematobium* infection in the area.

Access to safe drinking water and basic sanitation can have a strong positive effect on human health. In a meta analysis to assess the role of interventions programmes in the control of helminth infections, Asaolu et al. [60], found evidence to support the hypothesis that access to water supply and sanitation facilities reduces the risk of schistosomaisis infections in the intervention communities. The study shows that in both cohort and longitudinal intervention programmes, decreasing access to water supply and sanitation facilities increased the prevalence of schistosomiasis infections by over 39.3%, and helminths infections at rates ranging from 27% to as high as 194.5%. This latest findings highlights the importance of increasing access to portable water to reduce infection, as people will avoid having contact with permanent water bodies. However, as already reported elsewhere in this report piped water supply has been rolled out rapidly in the DSA and is possibly the main explanation factor, responsible for the low level of *S. haematobium* prevalence reported in the study.

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Clearly, the inherent nature of transmission of the disease (intermediate host) means that local neighbourhood effects are critical in the cycle of transmissions; it may be that determinants of transmission identified at the individual and household level are being modified by those at the community level. One author Barbosa FS, [61] has stated “While determinants are hierarchically ordered, the mediations between the macro and micro levels need to be better understood.” therefore a limitation could be that this study focuses mainly on individual risk factors and not community effects. However, both individual and structured variables of neighbourhood are important for targeting interventions. For complete elimination of the disease, especially re-infection with *S. haematobium*, there is the need for rapid roll out of piped water supply to the rural areas where the burden of the disease seems to be highest.

The results from the spatial analysis showed that higher prevalence occurred mostly in the eastern half of the DSA, moderate to low prevalence observed in the central part and the lowest prevalence in the western part of the DSA. This is in accordance with the widely accepted view of the focal distribution of schistosomaisis [15]. The reasons for this are varied, and reflect both human and environmental factors. GIS technology is particularly well suited to communicate the results of this type of analysis because it enables visualization of the geographical distribution of case prevalence via maps. Several factors may account for the spatial distribution of S. *haematobium* infection in the area. Prominent among them is the low altitudinal level of the south-eastern part of the DSA which provides a suitable breeding environment for *transmission*. Another possible explanation factor could the presence of streams and other water bodies across the eastern part of the DSA.

Household socioeconomic status was not significantly associated with schistosoma infection. Contrary to the findings in this study, other epidemiological studies focusing on the association between household socio-economic status and parasitic infection, has reported a significant association between poor household economic status and schistosoma infection [6, 11]. One other study, Ximenes R et al. [62] has also reported of a positive association between household income and schistosomiasis transmission. Their finding indicates that socioeconomic conditions operate through other intermediate mechanisms to influence the risk of infection, but not necessarily in a causal manner. The study found a strong relationship between socioeconomic covariates and schistosomiasis prevalence in an urban town in northeast Brazil. However, their findings could not be compared with rural areas, where there are generally weaker socioeconomic differentials.

Perhaps, if the study population were compared with a more affluent group, more relationships would become apparent between socioeconomic status and the diseases. In many cases, socio-economic status is probably the single most important indirect cause of diseases, because poverty has an intermediate effect on many of the other variables, including poor sanitation facilities and lack of access to piped water.

Recent studies have found correlations between household poverty and school children enrolment in the DSA. One study has established that household socio-economic status may affect school enrolment and attendance [38]. So it is possible that the school children who were absent from school on the day of testing fall within this disadvantage group. Similarly, the criteria for classifying communities into different wealth quintiles are not the same since different socio-economic variables have different values in different settings. Tshikuka JG et al. [63] stated in their study that "low socio-economic status are generally associated with ignorance, poverty, illiteracy and general deprivation in terms of good roads, health care facilities, alternative water supply, sanitation facilities and lack of good housing".

In building on the strengths of previous applications, this study has examined the risk factors associated with *S. haematobium* infection and the micro-geographic distribution of infection among grade five and six school children in rural KwaZulu-Natal, South Africa, using the compatibility of geographical information system to integrate parasitological, environmental and geographic variables to develop a disease map of *S. haematobium* transmission.

4.1 Limitations of the study

Although, almost all primary school children attend schools in the DSA [49] the consent rate of 67% may have introduced a selection bias into the analysis. For example, those infected with *S. haematobium* may be less likely to consent to testing. In addition, it is possible that children with high worm burdens are too sick to regularly attend schools and were therefore absent on the day of testing. This may cause an underestimate of the true prevalence in this population. In India, for example, it has been shown that on average a rich child was 31% more likely to be enrolled in school than a poor child [41]. It is possible that school children coming from poor home were absent on the day of testing. This may be one of the reasons for the lack of statistical significant association between wealth quintiles and infections.

One other limitation of the study is that because the results are based on a single urine sample we will miss a small number of light infections [39]. The true prevalence of infection is therefore probably closer to 20% rather than 16.5% reported in this study.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Controlling schistosomiasis infections requires knowledge of factors associated with infection and the spatial distribution of the disease. The objective of this study was to advance such knowledge by identifying factors associated with *S. haematobium* infection and delineating areas with high prevalence to guide schistosomiasis control in rural KwaZulu-Natal. This study has reported the current pattern, prevalence and intensity of *S. haematobium* infection among grade five and six school children in the Africa Centre DSA. The study also presented risk factors associated with *S. haematobium* infection and map out areas where the prevalence and intensity of infection is a problem and should be of great concern to the Department of Health and the Department of Education in the Umkhanyakude District of KwaZulu-Natal, South Africa.

The final model shows that age and sex of school children, access to piped water, homestead location, water contact behaviour, household altitude, and distances to permanent water bodies were the most significant factors associated with *S. haematobium* infection in the study area.

This study recommends that regular and consistent administration of chemotherapy be targeted at those areas with high infection rates in order to control re-infection in the DSA. It is suggested that school-age children living in areas where prevalence levels are above 30% should be targeted for mass treatment with prazinquntal (PZQ) once every year. This intervention strategy should be complemented with improved supply of PZQ to health facilities to make the drug available and accessible to other infected people in the communities.

Secondly, it is also recommended that school-age children living in areas with moderate prevalence levels (that is prevalence levels between 10% and 30%) should be target for treatment with chemotherapy once every two years. However, in areas with low prevalence levels (less than 10%) school-age children should be target for treatment twice during their primary school education (once on entry and one on leaving). These control programmes should be complemented with access to PZQ for passive case treatment in health facilities. Particular focus should be place on high risk-groups including school-age children, those out of school and pregnant women for systematic and regular treatment. However, the trend today is to combine schistosomiasis control with geohelminth control using a single school-based delivery system, this alternative control is well suitable for resource poor countries. It is also recommended that geospatial techniques such GIS and RS should be employed annually, as a rapid epidemiological tool to map and monitor high potential risk areas that require immediate action. This is because climatic conditions such as flooding and temperature are capable of changing the geographical location of transmission foci.

Health education is another effective control measure that could further raise awareness among school children about the epidemiology of schistosomiasis transmission and also encourage children showing signs and symptoms of the disease to visit their local clinic for testing and possible treatment. This study therefore recommends the development and integrations of health awareness into primary school curriculum in rural South Africa. The teaching of health education to school children should including posters, reading materials, slide shows and film shows. According to Asaolu S. O et al. [60] even though chemotherapy and good hygiene has remain the best option for morbidity control and to protect the uninfected, “Health education that is effective, simple and low-cost remains the only tool for creating the enabling environment for both chemotherapy and sanitation to thrive".

However, it will be difficult for health education alone to change children health seeking behaviour as long as such population remains poor and have to copy with other disease especially the burden of HIV/AIDS in the study area. It is therefore recommended that additional control activities such as the supply of portable water and good sanitation facilities, to all populations at risk of *S. haematobium* infection be continued in the study area.

A review of past experience in schistosomiasis control activities such as local environmental improvement and health education of population at risk of infection indicate that control activities are likely to achieve high coverage and low cost with active community participation [64]. Effective, community health education towards changing people's attitude towards contact with contaminated water and the use of good sanitary facilities throughout the DSA, especially in endemic communities is recommended. Community participation should also be considered as an integral part of the schistosomiasis control programme. This should include sensitising community members on the impact of the disease burden and soliciting for their cooperation with the local health authorities during the programme implementation. A community partnership in the control activities could easily be contacted to mobilized school-age children who are currently out of school for treatment.

In order to prevent re-infection and achieved a sustainable reduction in prevalence level over time, regular treatment and education should be supported by adequate supply of piped water and sanitation facilities. Lack of access to piped water couple with poor hygienic conditions is the underlying factors of schistosomiasis transmission in most populations [1, 2]. The government should provide piped water to rural areas to further reduce the observed rate of infection with schistosomiasis and other parasites that depend on contaminated water for transmission. An improvement in piped water supply appears to have a significant impact on health when improvement is secured close to the point of use (that is at the household level). Recent literature indicates that the provision of piped water at household level and household water treatment and safe storage are associated with significant health gains [65].

Hygiene and play habits make children vulnerable to schistosome infections; it is therefore recommended that school children be properly supervised during break hours to avoid contact with contaminated water. The proportion of school children who are heavily infected are often physically and intellectually compromised by anaemia and other diseases, leading to attention deficits, learning disabilities, school absenteeism and higher dropout rates [2, 16, 17]. Failure to effectively identify and treat these infected children will in the long-term hamper child development programmes initiated in the country.

With reference to the WHO guidelines for the control of schistosomiasis, the high proportion of heavy infection couple with levels prevalence levels reported in this study necessitates regular assessments and treatment of school children in the DSA. One treatment per year, applied after the end of summer [19] in high prevalence areas is sufficient to keep *S. haematobium* infection in the area at low levels.

The development of a national schistosomiasis control programme and a stronger national policy directed at providing good sanitary facilities and piped water, with a comprehensive and sustainable poverty alleviation strategy in rural KwaZulu-Natal is necessary to address the disease burden in the Africa Centre DSA and country as a whole.

The advantage of this study over preceding ones is that, it used statistical analysis to quantify the burden of schistosomiasis and identifies significant determinants of infection at the individual level. Moreover, it also employed GIS to map areas with high levels of infection to compliment the statistical analysis and identify areas at greatest need for sustained intervention.

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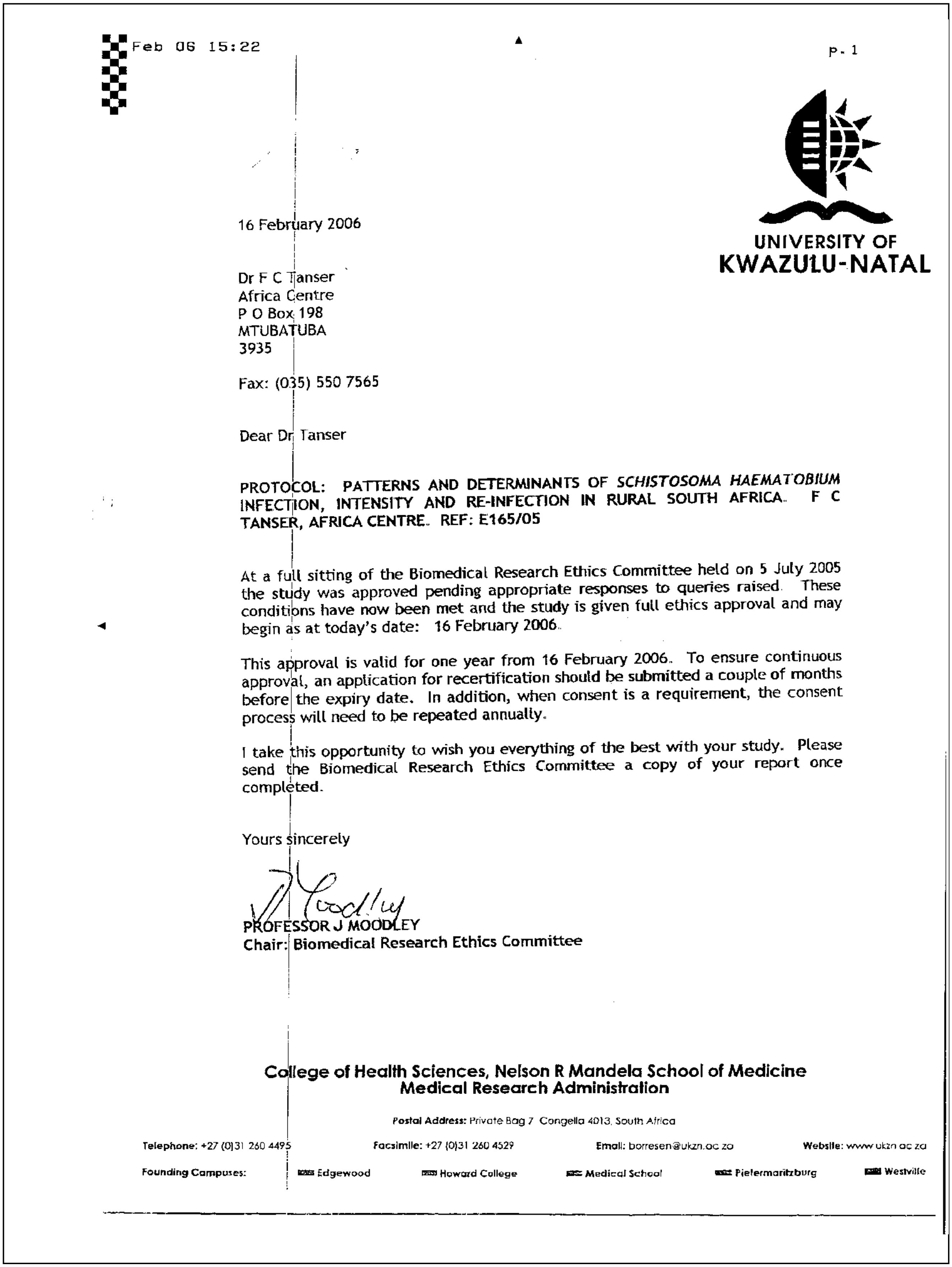
Environmental Burden of Disease Series No. 15.

Appendix 1



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p. 1

UNIVERSITY OF
KWAZULU-NATAL

16 February 2006

Dr F C Tanser
Africa Centre
P O Box 198
MTUBATUBA
3935

Fax: (035) 550 7565

Dear Dr Tanser

PROTOCOL: PATTERNS AND DETERMINANTS OF *SCHISTOSOMA HAEMATOBIUM* INFECTION, INTENSITY AND RE-INFECTION IN RURAL SOUTH AFRICA. F C TANSER, AFRICA CENTRE. REF: E165/05

At a full sitting of the Biomedical Research Ethics Committee held on 5 July 2005 the study was approved pending appropriate responses to queries raised. These conditions have now been met and the study is given full ethics approval and may begin as at today's date: 16 February 2006.

This approval is valid for one year from 16 February 2006. To ensure continuous approval, an application for recertification should be submitted a couple of months before the expiry date. In addition, when consent is a requirement, the consent process will need to be repeated annually.

I take this opportunity to wish you everything of the best with your study. Please send the Biomedical Research Ethics Committee a copy of your report once completed.

Yours sincerely

PROFESSOR J MOODLEY
Chair: Biomedical Research Ethics Committee

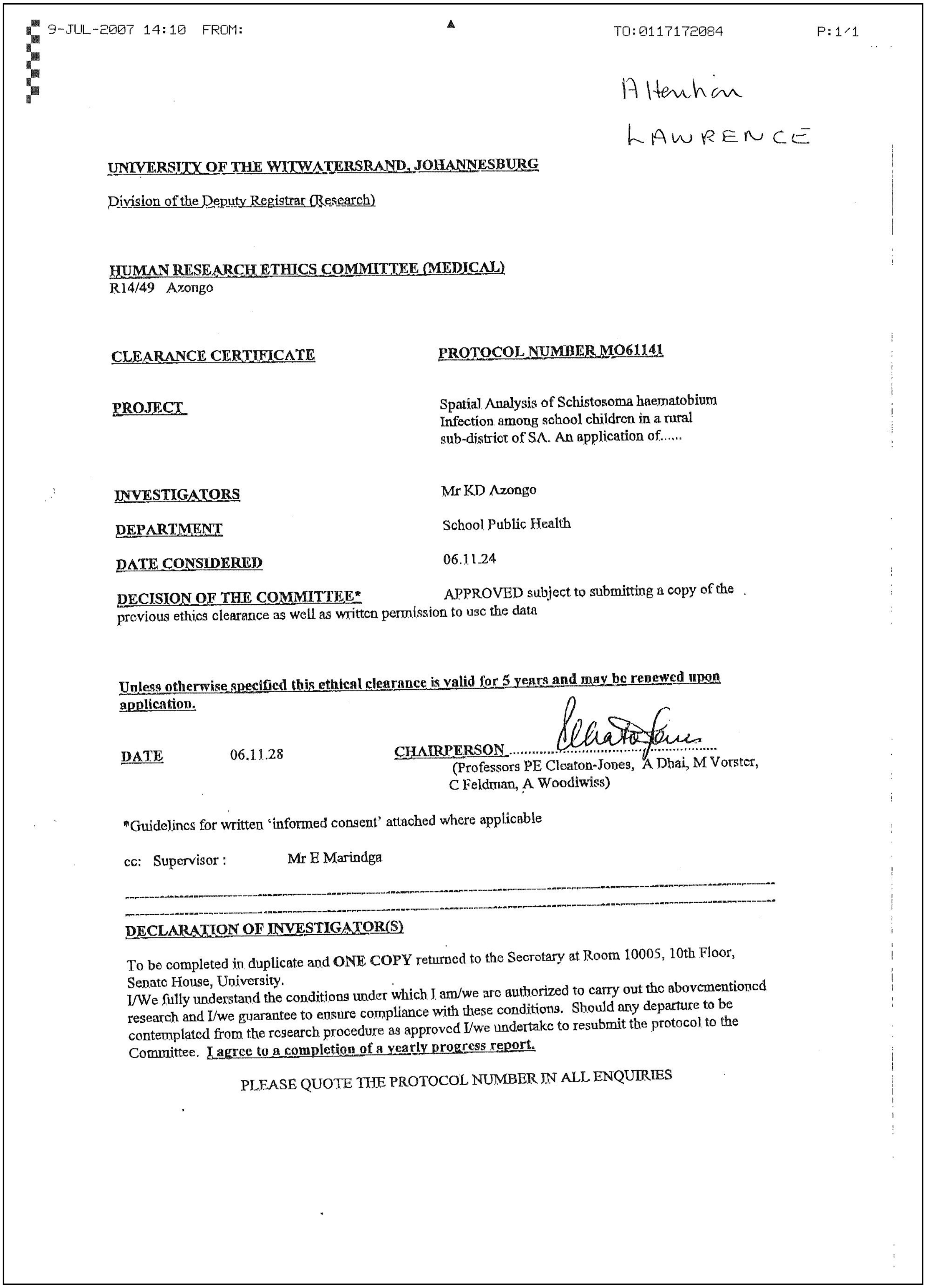
College of Health Sciences, Nelson R Mandela School of Medicine
Medical Research Administration

Postal Address: Private Bag 7 Congella 4013, South Africa

Telephone: +27 (0)31 260 4495 **Facsimile:** +27 (0)31 260 4529 **Email:** borresen@ukzn.ac.za **Website:** www.ukzn.ac.za

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix 2



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Attention
LAWRENCE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Azongo

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M061141**
PROJECT	Spatial Analysis of Schistosoma haematobium Infection among school children in a rural sub-district of SA. An application of......
INVESTIGATORS	Mr KD Azongo
DEPARTMENT	School Public Health
DATE CONSIDERED	06.11.24
DECISION OF THE COMMITTEE*	APPROVED subject to submitting a copy of the previous ethics clearance as well as written permission to use the data

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 06.11.28 **CHAIRPERSON** ....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Mr E Marindga

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES