Prevalence and intensity of *Schistosoma haematobium* in KwaZulu-Natal, South Africa

Investigating the prevalence and intensity of Urogenital Schistosomiasis among school going pupils in rural ILembe and UThungulu Health Districts, KwaZulu-Natal

by

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DECLARATION

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PREFACE

Urogenital schistosomiasis, a neglected tropical disease, has been receiving increased interest. This is because it could be associated with diseases such as the Human Immunodeficiency Virus, genital cancers, sexually transmitted diseases and liver diseases. Apart from the unpleasant symptoms associated with schistosomiasis, the prevalence of people infected with *Schistosoma haematobium*, the causative agent must be known and mass treatment programmes using praziquantel against the disease need to be implemented. In this dissertation the prevalence and intensity of urogenital schistosomiasis among school going pupils in rural ILembe and uThungulu Health Districts of KwaZulu-Natal province, South Africa is described. Knowledge of the current prevalence and intensity of urogenital schistosomiasis will provide useful information that enables policy makers and health care workers to take the necessary actions to control urogenital schistosomiasis.

DEDICATION

I would like to dedicate this dissertation to the children of Africa, my family and the University of KwaZulu-Natal for providing a platform for my education.

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ABSTRACT

Background and objectives: Urogenital schistosomiasis is a neglected tropical disease caused by the parasite *Schistosoma haematobium,* which is receiving increased attention, due to its reported association with diseases such as the Human Immunodeficiency Virus, genital cancers, sexually transmitted diseases and liver diseases. Symptoms of urogenital schistosomiasis include haematuria, frequent urination, tiredness and a decrease in the cognitive ability of children. The prevalence of S. *haematobium* infection needs to be known and mass treatment programmes against the disease implemented.

The aim of this study was to investigate the prevalence and intensity of *S. haematobium* infection. The objectives were to determine the prevalence and intensity of *S. haematobium* among school going children in ILembe and uThungulu Health Districts of KwaZulu-Natal province, to determine if there is an association between school location, sex, altitude, temperature and the prevalence of schistosomiasis and to assess the need for mass treatment campaigns.

Methods: In this study, 6265urine samples were collected for analysis using dipsticks from boys and girls attending rural public schools in these health districts. The prevalence and intensity of *S. haematobium* infection was calculated and thereafter associations with temperature, altitude and distance to the nearest river were investigated. Descriptive and analytical statistics were undertaken, the latter using a correlation coefficient and a linear regression ($p<0.05$) (Confidence Interval (CI) 95%).

Results: The prevalence of schistosomiasis for boys in ILembe was 40% and girls 39% and in uThungulu was 56% and 53% in girls and boys respectively. Most infection was the dominant intensity in both the Districts. There was a significant inverse relationship between prevalence of schistosomiasis and altitude ($p<0.05$). Associations between prevalence and distance of school to the nearest river were non-significant, and the average minimum summer temperature also showed a positive relationship but that was non-significant ($p>0.05$).

Conclusion: In both the Districts, the prevalence fell in the category that is recommended by the World Health Organisation for mass treatment. This information alerts health care workers to take the necessary actions to combat schistosomiasis infection and the transmission of urogenital schistosomiasis by providing mass treatment with praziquantel. Mass treatment in endemic communities impacted by schistosomiasis can significantly reduce the morbidity caused by the disease. Furthermore, treatment at an early age can help avoid complications that would predispose individuals to the risk of HIV. In endemic areas, public education about the disease should be prioritized. Furthermore clean water sources should be provided for communities at risk to prevent reinfection

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ACRONYMS AND ABBREVIATIONS

FGS	Female Genital Schistosomiasis
HIV	Human Immunodeficiency Virus
KZN	KwaZulu-Natal
MTC	Mass Treatment Campaign
PCR	Polymerase Chain Reaction
PZQ	Praziquantel
STI	Sexually Transmitted Infection
STH	Soil Transmitted Helminths
SAWS	South African Weather Services
TB	Tuberculosis
WHO	World Health Organisation

CHAPTER 1: INTRODUCTION

The neglected tropical disease, urogenital schistosomiasis, is a public health challenge in many developing countries. South Africa is one of the countries endemic for urogenital schistosomiasis, which affects both sexes [1]. An estimated 750 million people worldwide and 200 million people in Africa, including sub-Saharan Africa are infected and impacted on by this disease [2]. This water-borne disease, urogenital schistosomiasis is caused by the parasite *Schistosoma haematobium, which* is carried by a specific intermediate host snail [3]. People exposed to fresh water containing the intermediate snail are at risk of infection by schistosome miracidia, which enters the human host to cause schistosomiasis. Urogenital schistosomiasis has deleterious effects and symptoms, which include anaemia, a lack of ability to focus or process new information and other target organ dysfunctions [3-4]. Morbidity from urogenital schistosomiasis is manifested by haematuria, dysuria frequent urination [4]. Urogenital schistosomiasis is also regarded as a predisposing factor for infection with Human Immunodeficiency Virus (HIV) [5-6].

Schistosomiasis can be diagnosed microscopically (eggs in urine), or using Polymerase Chain Reaction (PCR), but haematuria is the most common symptom of urogenital schistosomiasis, and detection of blood in urine remains a standard method of diagnosing *S. haematobium* infections [2, 4, 11, 16 and 28]. Urine reagent strips are a good tool in the detection of haematuria as a proxy diagnostic method for *S. haematobium* infection [7]. The World Health Organisation (WHO) recommends mass treatment of schistosomiasis in regions of high prevalence and intensity, with a particular focus on children who usually exhibit the highest prevalence and intensity of infection [2, 10, 15 and 16]. Although there have been studies undertaken in the 1980s and 1990s [40-41], there exists at present little information available for the period of the past decade. It is documented that *S. haematobium* is more common in areas of low socioeconomic status [2-4]; since in poverty stricken areas health facilities are either remote or inadequate [15]. This poses a challenge for the rapid diagnosis, treatment and eradication of *S. haematobium* infection. However, the development of urine reagent strips (also called dipsticks) has revolutionized this challenge because they are easy to use, convenient and time efficient [14-15]. Poverty stricken areas are at risk of infection because they usually lack adequate safe water sources and people are thus compelled to live in contact with river or dam water contaminated by *S. haematobium* [2, 19]. Children and farm workers (involved in agricultural and fisheries industries) are at a heightened risk because of frequent contact with fresh water [16, 19 and 30].

Urogenital schistosomiasis is a latent and chronic disease, a factor resulting in the damage that it exerts develops relentlessly over time, a progression occurring due to adult worms producing eggs which disrupt various tissues and organs [4, 23]. The parasite eggs trapped in the body can induce an

adverse immunomodulatory effect that can favour the progression of other diseases [6, 22]. Such a process occurs because parasite eggs are small in size/dimension and can travel by blood and have the potential to breach membrane tissues that provide barrier protection against parasite, bacterial and viral entry [4-6, 22, 33 and 34]. Research now indicates that there exist several identifiable diseases such as cancer, HIV and sexually transmitted infections (STIs) associated with *S. haematobium* infection [5-6, 13 and 23].

This dissertation consists of the following chapters: Chapter 1. Introduction. Chapter 2. Literature review, Chapter 3. Methodology, Chapter 4. Results, Chapter 5. Discussion and Chapter 6. Conclusions and Recommendations. These chapters present the background of the current schistosomiasis situation, provide information about the Health Districts in which the data were collected and report on the current prevalence and intensity of urogenital schistosomiasis. It also presents and focuses upon the relationships that exist between the prevalence of *S. haematobium* infection and abiotic factors. Abiotic factors are those variables which are not alive but exert a substantial influence on the survival and reproduction of the intermediate host snail [8]. Abiotic factors that were explored in this study were the average summer temperature, altitude above sea level and distance from the school to the nearest river. It is crucially important to investigate the associated prevalence with abiotic factors because this can serve as bio indicators identify and to suggest specific areas that could be at risk of *S. haematobium* infection peaks [9].

AIM AND OBJECTIVES

The aim of this study was therefore to investigate the prevalence and intensity of *S. haematobium* in school going pupils in rural public schools in the ILembe and uThungulu Health Districts of KwaZulu-Natal in 2012/2013.

The objectives were:

- To determine the prevalence and intensity of urogenital schistosomiasis;
- To determine if there is an association between school location, sex, altitude, temperature and the prevalence of urogenital schistosomiasis; and
- To assess the need for a mass treatment campaign.

It is important to evaluate the prevalence and intensity of urogenital schistosomiasis because such an undertaking identifies and categorizes areas that are eligible for mass treatment [10].

CHAPTER 2: LITERATURE REVIEW

INTRODUCTION

Schistosomiasis is commonly referred to as bilharzia and is known as a disease associated with playing, swimming or other contact in contaminated fresh water. It is second most devastating parasitic infection in the world after malaria [11-12]. It has been estimated that the disease affects 750 million people worldwide, of whom 200 million reside in Africa, including sub-Saharan Africa [2].

Schistosomiasis is caused by schistosome flukes or worms found in infested water. Humans are vulnerable to contract the parasite by their being in contact with such water, either by swimming, crossing rivers, farming, irrigation and / or washing [2-3]. Upon contact with the infested water the parasite known at this stage as cercariae , the source which freely swim in the water bodies, requires an intermediate snail host specific for the schistosome species e.g. for *S. haematobium*, the snail, is *Bulinus africanus*. The parasite develops in the snail and emerges as a miracidium [3]. When the miracidia come in contact with the skin of the vertebrate host, it can easily penetrate the skin [4]. Once the parasite has invaded the host skin (human) it further migrates to any habitable location through either the movement of blood or the lymphatic system, where it will further undergo a series of maturation steps prior to invading the tissues of the organs [3, 11]. Depending on the schistosome species, the organ of choice could be the urinary tract (*Schistosoma haematobium*), or if *Schistosoma mansoni*, the liver, intestines, kidney or on rare occasions the spleen or cardiac muscle. Because the eggs of some species are so small, they are able to pass the blood brain barrier reaching the brain [3-4]. With *S. haematobium* infections, the parasite usually resides in the bladder and the genitals. Once the eggs have undergone full maturation into adult worms, the male and female worms mate to produce thousands more eggs. Direct disease related mortality is low and of the infected population, only 10% thereof progress to serious disease, although approximately 60% of those infected become mildly symptomatic [4]. The disease can be latent in a host. It is not the parasitic infection itself that causes disease but it is the host's immune response to the parasite that results in disease [4].

The morbidity caused by *S. haematobium* is manifested by haematuria, frequent urination and dysuria [4]. Loss of blood in urine can lead to anaemia [13]. There also occurs the occurrence of micro-damage to the genital organs, a factor which is attributed to the schistosome parasite and may indeed cause bleeding [5]. The World Health Organisation (WHO) fact sheets state that urogenital schistosomiasis may inflict both male and female infertility [44]. A reduced cognitive function has

been reported from patients with schistosomiasis [2]. Further children infected by schistosomiasis may also experience difficulties with learning and memory [2].

Since the presence of blood in urine is a common symptom of schistosomiasis, detection of haematuria serves as a standard method of diagnosing *S. haematobium* infections [7, 14 and 39]. Meents and Boyles, in 2010 reported that there was no significant difference between results obtained from reagent strip readings compared to those recorded in microscopy readings [39]. There are a number of commercially available urine reagent strips which measure the intensity of blood in urine [7]. After being dipped in urine for a minute, dipsticks change colour proportionate to the concentration of blood in the urine sample [7]. The observed colour change can then be matched with a standard colour chart from the supplier. This allows for the quantification of the concentration of blood in the sample and can be recorded as a numerical value [7]. The numerical value is informative if the sample proves negative (0) or positive (+) and, if positive, whether it is of a light intensity (1+) or heavy intensity (3+) infection [14].

The term urogenital schistosomiasis was ascribed to *S. haematobium* infection after it was observed that this infection involved both the urinary and genital tract [50]. The term has replaced the former designation urinary schistosomiasis, where it was previously believed that *S. haematobium* infection affected the urinary tract and the term genital schistosomiasis, where it was surmised that *S. haematobium* infection affected the genital tract. The term urogenital schistosomiasis was agreed upon because it acknowledges an infection that affects both the urinary and genital tracts [50].

EPIDEMIOLOGY AND CLASSIFICATION OF SCHISTOSOMIASIS

Of the over 200 million people currently infected in Africa and sub-Saharan Africa [2], approximately 80% of the infected individuals originate in under-developed/developing countries, where there may exist such factors as water scarcity and / or poor excreta disposal systems. Such a reality may be due to a lack of adequate latrine facilities [2, 11]. Schistosomiasis may affect a large percentage of school going children and in many such areas the egg excretion declines after the age of fourteen [2]. Schistosomiasis is caused by the parasite from the genus *Schistosoma*, which embraces a variety of species [4]. The family *Schistosoma* includes *S. haematobium,* a particular variant which is associated with urogenital schistosomiasis. The symptoms therefore usually include dysuria and haematuria (see page 8-9). It remains a common infection in India, Middle East, Africa and the Pacific [4]. *S. mansoni* is identifiable also in the family *Schistosoma* and is common in the Caribbean, South America, Egypt and Africa: it usually affects the intestines and the liver [4]

Brooker, in 2006 has stated that the major determinants of disease transmission are human behaviour, socioeconomic status and snail ecological factors. Stensgaard *et al.*, in 2013 also concurred with the fact that distribution of schistosomiasis is regulated by climate, ecological, economic and social factors. The parasite establishment and pattern of distribution is determined by the distribution and abundance of the intermediate host, the fresh water snail *Bulinus africanus* [3, 45]. The main etiological factor for schistosomiasis infection is the *Schistosoma* spp. and derives from it three intermediate host snails, namely *B. africanus, B. globusus* and *Biomphalaria pfeifferi* [45]. In an area where these intermediate host snails can reproduce and grow with no hindrance *S. haematobium* and *S. mansoni* will thrive and the infection rate might indeed be high [1-3, 16 and 45]. These snails have an optimal temperature for snail development and survival at around a temperature of 25^0 C and their survival is restricted above 30^0 C [3, 9 and 45]. Such a circumstance could be the reason that caused schistosomiasis to disappear from the southern parts of the Eastern Cape Province [12]. Due to the phenomenon of global weather change, the temperature in the southern parts of this particular region has increased by one or two degrees Celsius, a factor which has rendered it difficult for the snail to survive in such a geographical area [12]. Owing to such a development the transmission and prevalence of urogenital schistosomiasis infection was reduced in that zone. Global weather change is further likely to cause a reduction in the transmission of urogenital schistosomiasis in other areas, whilst in some areas it might additionally increase the transmission of urogenital schistosomiasis [43]. Stensgaard *et al.* reported that *B. pfeifferi* the intermediate snail host is likely to be favoured by the global weather change in some parts of South Africa. This may occur because this country contains a multitude of areas which are suitable for snail development, yet are not inhabited by *Biomphalaria* snails [43]. Joubert et al. showed that the survival of *B. africanus* and *B. pfeifferi* was of similar nature

and both these snails could not survive at very high temperatures (>39◦C), whilst *B. globusus* could continue to exist for a longer period even at high temperatures [45].

In South Africa, specifically in KwaZulu-Natal it is *S. haematobium* infections which feature as being the more prevalent and problematic concerning causes and schistosomiasis infection was perceived to occur at a rate higher among boys in the 5-10 years' age group [16-18]. The reason for such a factor could be due to young boys being more adventurous and willing to play in river water [19]. However, the prevalence of schistosomiasis identifiably increases among girls in the 10-15 years' age group [16]. The reason therefore could be due to girls in this age group being obliged to perform housekeeping chores such as washing and cleaning, hence increasing the chances of such females to more frequent contact with river water [19]. However, Der *et al.* in 2015 noted that it in Ghana the prevalence of urogenital schistosomiasis peaked between the ages of 20 and 29 years. Such a finding suggests that the prevalence of urogenital schistosomiasis differs between countries and provinces, possibly because of the existence of different social norms and lifestyles between specific ethnic groups living in different locations [20]. In a cross sectional study performed in southwestern Ethopia a 35.9% prevalence of *S.haematobium* was obtained among primary school children [56]. The study also reported children whom had a father working as a farmer had a 1.96 Odds Ratio to being infected with *S.haematobium* [56]. In a study performed in a small informal settlement in Kenya, schistosomiasis prevalence was 36% and yet community awareness about disease remained low [57].

ABIOTIC FACTORS

Liao *et al.* found the prevalence of *S. haematobium* to have a geographical preference. Areas at low altitude (±250 m) exhibited a higher prevalence of *S. haematobium* infection compared to areas at a high altitude (+1000 m) [18]. The reason therefore could be due to the life cycle of the intermediate host not being well adapted to survive in high altitude areas because of the factor of varying temperature [18]. In KwaZulu-Natal the helminth control programme found that schistosomiasis is prevalent at altitudes between 300-400 m [16, 42]. *S. haematobium* and other parasite infection prevalence were reported to be high in areas that are located near to the coast (approximately 50 m above sea level), but were significantly lower in areas of high altitude (1700 m) [42].

It has been demonstrated that the intermediate host of urogenital schistosomiasis, *B. africanus* retains a general preference to inhabit areas with cooler climatic conditions [8], hence the use of such a term as tropical disease. Oniya *et al.* in 2013 reported that schools in South Africa which were located near rivers reported a higher prevalence of *S. haematobium* compared to those schools that were situated further from the rivers [21]. *B. africanu*s has a notifiable preference for habitats with still and muddy water [8]. Other factors impacting on the snail distribution include the salinity and the chemical composition of the river or stream [9, 18]. The *B. pfeifferi* snail can thrive in water with a pH of 6.3-8.6, but as with other snails, it simply cannot survive for a long period in water contaminated with sewage and industrial waste [48]. De Kock in 1973 observed that there is an optimum temperature range which is required for snail survival and reproduction, which is identified to occur between 23 and 26°C [8]. It is thus important to understand the abiotic factors contributing to the survival and distribution of the host snail, because transmission of schistosomiasis is regulated by these factors.

PATHOPHYSIOLOGY & CLINICAL MANIFESTATIONS

The clinical manifestations of schistosomiasis are most likely caused by the host's immune response to the foreign material. The majority of these schistosome eggs are passed out in faeces and urine, whilst some are trapped within the host body, serving as antigens which arouse the host immune response. Activation of the non-specific and the specific immune response results in a series of changes which may lead to pulmonary or systemic acute manifestations [4]. Upon exposure to the parasite there are recognizable acute manifestations of such conditions as swimmers' itch and cercarial dermatitis. The latter is a short lived skin eruption due to the penetration of the parasite into the host [4]. Monokines attracting more cytokines lead to transient bronchial hyper-reactivity, referred to as bronchopneumonia [4]. As the worms mature, Katayama syndrome may develop, characterised by eosinophilia, fever, arthralgia and vasculitic skin eruptions [4]. The parasite antigens induce cell-mediated inflammation, an occurrence which leads to lesions causing granulomatous deposits [4, 22]. The eggs which are trapped inside the host body lead to the formation of schistosomal granulomata, a development which can obstruct the urethro-vesical junction, hence affecting the upper urinary tract. They can alternatively obstruct the porto-hepatic vein affecting the liver [4]. The anatomical location of where the deposited parasite eggs travel and reside, determines the extent of organ damage and level of organ dysfunction, a phenomenon resulting from the number of eggs trapped in that organ [20]. Urogenital schistosomiasis was noted as a risk factor for eliciting glomerular damage to the kidneys, and in such patients chronic kidney disease may develop [23].

The parasite eggs trapped in the tissues of organs can either remain viable or they can be calcified, with the subsequent attraction of effector cells of the immune response, which further results in the abnormal proliferation of blood vessels [22]. The integrity of blood vessels becomes challenged, and it has been reported that *S. haematobium* infection increases the vascularity of blood vessels in the genital mucosa [22]. The increased density of micro-blood vessels may lead to bleeding, which could increase and facilitate the transmission of HIV [13, 22].

Due to the design of the human body, excretion of normal waste and unwanted substances travel down to the kidney, pass through the ureters to the bladder and finally travel through the genital organs for excretion. When this process occurs there are a number of eggs which are carried by the blood and which become trapped in these areas [20]. Parasite eggs are generally found trapped in these lower body parts on post mortem histological slides, in areas such as the pelvis and the kidneys, as well as the male and female genital organs [20]. Parasite eggs trapped in the kidney may elicit an ongoing obstruction to the normal functioning of the kidney [23]. Such a process can result in the dysfunctioning or imbalance of the hydrostatic forces controlling kidney function and can cause kidney disease if this obstruction is sustained [23]. As a result of the disturbance of the normal kidney

function proteins may be lost in the urine; hence proteinuria and albuminuria may also develop among infected patients [23]. In a review done by Der *et al.* in 2015 it was observed that there was a significant development of prostate dysfunction (43%), scrotal carcinoma (29%) and sperm cord damage (29%) [20].

Parasite eggs trapped in the above organs of males may cause fibrosis, a phenomenon associated with male infertility. There were a few cases of iliac fossa (pain due to ova clogging the appendix) that were also reported from patients with *S. haematobium* infection [20].

Formation of granulomata in the kidneys can cause oedema and back pressure pains. In the liver eggs mature in the hepatic sinusoids causing lymphoid hyperplasia resulting in hepatomegaly [4]. The parasite and its eggs can migrate to ectopic organs. Although not prevalent in South Africa, *S. japonicum* eggs are so small in magnitude that they can migrate to the brain causing cerebral schistosomiasis; other parts of the brain that become infected are the basal ganglia, cortex, subcortical white matter and internal capsule [4]. If the infection is severe it can lead to neuro-schistosomiasis where there occurs increased intracranial pressure, multiple affected neurons and can cause disease of the spinal cord [4].

In endemic locations the parasite can migrate to genital organs causing genital schistosomiasis. Genital schistosomiasis results in characteristic lesions of the ovaries, fallopian tubes, epididymis, prostate and testicles which may cause infertility [4, 17]. Female genital schistosomiasis (FGS) is a clinical condition where the ova have been deposited in the ovaries or fallopian tubes, or alternatively in the lower genital tract, developments leading to ulcerative lesions around the cervix and vagina [5, 24]. Parasite eggs trapped in the ecto-cervix of the vagina can cause lesions where the area around the eggs form fibrous tissue leading to the formation of areas known as sandy patches [5].

Recently there has occurred an observation that the eosinophils around these tissues cause inflammation, the development where often retains a consistent populous appearance, referred to as rubbery papules [25]. There is growing evidence which suggests that FGS could increase the chances of an individual being infected with HIV if the genital barriers have been breached by parasite [5, 22, and 24]. However, the genital tract lesions caused by infection with *S. haematobium* were not reversed two years later after treatment with praziquantel tablets had been conducted [19]. *S. haematobium* is the most prevalent form of schistosomiasis in KwaZulu-Natal province [9, 15-16].

DIAGNOSIS

The major symptoms of schistosomiasis caused by *S. haematobium* are dysuria, frequent urination and haematuria [2, 4 & 26]. Diagnostic instruments for schistosomiasis either depend on detecting haematuria, a symptom of *S. haematobium* or detect the passing out of oval shaped schistosome eggs in urine. Furthermore, the usage of a questionnaire asking for these symptoms may serve as a diagnostic indicator [15, 27].

Surveys asking participants if they have experienced red urine or pain when urinating can be used to estimate the prevalence of *S. haematobium* [16]. However, the prevalence was approximately 40% less sensitive when compared with microscopy and dipstick results [16]. This can be due to the fact that if the disease intensity is low or at an early stage of infection, blood in urine may not be evident to the naked eye. This allows for the recording of false negatives. Therefore, questionnaires and surveys are a good tool only in the estimation of prevalence of disease in heavily infected areas and in large scale interventions [16]. The conduct of these can be used as a screening tool for the referral of patient for diagnosis and treatment at clinics [15].

Mild levels of disease infection cause dysuria and it is estimated that the incidence thereof can be associated with a passage of 10 eggs per 10 mL of urine. If there is visible passage of blood in urine, such a development is associated with more than 100 eggs per 10 mL urine [12]. Ibironke *et al.* in 2012 reported that Polymerase Chain Reaction (PCR) purportedly retains a 100% sensitivity and specificity for the identification of DNA for the parasite from urine [28]. However, even dead ova may create clinical problems and those are not detected by PCR [28].

SENSITIVITY AND SPECIFICITY OF DIAGNOSTIC INSTRUMENTS

The two most documented diagnostic instruments for urogenital schistosomiasis involve the egg count method using microscopy and the detection of haematuria using reagent strips. It may necessitate a certain period for the eggs to develop into adult worms once inside the host. So this makes the sensitivity of both these tests to be imperfect, especially at the onset of infection [14]. However, these instruments remain regarded as the gold standard tools for the diagnosis and the estimation of prevalence for urogenital schistosomiasis [14]. Appleton and Kvalsvig in 2006 reported a study in KwaZulu-Natal which showed that the prevalence of schistosomiasis estimated using microscopy reading or reagent strips did not significantly differ. Microscopy reading involves the counting of the number of eggs passed out in 10 ml of urine. Hence microscopy reading requires the presence of a

laboratory set up. The sensitivity of microscopy reading can be reduced if urine was not collected at the correct time of the circadian cycle [16]. This occurs between 10:00 am and 2:00 pm.

Urine reagent strips were estimated to have a sensitivity of 81% and a specificity of 89% in the detection of egg positive urines [14]. For active *S. haematobium* infection, the sensitivity and specificity of reagent strips was estimated to be 82 and 97% respectively [14]. The presence of haematuria detected using reagent strips serves as a faster indicator of active *S. haematobium* compared to egg detection [14]. The sensitivity was 71% higher for reagent strips when it was assumed that both instruments were imperfect measures of *S. haematobium* [14]. However, the sensitivity of reagent strips is less sensitive in low intensity infection areas and in areas that have received previous treatment for schistosomiasis [14]. Reagent strip performance is more sensitive in school-going pupils as compared with adults [14]. This factor could be due to the fact that adults have matured vascular, endothelial and epithelium tissue that may be less receptive to the parasite causing genital bleeding [7, 14].

Reagent strips possess receptors that react with the haeme group of haemoglobin in urine, thereby causing the specific block to change colour [7]. The observed colour change is then matched with a reference colour chart and the quantity of blood in urine can be expressed as a numerical value [7]. Hence reagent strips provide quantitative results [7]. The numerical values represent an estimation of the quantity of erythrocytes in urine [7].

The values can either be negative [-/0] (non-traceable levels of blood in urine; no haematuria), light positive [1+] (associated with the presence of 5-10 erythrocytes/µl of urine); moderate positive [2+] (presence of ±50 erythrocytes /µl) and highly positive [3+] (presence of ± 250 erythrocytes /µl of urine) [16].

For optimum reagent strip performance it is essential that they are stored, handled and read correctly, at the exact appropriate time [14]. The expiry date of the strips has to be checked before using them, operating instructions need to be strictly adhered to, and also avoidance of contaminating strips with additives or contaminants is essential for ultimate performance of the reagent strips [14].

Micro-haematuria detected using reagent strips serves as a useful estimator of the prevalence of urogenital schistosomiasis [14]. Prevalence proves a useful guide in determining the need for control intervention programmes [14]. Reagent strips are regarded as a gold standard diagnostic test in public health campaigns aimed at combating urogenital schistosomiasis [14-16]. They serve as an efficient rapid, inexpensive and employable with ease instrument for determining the prevalence of urogenital schistosomiasis [15-16]. False-positive reagent strip results could arise if subjects are going through the menstrual cycle, have sexually transmitted infections that may cause genital bleeding, or have

bacterial cystitis (which may cause epithelial tissue damage in the renal tract or the genitals) [14]. However, use of reagent strips for the control of urogenital schistosomiasis is an efficient diagnostic tool, especially in rural settlements where laboratory equipment may be scarce [15].

TREATMENT

Prevention and the eventual eradication of schistosomiasis can be achieved by the combined provision of clean water, proper sanitation, health education and mass drug treatment to school-going children [2]. The effective treatment of schistosomiasis is usually achieved by single dose oral administration of the drug praziquantel (PZQ) [40 mg/kg] [2]. The drug causes side effects such as vomiting, nausea or rash which are tolerably mild and possibly caused by the death of the worms and destruction of its eggs [12, 28].

The WHO recommends that all children from endemic communities be offered treatment if the prevalence and intensity of the disease is high (above 20%) [2]. Some poor communities still lack access to health facilities and may indeed remain uneducated about the disease and its sequel, rendering it difficult to eradicate the disease [11]. Odhiambo *et al*. revealed that community awareness about schistosomiasis was low despite high prevalence in Western Kenya [57]. In endemic areas such as the coastal regions of KwaZulu-Natal there are many communities which are at risk and are thus eligible for regular mass treatment with PZQ tablets [19]. Praziquantel is not effective for mature worms. Therefore, appropriate treatment should be undertaken after the summer period when water contact is optimal [29]. There exists a need for intervention strategies and school learners in endemic areas need to receive mass drug treatment because they are at a higher risk of contracting the infection of urogenital schistosomiasis, owing to their frequent contact with contaminated water. Depending on the prevalence and intensity in an area, it may be required that the community is treated once a year, in autumn for a period of three years or once a year for two years [10]. Prevalence also determines whether the entire community should receive treatment or if only the diagnosed cases ought to be treated [10].

Another control tactic for schistosomiasis entails the addition of a chemical or plant extract in a river/dam which can kill the intermediate host snail [58]. Chemicals and plant extracts which kill the intermediate host snail are called molluscicides. The use of molluscicides has been beneficial in the reduction of schistosomiasis for other countries; however there is little scientific literature available in South Africa to indicate its use [58].

Table 1: Categorization of schistosomiasis prevalence (adapted from WHO, 1998)

Category	Prevalence	Percentage	Recommendation
Ⅰ	High prevalence	≥ 50 %	Everyone in that particular community is subjected to treatment irrespective of status, age or sex (treatment once a year for a period of 3 years)
Ⅱ	Moderate prevalence	≥ 20 % ‹ 50 %	School-age children are subjected to treatment (treatment must be done 1-2 years)
Ⅲ	Low prevalence	< 20 %	Only positive cases are selected for treatment every two years

Source: WHO (1998)

ASSOCIATION OF SANITATION/ HYGIENE WITH SCHISTOSOMIASIS

Sanitation in public health concerns the provision and accessibility of clean water and adequate sewage disposal for all members of the community [2]. The availability of water in households was found to be associated with a decreased prevalence of schistosomiasis infection [26]. It was noted that in communities with proper latrine presence and an adequate water supply there occurred a decrease in the prevalence of infection of both water-borne and soil-borne diseases [2, 26]. Esrey *et al.* in 1991 reported that provision of piped water per household reduced the rate of disease infection by 30% in Saint Lucia. The WHO also confirmed that provision of clean water may reduce the prevalence of water-borne and soil-borne diseases [10].

The transmission and infection rate was significantly higher (by a two-fold measure) in those communities that lacked proper sewage disposal systems and had a scarcity of clean water [2, 26 & 30]. Among such poor communities there also occurred a high prevalence of spleen enlargement (splenomegaly) [4]. There is supporting evidence that a prolonged poor supply of water was a factor associated with severe schistosomiasis [11, 26].

Such observations emphasize that an improvement of water supply and sanitation can reduce the rate of morbidity and severity of diarrhoeal diseases, and hence enhance child survival and development [26]. Provision of praziquantel (for anti-schistosomiasis treatment) alone cannot eliminate the burden of schistosomiasis as it fails to prevent reinfection [2]. Such implementation of treatment should

preferably be accompanied by provision of access to safe water resources and proper sewage disposal systems [2, 15], together with the implementation of health promotion and health education being provided at the household, school and community levels to increase awareness of the existence of urogenital schistosomiasis [15].

In KwaZulu-Natal, ILembe and uThungulu Health Districts are typical rural communities where poverty and unemployment are prevalent [31-32]. The ILembe IDP of 2012 reported that there are proportions of inland rural communities that are solely dependent on river or pond water for survival [31]. In uThungulu Health District there features a figure of 55% of households which lack access to clean water and sanitation [32].

CO-INFECTION AND COMORBIDITY

Millions of people remain exposed to dangerous levels of biological and chemical contaminants [2]. People infected with urogenital schistosomiasis may also be infected with other parasitic (e.g. Soil transmitted helminths (STHs)), bacterial (e.g. Tuberculosis (TB)) or viral infections, such as Hepatitis C Virus (HCV), Human Papilloma Virus (HPV), Hepatitis B Virus (HBV) or Hepatitis A Virus (HAV) [13, 33]. The liver complications, hepatomegaly and albuminuria may be exacerbated in patients who are already infected with one disease [4]. Viral co-infection with HIV can place a heightened stress on the host immune system and can have negative effects on the patients [4]. The genital epithelium damaged (at microscope level) by the trapped ova affords opportunity of entry to other pathogens such as sexually transmitted infections (STIs) and HIV [20]. Communities that lack a proper supply of clean water are at a dual risk of schistosomiasis and soil transmitted infections [2]. Such a situation means that the morbidity from urogenital schistosomiasis may be more evident among those patients. This factor is due to more than one disease attacking the effector cells of the immune response. An example hereof is the fact that there occurred a general association of cancer and kidney disease development among patients impacted by schistosomiasis [20].

The combined effect and potency of co-infection in individuals affected by more than one neglected tropical disease is explained as follows [4, 13]. An appropriate analogy is the act of pushing a car in a straight road. We will assume that the car is a person and the number of people pushing the car is the number of diseases that a person can have. Now, if the car is pushed by one person, the car moves very slowly in relation to the force that is being applied to it. But if two or more people are pushing a car the resultant applied force will be greater to the extent that the car moves at a faster speed. The more people we have pushing the car the faster the car will travel. A similar scenario with co-infection is discernible if a patient has more than one infection the morbidity and burden of disease will be heightened putting the patient at an elevated risk of disease propagation and symptom manifestation. STHs infections are highly prevalent in communities that lack adequate sanitation and clean water and children are thus at risk of multiple infections [16]. Particular areas exist where there is more than one disease endemic in a location, and an example hereof is KwaZulu-Natal province where *S. haematobium* infections are endemic and HI Virus and TB are also endemic in the province. Although in northern KwaZulu-Natal near the Mozambique border malaria is endemic, this is not the case further south in uThungulu and ILembe Health Districts, where this study took place [51]. There is a geographical overlap between urogenital schistosomiasis, HIV/AIDS and TB [13]. Therefore, it is possible that one individual can be infected with more than one infection.

NECESSITY OF MONITORING PREVALENCE AND INTENSITY OF SCHISTOSOMIASIS

In epidemiology prevalence is defined as a measure of the proportion of people that present with a particular disease. It is a useful measure to detect the extent of disease and it usually expressed as a fraction, percentage or number of infected individuals per 10 000 or 100 000 [10]. Intensity is defined as the measure of the strength, degree, level or severity of disease [10]. It was reported that urogenital schistosomiasis is a parasitic infection with a high prevalence in endemic areas [27, 42].

From the explanation above it can be deduced that the prevalence of a disease is a potent indicator of the extent of the disease in a particular area. The prevalence and intensity of disease are determined by factors such as age, social behaviour (i.e. contact of individuals with contaminated water bodies), season (transmission usually occurs during the hot and humid summer) and the distribution of the intermediate snail host [19, 29]. These factors play a crucial role in determining the prevalence and intensity of urogenital schistosomiasis in a given community. Before mass drug treatment, campaigns are undertaken it is essential to monitor the prevalence and intensity of disease in order to determine the potential effectiveness of the intervention.

Saathoff et al. in 2004 stated that intensity is a reliable marker of treatment success and a good indicator of morbidity. Intensity of *S. haematobium* infection is expressed as the number of eggs per 10 ml of urine [29, 34]. It has been noted that heavy infections of urogenital schistosomiasis are associated with low levels of haemoglobin, a factor which subsequently results in a low red blood cell (RBC) count and, hence, anaemia [35]. There are many consequences of anaemia, such as tiredness and lack of ability to focus [2, 18, and 23].

To ascertain the presence and severity of micro haematuria possibly attributed to urogenital schistosomiasis, urine reagent strips are used to detect the presence of blood in urine [16, 35]. Reagent strips measure the quantity of blood in urine and, as described previously (see page 12), the results can be recorded in a semi-quantitative manner [7].

Once prevalence has been established it can be concluded whether or not a particular region requires a Mass Drug Treatment Campaign (MTC). In a study conducted by Koukounari *et al.* in 2007, it was observed that the prevalence of urogenital schistosomiasis was initially high prior to the MTC with praziquantel (PZQ) in Burkina Faso [46]. Consequently, intensity or level of schistosomiasis and level of anaemia and micro-haematuria can be reduced by large scale administration of PZQ. Hence, morbidity of disease is restricted by such treatment interventions [2]. Prevalence and intensity of

disease decrease and become significantly low in endemic regions that have been subjected to MTC over time for urogenital schistosomiasis. It is for these reasons mentioned above that the WHO recommends that regions that have a high prevalence and intensity of urogenital schistosomiasis are subjected to mass treatment campaigns. Learners that were treated for schistosomiasis displayed a decreased incidence and prevalence of haematuria and proteinuria [23]. Mass treatment should therefore continue to remain a priority to reduce the burden of schistosomiasis and its associated morbidity [23].

CONCLUSION

It is important to evaluate the prevalence and intensity of urogenital schistosomiasis because it provides information on the status of infection among communities. Urine reagent strips can be a good estimator of prevalence of the disease, which can help categorise a community according to the WHO guidelines for treatment. It would be expected that the prevalence and intensity of urogenital schistosomiasis will be high in communities in which the disease is prevalent and the community has not received any prior mass treatment with PZQ. However, if interventions such as mass treatment have been performed it would be expected that the prevalence of disease would consequently be decreased. These are useful measures to monitor the success of mass treatment and assist in determining the re-infection rate. Development of the water and sanitation infrastructure is necessary to improve access to clean water in endemic communities, and further reduce the need for contact with contaminated water. Bridges need to be built in communities where learners are exposed to crossing rivers before they reach school, since this will reduce the incidence of reinfection. Because school going learners are mostly at an elevated risk compared to adults, treatment should target them first. Mass treatment of learners at risk of schistosomiasis may consequently allow for a subsequent reduction in the transmission rate of HIV [2, 3, 13, 15, and 16].

CHAPTER 3: METHODOLOGY

STUDY DESIGN

A retrospective analytical study was undertaken. The urine samples were collected in 2013 from a total of 6265 high school learners. These learners came from schools randomly chosen from Department of Education school lists of the rural communities in ILembe and uThungulu Health Districts in the KwaZulu-Natal province of South Africa.

STUDY SETTING

KwaZulu-Natal is home to an estimated 10 015 990 population, making it the second most densely populated province in South Africa [32]. ILembe Health District is situated on the east coast of KwaZulu-Natal, north of Durban. UThungulu Health District is located in the north-eastern region of KwaZulu-Natal. Schools were stratified and randomly selected from the 20 wards of the ILembe and uThungulu Health Districts which are below 300-400m altitude. Urine samples were collected from high school learners of the rural ILembe and uThungulu Health District selected according to a specific grade (grade 8), as learners of this age group (14-15 years) are at high risk of contracting urogenital schistosomiasis [16].

a) ILembe Health District

The ILembe Health District population is estimated to include 805 239 people living in 145 907 households [31]. The District lies between two of South Africa's biggest ports, namely Durban and Richards Bay, located on the east coast of KwaZulu-Natal, north of Durban. ILembe Health District consists of four local sub-municipalities, which are Mandeni, KwaDukuza, Ndwedwe and Maphumulo local Health Districts. The water resources in the District include the presence of the Tugela, Umdloti, Emona, Tongati, Umhlali and Umvoti river systems. Cases of water-borne disease have been reported by the rural members of the community who are solely dependent on river water for survival [31]. The population of the District has 49% people under the age of 19 years old, whilst 72% of people are under the age of 34 years old [31]. Illiteracy is common across the District, which has further been affected by the devastating and crippling effect of the HIV/AIDS pandemic [31]. The District has a number of commercial farming hubs, with sugar-cane being the most prevalent crop for commercial farming and sustenance. Of the economically active population, 48% is unemployed. In the ILembe Health District it is mostly the rural, traditional areas of the District which are most affected by poverty and unemployment. Access to clean water and sanitation are challenges prominent in the inland rural parts of the District and the District council is aware of these difficulties, as plans for improvement are under implementation [31]. Other challenges in the District include lack of health facilities in rural areas, limited waste disposal management, and the depletion of wetlands due to the

development processes being undertaken in the District [31].

(b) UThungulu Health District

UThungulu Health District is located in the north-eastern region of KwaZulu-Natal province on the eastern seaboard of South Africa [32]. The District consist of six local municipalities; UMhlathuze, Ntambanana, uMlalazi, Mthonjaneni, Nkandla and Mbonambi. The District has the third highest population in the province after eThekwini Metro (Durban) and the uMgungundlovu District (Pietermaritzburg and surrounding areas). UThungulu Health District has the largest deep-water port on the African continent (Richards Bay), which imports the largest amount of bulk cargo of all African ports [32]. In uThungulu Health District 80% of the population is rural and 53% is aged between 0 and 19 years. Women make up 54% of the population due to migrating labour patterns [32]. The District possesses favourable agricultural conditions, with extremely fertile soils and has a good climate characterized by good rainfall and remains frost-free throughout the year [23]. The area has a number of wetlands, most notably the Lake Cubhu and the greater Mhlathuze wetland system south of Richards Bay at Esikhaleni. The Phobane Dam on the Mhlathuze River is the only major dam in the area. This dam is also used to supply the agricultural needs in the area (sugarcane, citrus and cotton are the major crops in the area). There are several rivers in the District, which are; Amatigulu River, Mhlathuze River, uMlalazi River, Mfule River, Nyalazi River and Mzingwenya River [32]. In the uThungulu Health District 15% of the population 20 years or older has had no schooling, 41% had no secondary schooling and 73% had not completed grade 12 [47]. The percentage of people with no schooling has dropped from 33% to 16% in uThungulu and from 30 to 15 in ILembe Health District [47].

In the uThungulu Health District there are an estimated 115 046 households, and 55% of these households were reported to lack access to clean water and adequate sanitation [32]. The province of KwaZulu-Natal carries the highest burden of disease associated with underdevelopment and poverty in the country. The HIV prevalence for uThungulu Health District is 38% and TB-HIV Co-infection is 86% [32]. The KwaZulu-Natal province has the highest HIV prevalence rate in the country. In 2009 of the 615 000 orphans, 83% were reported to be due to the HIV and AIDS pandemic [32].

The Health District has experienced recurring shortages in the delivery of infrastructure (backlogs) for provision of water and sanitation services, especially in the rural areas. The supply to rural areas is slow due to the high costs associated with the scattered settlement patterns in these areas [32]. The implementation of the 2009 uThungulu Water Services Development Plan has reduced the rural backlogs for water supply to the Reconstruction and Development Programme standards, from 82% in 2002 to 38% in 2011. With regards to sanitation the backlog has been reduced from 80% to 69% over

the same period [32]. These percentages show that there are a significant number of people (38% and 69%) who lack access to clean safe water and sanitation, respectively.

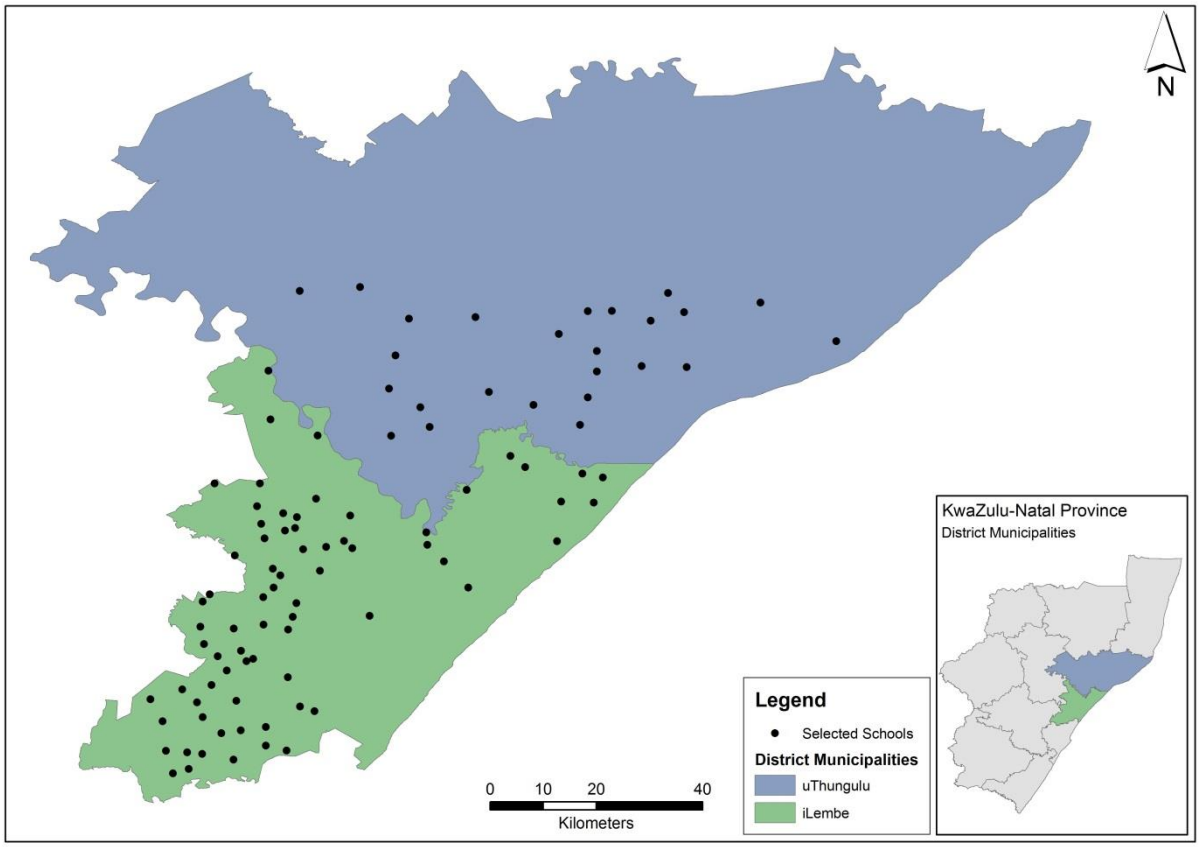

Figure 1:Locality map showing the ILembe and uThungulu Health Districts of the KwaZulu−Natal province, the black dots on the map show the location of schools which were selected for this study, n=96.Source [56].

TARGET POPULATION

School going learners in public high schools of the rural ILembe and uThungulu Health Districts were targeted. The target group is estimated to be between the ages of 12-19 years. In ILembe District 49% of the population is under the age of 19 years old [31]. In uThungulu District 53% of the population is under the age of 19 years old [32].

STUDY POPULATION

Grade 8 high school learners of the rural ILembe and uThungulu Health Districts were chosen. This grade was selected because it was reported that the prevalence of schistosomiasis peaks at 14 years of age, and learners of this age group are most likely to be in grade 8 [16, 19].

INCLUSION / EXCLUSION CRITERIA

For the dipstick collection, female learners were asked by an experienced female fieldworker if they are currently in their menstrual period. If so, the sample was not collected because this would interfere

with the results. Only students that provided written parental informed consent and who assented were included in this study.

STUDY SAMPLE

METHOD OF SELECTING SAMPLE

From the list of high schools provided by the KwaZulu-Natal Department of Education (DoE) for ILembe and uThungulu Health Districts, a random selection of 100 schools was undertaken in areas that are endemic for schistosomiasis. The schools involved in this study were stratified by the wards in ILembe and uThungulu Health Districts and included schools situated in medium and low altitude, an altitude conducive to snail development [8]. This provided a good representation of schistosomiasis endemic areas in the two Health Districts, ILembe and uThungulu.

SIZE OF SAMPLE

The school was the unit of the study. There were a total of 96 high schools included in the study, 71 schools from ILembe Health District and 25 schools from uThungulu Health District. From the schools selected the results of four schools could not be used in this study because these schools were located outside the Districts of focus (ILembe and uThungulu) for this study. The schools chosen are from the municipalities at low and medium (0-1100 m) altitude (above sea level) and situated nearest to the coast. All learners (both sexes) in the selected schools registered for grade 8 were invited to participate in the study. A total of 2940 girls and 3228 boys who were present on the day of sample collection participated in this study. The learners were not aware of the date when the data collection team would be visiting their school.

DATA SOURCES

The data base for the MTC undertaken by the KwaZulu-Natal Department of Health, assisted by the Female Genital Bilharzia study, was used to obtain the data for this study. Permission was granted by the principal investigator for the student to use this data (see Appendix B). The data were collected by well-trained fieldworkers (research assistants) using the data collection methods described below. The temperature data used in this study was obtained from the records of the South African Weather Services (SAWS). The SAWS' offices are located in Pretoria and they have the records of past temperature ranges for the whole country. In this study the average (maximum and minimum) summer temperature for the year 2013 (when the samples were collected) were requested for use in this study (see Appendix E). The altitude and the distance of a school to the nearest river was calculated using a Geographical Information System (GIS) programme called ArcMap 9.2, by a professional GIS consultant from "EDUACTION", using the geographical co-ordinates for each school provided by the Department of Education website [52].

MEASUREMENT INSTRUMENTS / DATA COLLECTION TECHNIQUES

Trained research assistants visited the schools first to explain the study and provide the documents required for parental informed consent. At a subsequent visit, male and female learners were assigned an ID number which was recorded on the honey jar used for urine collection. The circadian cycle of schistosomiasis peaks during mid-noon and therefore the urine samples were collected between 10 am and 2 pm [2]. [In practising human health ethics the names of the learners were not recorded in the lab books but the ID numbers were used instead].

Urine samples were collected in the following manner to estimate the prevalence of *S. haematobium* in school going learners from ILembe and uThungulu Health Districts, initially school going learners from high schools around ILembe and uThungulu Health Districts were presented with a lecture. The lecture highlighted both basic and general information about schistosomiasis, the disease pathology and mode of transmission, associated morbidity and the benefits of available treatment. School learners were given informed consent forms (information and consent forms) to give to their parents to sign in order to obtain permission to be part of the study. (In cases where the child wanted to participate but could not reach the parent due to unforeseen circumstances, the class teacher was given the right to grant consent for the child). Only learners that provided positive feedback (each learner assented) with signed parental consent forms were included in this study. To estimate the prevalence of urogenital schistosomiasis, urine reagent strips from Neotest 4 © (Kendon Medical supplies, Johannesburg) were used. Urine jars (with a given ID number recorded for that child only) were given to the participating learners and they were sent to go and urinate in the toilets, where a research assistant monitored that it was done in an orderly manner. Micro and macro haematuria were then explored.

Reagent strips measure the quantity of blood in urine and the results can be recorded in a semi-quantitative manner [7]. Urine reagent strips give results that can either be interpreted as negative (-), light intensity infection (+), moderate intensity infection (++) or heavy intensity infection (+++) [10, 33-34]. Reagent strips are a potent instrument to measure how severe the disease is, they are portable and easy to carry and interpret. The severity of urogenital schistosomiasis among the high school going population thereafter could be calculated and expressed as a percentage [10].

The values can either be negative [-/0] (non-traceable levels of blood in urine; no haematuria), light positive [1+] (associated with the presence of 5-10 erythrocytes/µl of urine); moderate positive [2+] (presence of ±50 erythrocytes /µl) and highly positive or severe infection [3+] (presence of ± 250 erythrocytes /µl of urine) [16]. The results can then be categorised to be of light intensity infection (1+ to < 2+) or heavy intensity infection (≥2+ to 3+) of urogenital schistosomiasis [16]. The results were recorded as follows: 0. -.Negative strips; 1. + visible on strip; 2. ++ visible on the strip and 3. +++

Visible on the strip. For each reagent strip analysed the observed colour change was confirmed by a trained co-field worker, before the data were recorded. All urinalysis was performed exactly after one minute of dipping the strips in urine inside the honey jar (a stop watch was used).

The prevalence of urogenital schistosomiasis is an important indicator of the number of infected people in a given population [10].To calculate the prevalence of urogenital schistosomiasis infection, this formula was used (adopted from [10])

(a) Prevalence = $\frac{\text{Number of subjects testing positive}}{\text{Number of subjects investigated}} \times 100$

(b) Categorization of Intensity

Table 2: Intensity of urogenital schistosomiasis can be classified as follows[10].

S. haematobium	Light intensity infection	Heavy intensity infection
Reagent strip reading	1+ < 2+	>2+ = 3+

Source: WHO, (1998)

The data were then entered into a Microsoft Excel file which was exported into the software package Statistical Package for the Social Sciences (SPSS 21) for statistical analysis.

METHOD OF EXTRACTING ALTITUDE AND DISTANCE VALUES FOR EACH SCHOOL

In the spatial analysis for this study, a Geographical Information System (GIS) program called ArcMap 9.2 was used.

Calculating the altitude and spatial location of the schools:

A 20m digital elevation model (DEM) of KwaZulu-Natal was used to calculate the altitude of each school in the country. In ArcMap, a point shapefile of the schools of iLembe and uThungulu Health Districts were added into the programme as well as the DEM. Each point represents the specific location of a school. The DEM is a raster image which stores elevation values for specific areas and is used to help model the relief of landscapes [56].

Using the Extract Values to Points Spatial Analyst Tool in ArcMap, the GIS extracted the elevation points from the raster image and added them to each of the school points. The elevation value given to a school depended on its position in relation to where it overlaid the DEM. The elevation value represents the altitude above sea level in metres.

Calculating distances of schools to rivers:

In order to calculate the distance from each school to its closest perennial river, a spatial join was performed in ArcMap between the rivers' (1:50 000 Perennial Rivers' Dataset) and Schools' shapefiles. A spatial join joins the data from the River`s shapefile to the data of the school that is closest to it, and it calculates and adds a distance field to the attribute table. This distance (in metres) indicates the distance from the school to the closest river segment in the GIS [56].

Calculating the average summer temperature:

Daily and average temperature data (in °C) of four weather stations surrounding the study area was provided by the South African Weather Service (SAWS). The average maximum and minimum summer temperatures for each weather station was calculated by taking the average temperatures for the months of summer (December, January and February) and averaging them.

These values were then used to interpolate average maximum and minimum summer raster coverages for the study area. The Inverse Distance Weighted (IDW) spatial analyst technique was used in ArcGIS in order to interpolate the results. Each raster coverage contains interpolated pixel values of the average maximum or minimum temperature that the spatial analysis has calculated from the given weather station temperature points.

MEASURES TO ENSURE VALIDITY

INTERNAL

Internal validity was optimised by the fact that the research assistants were well trained in the collection and handling of urine samples. Before work was carried out there was an intensive training of the experienced research assistants which aimed to ensure that appropriate data collecting techniques were applied. The time frame for data collection was kept strictly within the designated period for reading the strips (after 60 seconds). The female research assistants asked female learners in a polite discrete manner if they were currently going through menstruation before collecting their urine, to ensure valid results.

REDUCTION OF BIAS

The random selection of schools in iLembe and uThungulu Health Districts that are below the altitude of 300-400 m, and the fact that all learners in each class of the chosen grade were invited to participate in the study reduced any potential bias. These criteria were kept constant throughout the study. Samples were collected from grade 8 learners in all the schools in the study. It ensured that the schools involved in the study are a representation of the rural schools of the ILembe and uThungulu Health District areas. Furthermore fieldworkers who read the urine dipsticks were allowed to take five minute

breaks between the reading processes to reduce human error due to tiredness of the eyes. The samples were only collected between 10 am and 2 pm to optimise accuracy.

SELECTION BIAS

To reduce bias a stratified random selection of schools from the KwaZulu-Natal Department of Education (DoE) school list was undertaken and all grade 8 learners in iLembe and uThungulu Health Districts whose schools were below 1100 m altitude (above sea level) were included to avoid selection bias.

INFORMATION BIAS

For the reagent strips the observed colour change was confirmed by a trained co-field worker, before the data were recorded.

EXTERNAL VALIDITY / GENERALIZABILITY

ILembe and uThungulu Health Districts are similar and adjacent Districts located in the subtropical region of KwaZulu-Natal. They are Health Districts along the coast of KwaZulu-Natal which are facing similar climate and soil conditions, and also lack basic facilities such as safe water, proper latrines and electricity. ILembe and uThungulu Health Districts are typical of other geographical locations in KwaZulu-Natal and share cultural practices with other Districts in the province of KwaZulu-Natal, and the results may thus be generalized to other similar coastal areas.

DATA COLLECTION

As described previously, data were collected by trained research assistants and checked. The data sheets were then photostated and then cross checked before being transferred into an Excel file. It was also checked that no alterations to the collected data were made.

STATISTICAL METHODS

DESCRIPTIVE STATISTICS

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) 21. Univariate analysis was performed to describe the study sample. A frequency graph was used to present the prevalence and intensity of urogenital schistosomiasis in the District. Descriptive statistics were undertaken for all the variables/data in the study.

ANALYTICAL STATISTICS

The differences in the mean prevalence of urogenital schistosomiasis were compared for boys and girls, using school, class per grade, District /ward, altitude and distance from the nearest river/s. To establish if there is a relationship and the strength of the relationship between these variables, the Spearman's correlation coefficient (r) was calculated. Where a (r) value was close to +1 this indicated a strong relationship between measured or observed variables, whilst that close to zero indicated a weak relationship. A one tailed t test was performed to explore the difference between the mean prevalence in boys and girls of ILembe and uThungulu Health Districts. A two tailed t test was performed to evaluate if the difference observed between boys and girls was significant between the two Health Districts. A p value of $p<0.05$ was used as the level of significance and a 95% confidence interval was calculated.

A simple linear regression was performed to explore the relationship between prevalence versus altitude versus school distance versus temperature (each one pair at a time). For this test conclusions were made based on the gradient of the slope that was obtained between each tested variable to determine whether a positive relationship, negative relationship or no relationship exists.

ETHICAL CONSIDERATIONS

Permission to conduct the current study was provided by the principal investigators of the Child Development Research Unit/ VIBE youth clinic. The Department of Education (DoE) and the Department of Health (ILembe and uThungulu Health Districts) gave permission to conduct this research (Reference number 2/4/8/66; HRKM008/10). Permission for the study was obtained from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal. The BREC reference number: BE165/15 (see Appendix C). Written informed consent was obtained from the parents of participating learners and learners provided assent, no names of schools are included but each school was allocated a number to ensure confidentiality.

LIMITATIONS

Using the difference between the number of learners registered for grade 8 and those who participated in this study; 1015 boys and 847 girls in schools of ILembe Health District and 901 boys and 734 girls in schools of uThungulu Health District could not participate in this study. These learners were either absent on the day of sample collection, registered for grade 8 in that school but were not schooling there anymore or could not provide parental consent. Out of the 100 randomly selected schools, four schools could not be included in the final analysis because they are in uMgungundlovu Heath District not ILembe and uThungulu Health Districts, and this was outside our targeted scope area. The raster

interpolation result was not an accurate representation of the study area, as the weather station points used in the analysis were not well spread out over the study area, and there were few of them. The Extract Values to Points Spatial Analyst Tool in ArcMap was used to extract the average maximum and minimum temperature values from the raster image to each school point. The age of learners participating was not recorded.

CHAPTER 4: RESULTS

In the results chapter I have presented the prevalence and intensity of urogenital schistosomiasis in boys and girls in schools of ILembe and uThungulu Health Districts. The prevalence for each school was then associated with abiotic factors and the correlations are shown as well.

Prevalence of urogenital schistosomiasis

In ILembe Health District 71 schools participated in the survey and in uThungulu there were 25 schools. In ILembe the number of participants at each school ranged from 9 to176 and in uThungulu from 24 to154. The mean prevalence of urinary schistosomiasis in ILembe was 39% and in uThungulu 55% in 2013. The range of urogenital schistosomiasis prevalence in ILembe was 0 to 100% and in uThungulu 0 to 88%. For the sexes the mean prevalence of schistosomiasis amongst girls in ILembe was 39% and boys 40% whereas for uThungulu girls 56% and boys 53%.

Table 3: Comparison of the mean Prevalence [%] of Urogenital Schistosomiasis in schools in ILembe and uThungulu Health Districts of KwaZulu−Natal, South Africa. Showing the total number of learners participating in each school and the total mean prevalence of urogenital schistosomiasis. The schools visited are represented by a number on the first column.

(a) ILembe District

Table 3:Prevalence of urogenital schistosomiasis in boys and girls in schools of Ilembe Health Distric 2012.

School	Number of girls	Prevalence (girls)	Number of boys	Prevalence (boys)	Total n	Total Prevalence
1	60	23%	95	28%	155	26%
2	25	16%	10	20%	35	18%
3	19	26%	20	20%	39	23%
4	27	48%	38	61%	65	54%
5	62	32%	77	34%	139	33%
6	14	71%	11	64%	25	68%
7	7	71%	11	55%	18	63%
8	11	91%	14	86%	25	88%
9	18	89%	14	86%	32	87%
10	19	47%	20	50%	39	49%
11	8	63%	10	0%	18	31%
12	42	10%	34	29%	76	19%
12	38	68%	37	78%	75	73%
13	15	47%	24	21%	39	34%
14	21	48%	7	57%	28	52%
15	10	60%	2	100%	12	80%
16	29	0%	41	5%	70	2%
17	39	51%	38	55%	77	53%
18	25	48%	28	36%	53	42%
19	21	38%	22	55%	43	46%
20	12	17%	21	29%	33	23%
22	2	100%	7	100%	9	100%
21	39	49%	30	63%	69	56%
22	29	62%	36	44%	65	53%
23	16	38%	20	50%	36	44%
24	3	33%	14	36%	17	35%
26	19	11%	18	33%	37	22%
27	24	29%	40	43%	64	36%
28	26	15%	17	0%	43	8%
29	20	65%	23	70%	43	67%
30	43	26%	123	15%	166	21%
31	4	25%	7	71%	11	48%
32	34	56%	42	48%	76	52%
33	31	26%	38	47%	69	37%
36	23	65%	12	33%	35	49%
34	16	6%	23	0%	39	3%
35	18	67%	14	57%	32	62%
36	23	30%	22	55%	45	42%
37	5	40%	11	55%	16	47%
38	22	64%	17	47%	39	55%
39	33	42%	40	45%	73	44%

School	Number of girls	Prevalence (girls)	Number of boys	Prevalence (boys)	Total n	Total Prevalence
41	21	14%	20	30%	41	22%
45	40	60%	26	50%	66	55%
42	100	31%	111	26%	211	29%
43	12	83%	7	57%	19	70%
44	25	24%	13	0%	38	12%
45	22	36%	20	40%	42	38%
46	26	54%	25	64%	51	59%
47	21	33%	40	33%	61	33%
52	44	73%	35	86%	79	79%
48	20	15%	27	26%	47	20%
49	19	53%	17	53%	36	53%
50	32	53%	32	50%	64	52%
51	31	26%	27	30%	58	28%
52	44	36%	43	30%	87	33%
58	27	59%	42	67%	69	63%
53	24	21%	46	15%	70	18%
54	29	34%	22	55%	51	45%
55	36	56%	29	45%	65	50%
56	28	29%	23	17%	51	23%
57	14	57%	13	62%	27	59%
58	23	0%	27	4%	50	2%
59	92	15%	32	13%	124	14%
60	26	73%	27	81%	53	77%
61	21	14%	40	40%	61	27%
62	15	27%	11	55%	26	41%
63	83	36%	93	46%	176	41%
64	11	45%	16	38%	27	41%
65	13	0%	17	0%	24	27%
66	35	69%	45	82%	80	75%
67	9	56%	22	32%	54	69%
68	55	65%	46	57%	101	61%
69	42	10%	34	29%	88	33%
70	22	45%	29	41%	51	49%
72	26	92%	40	81%	66	87%
73	35	91%	44	68%	79	80%
Totals	**1888**	**39%**	**2017**	**40%**	**3905**	**40%**

Table 4: Prevalence of urinary schistosomiasis in boys and girls in schools of UThungulu Health Distric 2012.

Schools	Number of girls	Prevalence (girls)	Number of boys	Prevalence (boys)	Total n	Total Prevalence
1	39	46%	21	71%	60	59%
2	31	68%	36	72%	52	70%
3	35	57%	67	54%	102	55%
4	30	67%	90	9%	97	60%
5	53	36%	35	43%	88	39%
6	16	75%	33	45%	51	59%
7	50	52%	48	67%	98	59%
8	20	90%	42	50%	68	78%
9	22	59%	17	41%	39	50%
10	21	62%	22	73%	38	52%
11	35	51%	21	43%	56	47%
12	26	23%	37	24%	47	33%
13	21	62%	43	33%	64	47%
15	31	55%	30	50%	61	52%
16	12	83%	31	94%	42	89%
17	44	64%	34	56%	78	60%
18	22	68%	14	71%	56	69%
19	75	87%	79	89%	154	88%
20	10	30%	7	57%	89	44%
21	93	32%	83	52%	176	52%
21	32	69%	32	34%	115	66%
22	30	63%	19	68%	49	50%
24	16	19%	14	14%	30	18%
25	19	58%	30	83%	33	71%
26	16	31%	11	55%	27	43%
27	13	0%	17	0%	24	0%
Total	**1112**	**56%**	**1248**	**53%**	**2360**	**55**

The prevalence of urogenital schistosomiasis in uThungulu schools (54%) was significantly higher than that of ILembe (39%) district ($p<0.001$) (Table 3 & 4). There was no significant difference in the prevalence of urogenital schistosomiasis between boys (40%, 95% CI: 38-42) and girls (39%, 95% CI: 37-41) ($p=0.72$) in ILembe district). However, the prevalence of infection is higher in uThungulu Health District where girls showed a higher but no significant prevalence (56%, 95% CI: 53-59) compared to boys (53%, 95% CI: 50-56) ($p=0.83$). Total sample size equalled 6265, which was a large sample.

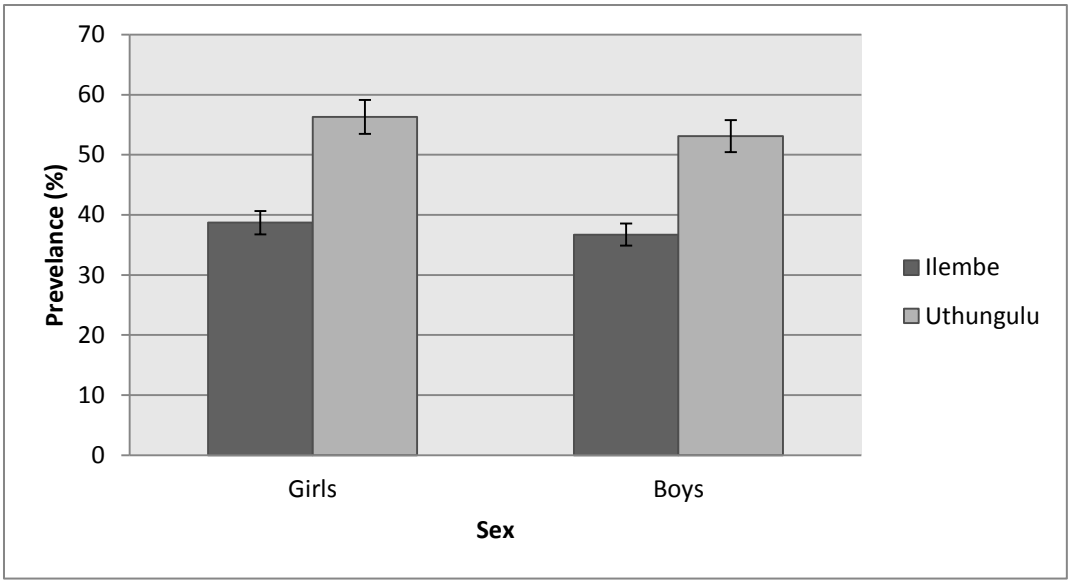

Figure 2: Prevalence of urogenital schistosomiasis by sex in grade 8 school going learners in ILembe and uThungulu Health Districts, KwaZulu-Natal (girls =3000; (boys =3265).

(a) Intensity of urogenital schistosomiasis

When using urine reagent strips to test for haematuria , there can be a sample with no haemturia (negative) or the sample can have haematuria (positive). The positive samples can further be categorized as either light, moderate or heavy haematuria. The following code was used: Light infection-equivalent to a 1+ observed on a reagent strip, Moderate infection- equivalent to a 2++ observed on a reagent strip and Heavy infection- equivalent to a 3+++ observed on a reagent strip.

In ILembe Health District more learners were negative (61% girls and 60% boys) than positive (38% girls and 40% boys). In uThungulu Health District more learners were positive (56% girls and 53% boys) than negative (44% girls and 47% boys). N=6265

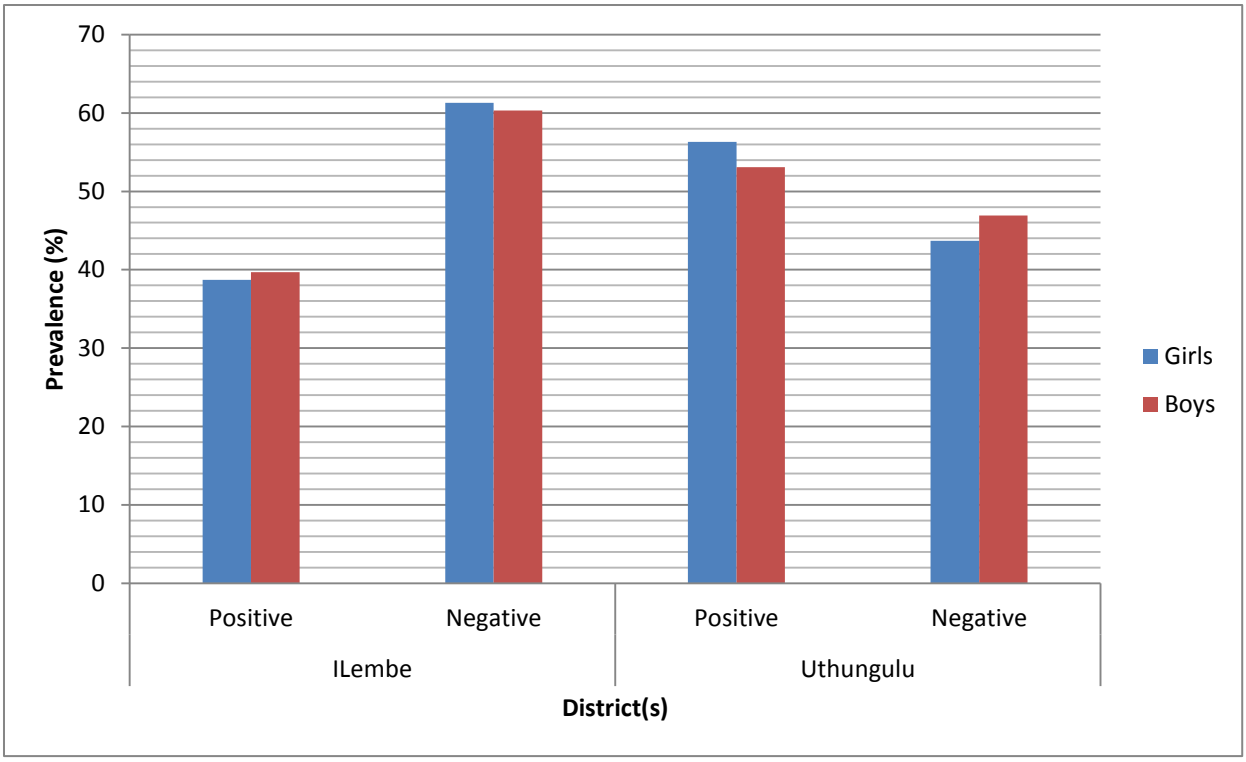

Figure 3: Comparing negative and positive learners in schools of ILembe and uThungulu Health Districts.

There were no significant differences between the sexes in the low, moderate or high levels of haematuria (p=0.13). The intensity/level of haematuria was non-significantly different between the Health Districts (p>0.05). Although the intensity of haematuria followed the same trend for girls in uThungulu (1+ = 40%, 2+ = 30%, 3+ = 30%) and ILembe (1+ = 41%, 2+ = 28%, 3+ = 30%), the uThungulu boys (1+ = 35%, 2+ =28%, 3+ = 36%) had a slightly higher percentage of heavy intensity haematuria compared to boys in ILembe (1+ = 41%, 2+ = 28%, 3+ = 31%) although this difference was not statistically significant (p =0.17).

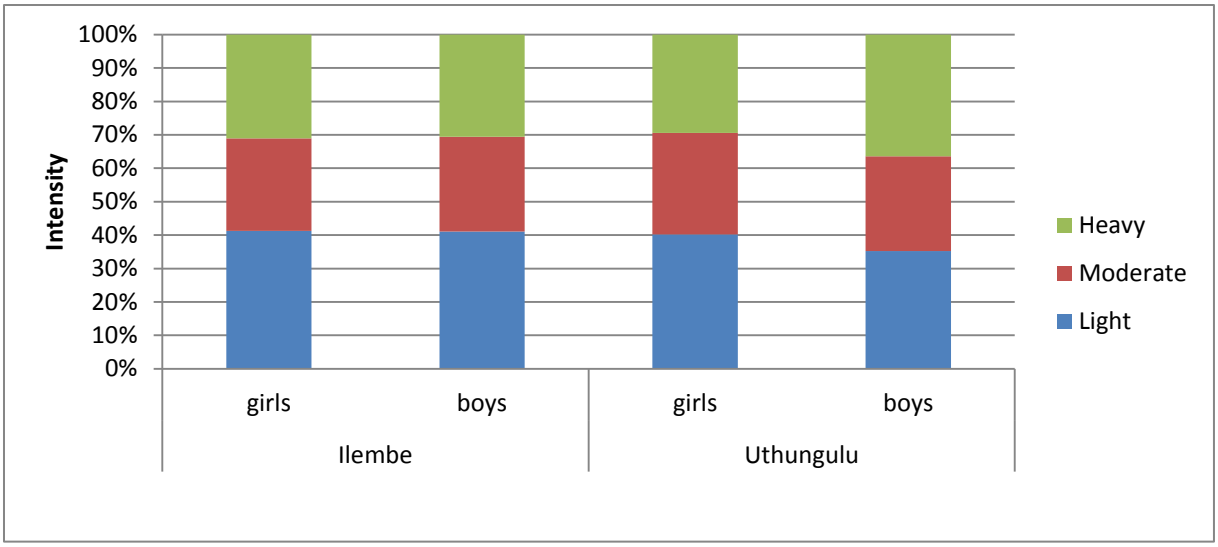

Figure 4: Mean intensity of urogenital schistosomiasis infection obtained from reagent strips at rural schools (n=96) of ILembe (71 schools) and uThungulu (25 schools) Health Districts (total n=2820 infected learners; n (girls=1362), n (boys=1458))

(b) Association of prevalence of urogenital schistosomiasis with abiotic factors

The Figure below shows that most of the schools that were included in this study are in the ILembe Health District. In uThungulu Health District two schools are in the less than 20% prevalence category, and they are at a 0-300m altitude range. There are eight schools in uThungulu that fall in the >20% & <50% prevalence category; these schools are at a 0-300m and 300-800m altitude range. In ILembe three schools which have less than 20 % prevalence are in the 0-300m altitude range, but this altitude range also has 18 schools that have urogenital schistosomiasis prevalence greater than 50%. ILembe district has the most (21) schools with a low urogenital schistosomiasis prevalence, less than 20%. These schools are located in the 300-800m altitude above sea level. Whilst schools that are located in the altitude range of 0-300m show a tendency to have a urogenital schistosomiasis infection percentage higher than 20% and up to 50%.

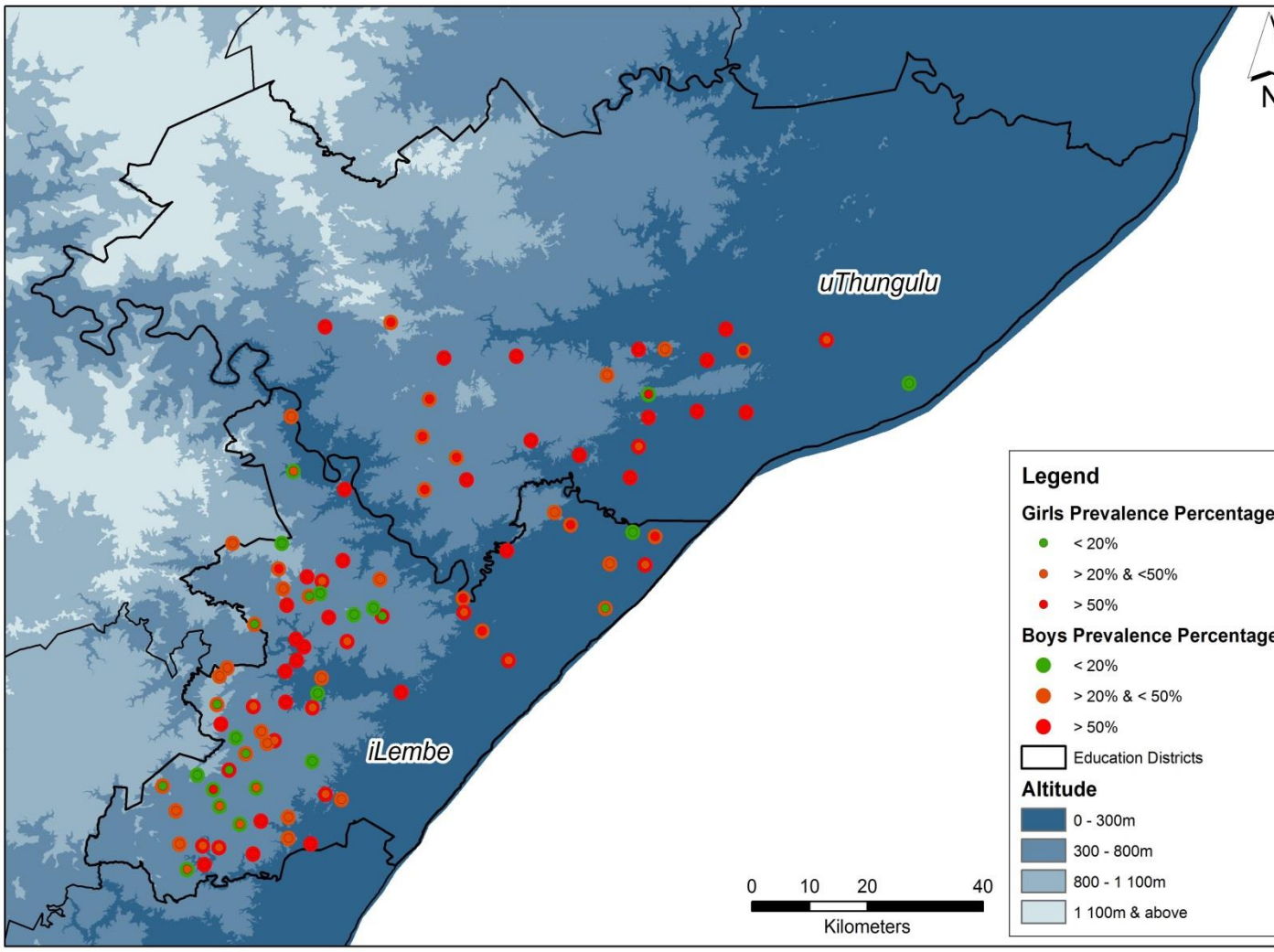

Figure 5: Map of uThungulu and ILembe Health Districts, categorizing the prevalence (<20%, >20-50% and >50%) of selected school and the assiciated altitude (m).[56]

The girls and boys prevalence is represented by colour coded dots (small for girls and bigger for boys) placed on each of the chosen school location, where green indicates prevalence less than 20%, orange indicates prevalence greater than 20% but less than 50% and red indicates prevalence greater than 50%.

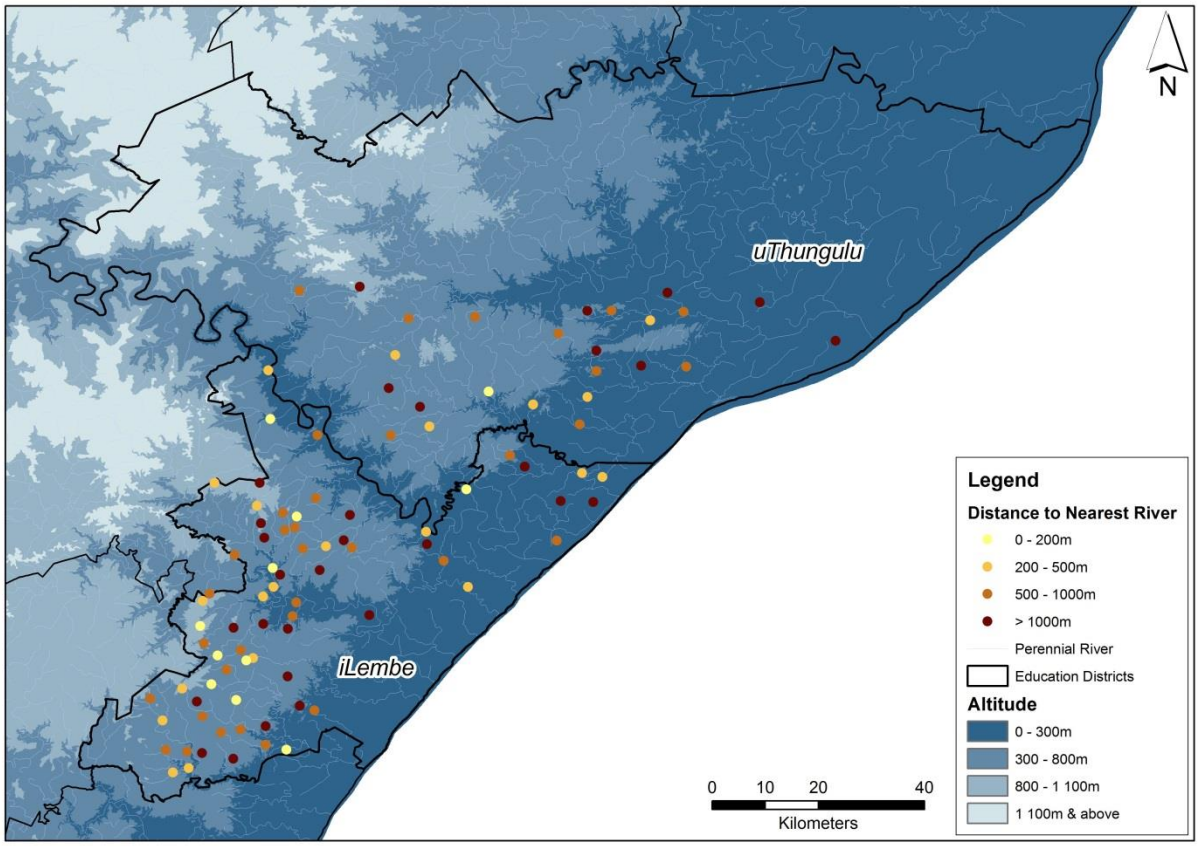

Figure 6: Relative distance (m) to the nearest river contact point for each of the chosen school and the altitude (m) above sea level for ILembe and uThungulu schools, n=96 [56]

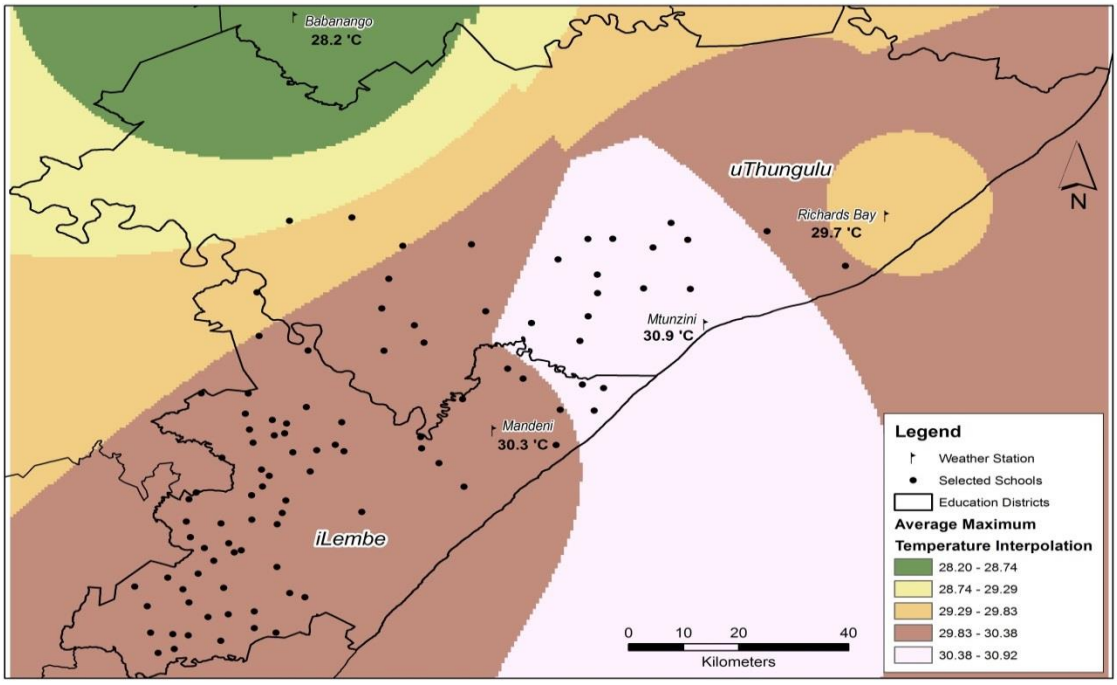

Figure 7: Average maximum summer temperature (C) of the areas surrounding the schools that were selected in the study [56]

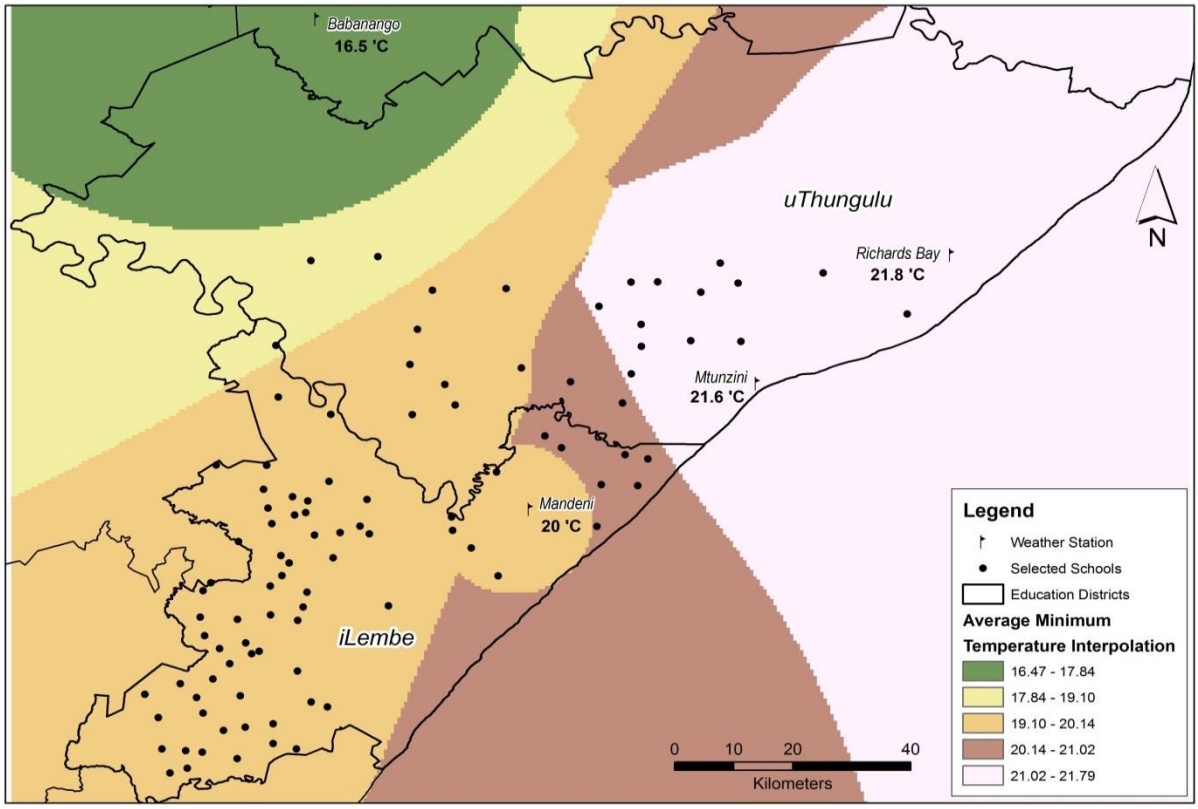

Figure 8: Representing the average minimum summer temperature (C) of the areas surrounding the schools (n=96) that were randomly selected for this study [56]

Table **5.** The values for the altitude above sea level (m), the distance to the nearest river (m), the average summer maximum and minimum temperature (◦C), with the associated prevalence for boys and girls in schools in ILembe and uThungulu Health Districts.

(a) Arc Map 9.2 calculated the altitude above sea level (m) and the distance to the nearest river (m), the resulting values for each school are presented on a table below together with the average summer maximum and minimum temperature and the prevalence (for boys and girls) in each of the schools chosen in the study.

Table 5: (a) Altitude (m's) distance to river and aveage summer temperature with schistosomiasis prevalence in girls and boys in ILembe Health District

Schools	Altitude above sea level (m)	Distance to nearest river (m)	Average Summer Maximum (°C)	Average Summer Minimum (°C)	Girls Prevalence %	Boys Prevalence %
1	558	566	30.1	19.7	45%	38%
2	723	538	30.1	19.7	71%	64%
3	935	777	30.0	19.7	43%	48%
4	61	418	30.3	20.1	38%	55%
5	647	1459	30.1	19.7	63%	0%
6	863	566	30.0	19.7	12%	24%
7	746	1272	30.2	19.9	42%	45%
8	220	294	29.7	19.0	36%	30%
9	140	125	30.2	19.9	65%	70%
10	582	1491	30.2	19.9	30%	55%
11	243	558	30.3	20.1	56%	45%
12	80	361	30.6	20.8	64%	47%
13	948	1791	29.0	19.6	6%	0%
14	642	643	30.2	19.9	0%	100%
15	499	905	30.1	19.7	29%	17%
16	311	3060	30.2	19.9	40%	55%
17	20	359	30.9	20.6	15%	13%
18	684	769	30.1	19.8	51%	55%
19	798	977	30.0	19.6	17%	29%
20	618	327	30.1	19.8	38%	50%
21	174	1125	30.3	20.0	57%	62%
22	618	133	30.1	19.7	0%	4%
23	622	1406	30.1	19.8	48%	61%
24	582	1763	30.1	19.7	91%	86%
25	643	497	30.0	19.7	36%	40%
26	643	641	30.1	19.7	11%	33%
27	639	47	30.1	19.7	8%	50%
28	466	1283	30.1	19.8	89%	86%
29	362	1401	30.1	19.8	31%	26%
30	101	1438	30.4	20.3	36%	46%
31	260	89	29.9	19.3	21%	15%
32	239	197	30.3	20.0	53%	50%
33	403	473	30.1	19.7	26%	20%
34	203	1450	30.4	20.2	100%	43%

schools	Altitude above sea level (m)	Distance to nearest river (m)	Average Summer Maximum (°C)	Average Summer Minimum (°C)	Girls Prevalence %	Boys Prevalence %
35	670	183	30.1	19.8	24%	0%
36	1060	473	29.9	19.4	47%	21%
37	784	1677	30.1	19.7	49%	63%
39	336	809	30.1	19.7	47%	50%
40	505	1166	30.7	19.9	37%	83%
41	423	876	30.4	20.2	32%	34%
42	643	655	30.1	19.8	26%	15%
43	402	1137	30.1	19.8	60%	50%
44	545	505	30.1	19.8	33%	36%
45	76	275	30.3	20.0	56%	48%
47	926	404	30.0	19.7	26%	30%
48	645	104	30.1	19.8	26%	47%
49	240	909	30.2	19.9	29%	43%
50	405	1370	30.1	19.8	54%	64%
51	585	937	30.1	19.8	16%	20%
52	213	502	30.2	19.8	33%	33%
53	287	1523	30.3	20.0	25%	71%
54	331	934	30.1	19.8	83%	57%
55	867	1052	30.1	19.7	48%	36%
56	81	952	30.3	20.2	15%	26%
57	822	541	30.1	19.7	53%	53%
58	564	642	30.2	19.8	71%	55%
59	408	2283	30.2	19.9	0%	0%
60	344	929	30.1	19.8	23%	28%
61	885	135	30.0	19.7	14%	40%
62	422	423	30.1	19.8	100%	100%
63	82	2127	30.5	20.6	27%	55%
64	907	382	30.1	19.7	15%	0%
65	986	357	30.0	19.6	62%	44%
66	219	330	30.1	19.8	60%	100%
67	253	239	30.1	19.7	73%	81%
69	624	1063	30.2	19.9	0%	5%
70	164	547	30.0	19.6	67%	57%
71	240	170	30.1	19.8	68%	78%
72	646	114	30.1	19.8	48%	57%
73	559	728	30.1	19.7	14%	30%

In ILembe and uThungulu Health Districts the average summer temperatures are high. In ILembe schools with urogenital schistosomiasis prevalence above 50% for boys and girls were from 124-1762 m distance to the nearest river. In uThungulu schools distance to the nearest river from school location ranged from 54– 2052 m. In ILembe schools distance to the nearest river from school location ranged from 47-1791 m. In ILembe schools altitude above sea level ranged from 61-1166 m. In uThungulu schools altitude above sea level ranged from 12-1004 m.

The altitude above sea level for the schools was significantly related to the prevalence of urogenital schistosomiasis for both girls and boys and the negative correlation coefficient indicates that increasing altitude was associated with a decrease in the prevalence of infection. For girls there was a statistically significant association between the prevalence of urogenital schistosomiasis and the maximum mean summer temperature (p=0.017) but this was not found in boys. The distance from the school to the nearest river was found to not influence the prevalence of urogenital schistosomiasis in either sex.

(b) Altitude (m), distance to nearest river and average summer temperature with urogenital schistosomiasis prevalence in girls and boys in uThungulu Health District

Schools	Altitude above sea level (m)	Distance to nearest river (m)	Average Summer Maximum (◦C)	Average Summer Minimum (◦C)	Girls Prevalence %	Boys Prevalence %
1	302	54	30.3	20.1	90%	50%
2	353	821	30.6	21.3	23%	24%
3	1005	1229	29.4	18.6	56%	32%
4	123	205	30.7	21.4	69%	82%
5	403	728	30.6	21.1	36%	43%
6	619	1559	30.2	19.9	51%	43%
7	501	547	29.8	19.3	59%	80%
8	409	653	29.3	18.3	62%	73%
9	120	281	30.6	21.1	46%	71%
10	81	880	30.9	21.5	68%	72%
11	348	1565	30.6	21.2	64%	56%
12	561	2052	30.1	19.7	59%	41%
13	192	1282	30.6	21.4	57%	54%
14	167	607	30.7	21.5	69%	34%
15	13	1454	29.9	21.7	19%	14%
16	667	493	30.0	19.5	62%	33%
17	656	221	30.2	20.0	55%	50%
18	79	595	30.6	20.9	68%	71%
19	362	1066	30.7	21.2	67%	9%
20	743	735	30.2	19.9	75%	45%
21	224	423	30.5	20.7	52%	67%
22	305	809	30.7	21.2	83%	94%
23	83	1021	30.3	21.6	30%	57%
24	510	918	30.1	19.8	65%	57%
25	230	1684	30.8	21.4	87%	89%

Table 6: Correlation coefficients (r) of urogenital schistosomiasis prevalence and associated abiotic factors (altitude above sea level, distance to the nearest river contact point and the maximum and minimum average summer temperature). n = 6265

Prevalence	Factor	Correlation coefficient (r)	P value
Girls	Altitude above sea level	-0.215	0.035*
Boys		-0.283	0.005*
Both		-0.262	0.009*
Girls	Distance to nearest river	0.010	0.922
Boys		-0.007	0.947
Both		0.008	0.936
Girls	Average summer	0.244	0.017*
Boys	maximum (°C)	0.150	0.145
Both		0.234	0.021*
Girls	Average summer	0.121	0.242
Boys	minimum (°C)	0.146	0.156
Both		0.174	0.088

*Correlation is Significant at $p<0.05$ (at a 95% confidence interval)

CHAPTER 5

Discussion

Prevalence and Intensity of urogenital schistosomiasis in ILembe and uThungulu Health Districts

The results of this study report that the prevalence of infection with *S. haematobium* is high among school going pupils. In ILembe Health District a total of 71 rural public schools were visited and a total of 1888 girls and 2017 boys tested for presence of blood in urine using reagent strips. Out of the learners that gave urine samples a total of 801 boys (37%) and 730 girls (39%) were found to have haematuria. In uThungulu Health District a total of 1112 girls and 1248 boys consented to participate in the study. A total of 626 girls (56%) and 663 boys (53%) were found to have blood in urine. From the percentage of urogenital schistosomiasis infection in both the Health Districts, it can be discerned that infection is significantly higher ($p<0.001$) in the uThungulu Health District learners compared to ILembe Health District learners. This study also found that the prevalence of urogenital schistosomiasis is higher among girls than boys in the uThungulu Health Districts, whereas in ILembe Health District boys showed a prevalence of infection that is higher compared to girls ($p=0.72$). These results concur with other scientific research findings [15, 16 and 19].

Learners studying grade eight in the KZN province are usually between the ages of 13-14 years old, and are more likely to be swimming or playing in rivers due to the hot weather and lack of recreational facilities [19]. Morgas et al. in 2010 noted the possibility of infection being high among girls in this age group, due to the factor of girls being responsible for most housekeeping chores, which could include washing of dishes or clothes in the river and fetching water from the river for other uses [19]. The high prevalence of schistosomiasis infection observed in boys of this age group could be accounted for by the fact that boys in this age group are still at an explorative stage and more willing to play or swim in river water.

The prevalence of urogenital schistosomiasis infection in ILembe Health District falls under category 2 (moderate prevalence) according to the WHO classification system [10]. In ILembe Health District the prevalence was higher than 20% but less than 50%, for both sexes. The prevalence of urogenital schistosomiasis infection in uThungulu Health District falls under category 1 (high prevalence), that is above 50% for both sexes [10]. According to the WHO guidelines it would be recommended that all school age learners receive praziquantel (PZQ) treatment in the ILembe Health District and mass treatment must be done for at least one to two years [10]. For uThungulu Health District which falls under category 1, the recommendation would be that everyone in the community receives treatment

irrespective of status, age or sex and mass treatment should be provided once a year for a period of 3 years [10, 32].

The results presented in this study thus emphasize that there are a large number of school going learners who require treatment with the anti-schistosomal drug, PZQ. Provision of mass treatment to school learners that are infected by *S. haematobium* can have other health benefits. Treatment of *S. haematobium* can result in profound health improvements as destruction of adult worms by the drug can reduce the number of circulating parasite eggs, Mass treatment holds the potential to reduce the burden of disease and its complications [35]. From the association reported between FGS and HIV infection, treatment of urogenital schistosomiasis may reduce the likelihood of transmission of HIV [5, 6 & 13]. The reduction of circulating parasite eggs in the body can reduce the damage caused by the development of sandy patches in the cervix and may reduce the development of cancer in the genitals and kidneys, which could be due to tissue obstruction by the trapped eggs [5, 13, 19 and 22].

The prevalence and distribution of urogenital schistosomiasis is governed by abiotic factors that influence the survival and replication of the intermediate host snail *B. africanus* [9]. *B. africanus* is in the family Planorbidae, and another snail that is included in this family is *B. pfeifferi* and this snail has been shown to act as an intermediate host snail for both *S. mansoni* and *S. haematobium* infection. Both these snails have similar habitat preferences and can survive under similar conditions [9, 45]. Where scientific data is lacking for *B. africanus*, information on *B. pfeifferi* is considered, as it has been proven that both these snails can survive in similar conditions [45]. Abiotic factors of importance are the temperature range, altitude range, turbidity range, salinity range and flow rate of water in water streams [9]. In the results obtained in this study the prevalence of urogenital schistosomiasis is high in both Districts. This suggests that the ILembe and uThungulu Health Districts have temperature and altitude regimens which are conducive for the survival and production of the intermediate host snail. Owing to the fact that the snail is important for the transmission of *S. haematobium* infection, factors favouring or limiting its survival consequently impact on *S. haematobium* transmission and infection.

Urogenital schistosomiasis prevalence was found to be significantly different ($p<0.001$) between the two Districts (uThungulu Health District schools showed a higher prevalence than those in ILembe Health District). Urogenital schistosomiasis prevalence was not shown to be significantly different ($p=0.72$) among boys and girls in both districts. Such a finding suggests that there existed no significant differences regarding the prevalence of infection which are due to the factor of sex.

In Figure 5 it can be observed that most of the schools that were included in this study are in the ILembe district. ILembe district contains the most (21) schools with a low urogenital schistosomiasis prevalence, less than 20%. These schools are located in the 300-800m altitude above sea level. On the

other hand, schools that are located in the altitude range of 0-300m show a tendency to have a urogenital schistosomiasis infection percentage higher than 20% and up to 50% (see Figure 5). The majority of schools in uThungulu Health District displayed infection percentages greater than 20% and some schools greater than 50 %. These schools are found in the 0-300 and 300-800 m altitude above sea level. Such a fact suggests that there are a large number of learners that are infected with *S. haematobium* in uThungulu Health District, despite the district having a lower sample size than its counterpart. In comparison, Wolmarans *et al*. in 2001 found the prevalence of urogenital schistosomiasis in the Limpopo province to be 70% in pupils younger than 14 years of age, but the sample size was smaller (n=420) [8, 49]. In Mpumalanga province an epidemiological study revealed a prevalence of 35% among primary school pupils in 30 schools [8].

The intensity of urogenital schistosomiasis was seen in this study to follow the same trend as the prevalence. Sex did not have a significant impact on the intensity of urogenital schistosomiasis infection (see Figure 4). The intensity of infection (light, moderate or heavy) was not affected by sex differences. Intensity of infection was however observed to be significantly impacted by the Health Districts and the results show that the intensity of urogenital schistosomiasis infection remains higher in schools located in uThungulu compared to schools in ILembe Health District ($p<0.005$). The intensity of urogenital schistosomiasis infection was not significantly different ($p>0.05$) in respect of light, moderate and heavy infections in boys and girls of ILembe Health District. In uThungulu Health District, boys exhibited a higher tendency towards heavy infection, whilst girls in this District have a higher distribution in the light infection category (see Figure 4). These results could be due to the fact that uThungulu Health District has a plethora of rivers compared to ILembe Health District [31, 32]. A light intensity indicates a 1+ reading on a reagent strip, and it was reported that this is equivalent to a release of 5-10 erythrocytes per micro litre of urine [16]. This means that the majority of learners in ILembe Health District are losing 5-10 erythrocytes /uḷ of urine, which can be a significant number of lost red blood cells considering that the factor of urinating is a process that happens regularly every day. In uThungulu Health District boys lose many red blood cells in urine (±250 erythrocytes /uḷ of urine) because the results show that they have a higher percentage of heavy intensity of infection. Loss of blood in urine can have serious health complications, such as anaemia and tiredness [3-4].

Campaigns that provide mass treatment to school age girls and boys are strongly encouraged, especially in areas where *S. haematobium* is endemic and prevalent [25]. Treatment of school pupils vulnerable to *S. haematobium* infection is required because genital lesions and other defects caused by trapped parasite eggs can be minimized if treatment is offered early. This may be an effective strategy in areas co-endemic for schistosomiasis and HIV [13].

Early treatment of urogenital schistosomiasis may reduce the transmission and potentiation of transmission of HIV. Treatment is required, however, for both sexes, as both boys and girls appear to be similarly infected and do require treatment. It is also advisable to treat at a young age before the parasite has exerted any non-reversible defects (sandy patches, changes in blood vessels) and complications [25, 35]. The WHO has advised the joint drug delivery for schistosomiasis and soil transmitted helminth infections, after it was observed that these two infections threaten similar communities and are most likely to be found where there is inadequate sanitation [53]. With school going learners being the category most at risk, early treatment is advised because this can be beneficial for possibly anaemic students who are always tired and lack the ability to focus at school due to infection with *S. haematobium* and this can improve the rate of absenteeism among infected learners [53]. The high percentage of infected learners in this study is an indication that urogenital schistosomiasis really is a neglected disease, which is why it is important to offer mass treatment to schools at risk. Since this is a neglected disease local health facilities in rural areas might lack equipment for diagnosis and sometimes even the drug PZQ might not be available at Primary Healthcare Clinics. For these reasons mass treatment with PZQ at schools endemic for *S. haematobium* infection is encouraged [2, 11 and 53]. The use of urine reagent strips in remote communities and schools can help hasten the diagnosis process; hence treatment delivery can be prioritized [15].

In this study the prevalence and intensity of urogenital schistosomiasis was monitored using urine reagent strips. Urine reagent strips are estimated to have a sensitivity and specificity of 82% and 97% respectively for an active *S. haematobium* infection [14]. This suggests that the results presented here are valid, reliable and can be trusted, as there is no significant difference between infection levels detected with microscopy or urine reagent strip [16]. Meents and Boyles, in 2010 reported congruence in that there were no significant differences between the results obtained from reagent strip readings compared to those obtained from microscopy readings [39]. However, false positives could have arisen from other bacterial or viral infection(s) that lead to haematuria and also if some girls were going through their menstrual cycle but never the less did eventually supply a sample [7, 14].

We were expecting to obtain a relatively high prevalence of urogenital schistosomiasis in the present study, because the chosen districts are in the coastal area, and have areas that still lack adequate sanitation and water supply [31, 32]. The many schools were at around 300-400 m altitude above sea level, an altitude that permits the survival and reproduction of the intermediate host snails [18]. These are factors which have been reported to permit or promote the transmission of *S. haematobium* infection and areas with such conditions can be indicative of displaying a high prevalence of urogenital schistosomiasis [15-16, 19].

Relationship between prevalence and abiotic factors

The prevalence of urogenital schistosomiasis in boys and girls showed a significant (p=0.035 and p=0.005, respectively) negative relationship with altitude above sea level (see table 4). The Spearman's rank correlation showed a weak negative correlation coefficient (r) (-0.215 and -0.283) for the relationship between altitude and prevalence of infection for girls and boys. This factor means that when the prevalence is high, urogenital schistosomiasis is likely associated with a low altitude range, and where altitudinal zones are high (i.e. 800-1100m) prevalence would likely be low. This finding is congruent with what other researchers have established the fact that locations of low altitude (±250 m) are conducive for the survival and production of the intermediate snail host, and hence such areas have a high prevalence of *S. haematobium* [18]. Alternatively, locations at high altitude (+1000 m) are not conducive for the host snail's survival and infection rates remain low [18]. The prevalence in such locations is low due to the environment not being conducive to the survival of the intermediate host snail [18]. Appleton and Kvalsvig, in 2006 reported that areas that had an altitude >800m had low risk of soil transmitted helminth transmission, whilst areas at <300m altitude reflect the opposite [16].

The relationship between prevalence of urogenital schistosomiasis and distance to the nearest river was not significant (p=0.922 and 0.947) for both girls and boys. Girls showed a low (weak) r value = 0.010, suggesting that there exists a weak positive relationship between these parameters, but this was non-significant. Boys reflected a weak negative r value =0.007. This information implied that a short distance to the nearest river could be associated with a high prevalence for urogenital schistosomiasis infection among boys, whilst in girls the opposite is true. However, the relationship between prevalence and distance to the nearest river could be regarded as non-conclusive in this study, as the r values are low (weak correlation) and the p values are non-significant. In Figures 4 and 5 it can be observed from the maps that schools that have short distances to the nearest river (0-200m & 200-500m) tend to have an infection percentage greater than 20 and 50%. However there are a few schools which have a 500-1000m distance to the nearest river but still showed infection percentages greater than 20 and 50 %. This suggests that learners may be living in areas with rivers near their homes or may encounter rivers *en route* to/from school. In our study there is no statistically significant association between school location and the distance to the nearest river and urogenital schistosomiasis prevalence, However one study reported that schools which were close to river(s)/dam(s) tended to record a higher prevalence of *S. haematobium* infection than those that are further away [21].

Due to the competition for jobs, education and other valuable human resources there has been an increase in migration to nearby cities, communities and towns [31, 32 and 47]. As a result there are a number of people who are constantly travelling from one place to another in search of a better living.

This may have an effect on the distribution pattern of *S. haematobium*, as some individuals could be infected by *S. haematobium* already and by travelling to another community could introduce the parasite to rivers that did not contain the parasite before. If conditions are favourable and the intermediate host snails are present there would be a rise in the prevalence of *S. haematobium* infection. Safe water availability in any given community is the driving force for the prevalence of *S. haematobium* infection, because this is a water-borne disease [1, 3, 17 and 26]. In South Africa, in 2015 there was a reported national drought, which affected agriculture and communities with a scarcity of safe water availability [53]. This suggests that remote rural communities might be compelled to use fresh river water for their daily needs, and further means an increased frequency of contact with river water and could favour the transmission of urogenital schistosomiasis. In this study, we measured the distance from the school to the nearest river, but boys and girls probably access river water near their homes and this was not measured in the study.

Associations between average summer maximum (◦C) temperature and prevalence of urogenital schistosomiasis showed a weak positive relationship for both girls and boys (r =0.244 and 0.150 respectively). This relationship was considered significant for girls (p=0.017) but it is non-significant for boys (p=0.145). The average summer minimum temperature (◦C) also showed a weak positive relationship (r =0.121 and 0.146 respectively) with urogenital schistosomiasis prevalence. However, the p values for this correlation are non-significant (p = 0.242 and 0.156 respectively). The majority (63) of schools in ILembe District fall in the range of average maximum summer temperature of 29.8-30.3◦C (see Figure 5). The majority (13) of schools in uThungulu Health District fall in the maximum summer temperature of 30.4- 31.0 ◦C. For the average minimum summer temperature, the majority (56) of the schools in ILembe fall in the range of 19.1-20.1 ◦C, but in uThungulu only eight schools fall in this temperature range. The warmer temperatures in uThungulu may contribute to the increased prevalence of infection. The remainder of the schools (13) have an average minimum temperature of 21.0-21.8 ◦C (see Figure 6 and 7). The average temperatures in the ILembe and uThungulu Health District are conducive to the survival and reproduction of the intermediate host snail, and even when temperatures are high, it is a sub-tropical area and, therefore, there exist a covering of plenty of trees which shade the water streams from extreme temperatures.

It has been reported that *B. pfeifferi* and *B. africanus* have a general preference of inhabiting locations with a temperature range of 18.0 ◦C to 32.0 ◦C and the snail develops and reproduces well in temperatures between 20.0 ◦C and 27.0 ◦C [9]. *B. pfeifferi* and *B. africanus* survival was found to be influenced by temperature and it was observed that adequate temperature ranges can promote fecundity and aestivation of the snail [9]. The optimal survival temperature ranges of *B. pfeifferi* and *B. africanus* are those that are typically found in the ILembe and uThungulu Health District, hence the prevalence of *S. haematobium* was observed to be high. *B. pfeifferi* was reported to transmit *S.*

mansoni and *B. africanus* to transmit *S. haematobium*; poly-parasitism with both these parasites is common in areas near the coast and the prevalence of both was found to be similar [40-43]. This is possibly due to both the intermediate host snails being adapted to survive under similar conditions, as both species of snails cannot survive temperatures higher than 39.0 ◦C and both snails cannot reproduce and survive in areas of high altitude (+1100m) [42, 48]. A limitation with the temperature data concerned the fact that although data from various stations were available and used, nonetheless for some areas only an extrapolated value was used.

CHAPTER 6

Conclusions and Recommendations

The prevalence and intensity of urogenital schistosomiasis provides a useful estimate for the status of *S. haematobium* infection in a community. In ILembe and uThungulu Health District the prevalence of urogenital schistosomiasis (39 and 55% respectively) was found to fall in the categories in which the WHO recommends immediate treatment to avert the burden caused by the disease. If the prevalence of *S. haematobium* is neglected at the onset, since schistosomiasis is a latent disease the ramifications in later life can be profound and may result in a high burden of disease. It therefore would be of advantage to the public health sector if treatment could be offered at the school level to reduce transmission and avoid the complexity of the disease in later adult life. This will prevent the development of genital lesions (which are non-reversible) and kidney damage due to the calcification of eggs and formation of fibrous tissue in the kidneys [23]. This may contribute to improvements in the current status of communicable (such as HIV) and non-communicable diseases (such as cancer). The abiotic factors explored in this study (temperature, altitude and distance) can serve as useful bio indicators in the identification of areas at risk for urogenital schistosomiasis. Temperatures and altitudes that are conducive to the survival and reproduction of the intermediate snail host are therefore conducive for the distribution and transmission of *S. haematobium* infection.

Stothard, in 2013 reported a 82% reduction in the number of circulating parasite eggs after treatment with PZQ. Praziquantel is effective in destroying the schistosomules and adult worms but not the parasite eggs. However, this is beneficial, because it stops further production of parasite eggs [53]. It is for these reasons that in heavily infected populations it is recommended that treatment be offered for a period of two or three years [2, 10 and 53]. It has been determined that the extent of tissue or organ damage due to parasite invasion is directly proportional to the number of eggs in the host [4, 23]. Therefore, the reduction in the number of circulating parasites through provision of PZQ can subsequently reduce or delay tissue and organ damage. The prevalence and intensity of urogenital schistosomiasis was significantly reduced after mass treatment in Burkina Faso and the associated morbidity (anaemia and micro haematuria) was also halted by such measures [46]. However another study observed that the prevalence of *S. haematobium* infection remained high despite mass treatment with PZQ provision in a rural village in South Africa [49]. The reason therefore could have been due to reinfection owing to the fact that safe water access was not being made available for the community and additionally to the lack of anthelminthic drugs at the local clinic [49]. This emphasized the need for proper piped water delivery to such communities so that members of these communities can stop using river water for their daily water requirements [26, 49].

Recommendations: From the results obtained in this study it is suggested that school age pupils be offered treatment for 2 years in the ILembe Health District and everyone (irrespective of age, sex or status) in the uThungulu Health District be offered treatment once a year for a period of 3 years in line with WHO recommendations [2, 10]. As a further precautionary measure to ensure sustainability, it should also be considered that these communities will be at constant risk of re-infection unless safe drinking water, adequate sanitation and recreational facilities are made available to such communities. This should, of course, be accompanied by increasing community awareness through educational ventures that teach community members about the cause, transmission and the sequelae of the disease. Health education can be part of the school curriculum to alert learners about the potential dangers of being in contact with unsafe water and the importance of personal hygiene. Health education and health promotion can change the way in which school learners and community members think or feel about being in contact with fresh river water [49]. However such interventions will be in vain if the people in those communities have no other source of water because dirty/contaminated water remains better than no water at all and water itself is vital for life and, if these people lack a proper supply of clean water, they will be left with no choice but to use the water from the rivers. There are millions of girls and boys who are at risk of urogenital schistosomiasis in Africa, and it is necessary for the national health authorities to act immediately to avoid any further complications that arise from *S. haematobium* infection [49]. There is a series of scientific researchers and authors that advocate for the early treatment of *S. haematobium* infection [6, 11, 13, 23, 24, 34, 37-39, 46, 49 and 53]. This could be because treatment at an early age attenuates proteinuria, albuminuria and anaemia among infected individuals [37]. Early treatment of urogenital schistosomiasis infection can stop the development of granulomatous and fibrous tissue (from trapped eggs) and, hence, reduce the development of complex lesions in the genital tracts of infected individuals; this may in-turn reduce the transmission of HIV and STI infections that would be transmitted easily due to the breached epithelial membranes [5, 13]. Diagnosing and treating diseases such as FGS, kidney dysfunction and STIs can be a complex process requiring resources and time [5, 23]. There is an association with increased susceptibility and the development of these diseases among individuals who were infected by *S. haematobium* at a young age but not treated [13]. To avoid having to deal with diseases associated with *S. haematobium* infection in later life stages, it is important that we treat school learners now and implement strategies that will prevent reinfection and further transmission of *S. haematobium*.

REFERENCES

1. Johnson CC, Appleton CC. (2005) Urban schistosomiasis transmission in Pietermaritzburg, South African Journal of Epidemiology and Infection. Vol 20 (3); 103-107.
2. WHO. (2012) Prevention and Control of schistosomiasis in endemic areas. Technical report series .87: 81-89.
3. Brooker S. (2007) Spatial epidemiology of human schistosomiasis in Africa: risk models, transmission, dynamics and control. Trans R Soc Tropical Medicine Hygiene. 101 (1): 1-8. diol: 10.1016/j.trstmh.2006.08.004.
4. Barsoum RS, Esmat G, El-Baz T. (2013) Human schistosomiasis: Clinical perspective, Journal of Advanced Research. http://dx.doi.org/10.1016.j.jare.2013.01.005. accessed on 15-08-2013
5. Kjetland EF, Leutscher PDC, Ndhlovu PD. (2012) A review of female genital schistosomiasis.Trends in parasitology. 28(2);1-4.
6. Mbabazi PS, Andan O, Fitzgerald DW, Chitsulo L, Engels D, et al. (2011) Examining the Relationship between Urogenital Schistosomiasis and HIV Infection. PLoS Neglected Tropical Diseases 5(12): e1396. Doi:10.1371/journal.pntd.0001396
7. Pugia MJ. (2000) Technology behind diagnostic reagent strips. Laboratory medicine. CE Update –INSTRUMENTATION ii. 31 (2): 46515-0070.
8. De Kock KN, Wolmarans CT. (2004) Distribution and habitats of the Bulinus africanus species group, snail intermediate hosts of Schistosoma haematobium and S. mattheei in South Africa. Water SA. 31(1): 117-127.
9. Appleton CC. (1978) Review on Abiotic factors influencing the distribution and the life cycles of Bilharziasis host snail. Malacological Review. 11: 1-25.
10. WHO. (1998) Prevention and control of schistosomiasis and soil transmitted helminths. Technical report 813-911 Geneva: World Health Organisation.
11. Amazigo UV, Leak SGA, Zoure HGM, Njepuome N, Dikassa PSL. (2012) Community-driven interventions can revolutionise control of neglected tropical diseases. Journal of Neglected Tropical Diseases. 26 (6):231-238.

12. Appleton CC, Naidoo I. (2012) Why did schistosomiasis disappear from the southern part of the Eastern Cape? South African Journal of Science. 108: 1-11.
13. Hotez JP, Molyneux DH, Fenwick A, Ottesen E, Sachs SE. (2006) Incorporating a Rapid-Impact package for Neglected Tropical Diseases with programs for HIV/AIDS, Tuberculosis, and malaria. A comprehensive pro-poor health policy and strategy for the developing world. Plos Medicine. 3(5); 0576-0584.
14. King CH, Bertsch D. (2013) Meta-analysis of urine heme dipstick diagnosis of schistosoma heamatobium infections including low prevalence and previously-treated populations. Plos Neglected Tropical Diseases. 7 (9): e2431. Dol; 10.17371/ Journal.pntd0002431.
15. Taylor M, Jinabhai CC, Naidoo K, Dlamini SB, Sullivan KR. (2004) The epidemiology of schistosomiasis among Zulu children in a rural district in South Africa: determining appropriate community-based diagnostic tools. The South African Journal of Epidemiology and Infection. 19(3,4): 90-95.
16. Appleton CC, Kvalsvig JD. (2006) A school based helminth control program successfully implemented in Kwa-Zulu-Natal. The South African Journal of Epidemiology and Infection. 21 (2): 55-67.
17. Risikat SA, Ayoade AA. (2012) Correlation analysis between the prevalence of Schistosoma Haematobium and water conditions: A case study among the school pupils in South Western Nigeria. 13(1): 160-169.
18. Liao CW, Sukati H, Nara T, Tsubouchi A, et al. (2011) Prevalence of Schistosoma haematobium infection among school children in remote areas devoid of sanitation in North Western Swaziland, Southern Africa. Japan Journal of Infection and Disease. 64: 322-326
19. Morgas DE, Kvalsvig JD, Gunderson SG, Taylor M, Kjetland EF. (2010) Schistosomiasis and water-related practices in school girls in rural kwaZulu-Natal, South Africa. 25 (4): 31-33.
20. Der EM, Quayson SE, Mensah JE, Tettey Y. (2015) Tissue schistosomisis in accra ghana: a retrospective histopathologic review at the korle-bu teaching hospital (2004-2011). Pathology Discovery. 3 (1): 1-6. Doi: 10.7243/2052-7896-3-1.
21. Oniya MO, Ishola MA, Jayeoba OD. (2013) Schistosomiasis in Ipogun: Update Assessment on Endemicity and Efficacy of Praziquantel in

Chemotherapy. International Journal of Tropical Disease and Health. 3(1): 37-44.

22. Jourdan PM, Roald B, Poggensee G, Gunderson SG, Kjetland EF. (2011) Increased Vascularity in Cervicovaginal Mucosa with *Schistosoma haematobium* infection. PLos Neglected Tropical Diseases. 5(6): 1-6 e1170.
23. Kayange NM, Smart LR, Tallman JE, Chu EY, et al. (2015) Kidney disease among children in sub-Saharan Africa. Systemic Review. Paediatric research. 77(2): 85-189. Dio: 101038/pr2015.189.
24. Ndeffo ML, Mbaha I, Kjetland EF, Atkinsa C, *et al*. (2013) Cost-effectiveness of a community-based intervention for reducing the transmission of *schistosoma haematobium* and HIV in Africa. Population Science and Economic sciences. 110 (19); 7952-7957. Doi:10.1073.pnas.1221396110.
25. Randrianasolo BS, Jourdan PM, Ravoniarimbinina P, Ramarokoto CE. (2015) Gynecological manifestations, Histopathological findings and schistosoma-specefic PCR results among women with schistosona haematobium infection: A cross-sectional study in Madacascar. FGS histopathology and PCR. Journal of Infectious diseases: 212-275. Diol: 101093/infdis/jiv035.

26. Esrey SA, Potash JB, Roberts L, Schiff C. (1991) Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasts, hookworm infection, schistosomiasis and trachoma. Bulletin of the World Health Organisation. 69(5): 609-621.
27. Clements AC, Garba A, Sacko M, Toure S, et al. (2008) Mapping the probability of schistosomiasis and associated uncertainty in West-Africa. Journal of Emerging Infectious Disease 14(10): 1629-1632 . Diol 10.3201/eid1410.080366.
28. Ibironke O, Koukounari A, Moustaki I, Shiff C. (2012) Validation of a new test for *schistosoma heamatobium* based on detection of DNA fragments in Urine: Evaluation through latent class analysis. Plos Neglected Tropical Diseases 6(1) e1464 doi: 10.1371/journal.pntd.0001464.
29. Saathoff E, Olsen A, Magnessen P, Kvalsvig JD, et al. (2004) Patterns of *S. haematobium* infection, impact of PZQ treatment and re-infection after

treatment in a cohort of school children from rural KwaZulu-Natal BMC infectious disease, 4;40 diol:10.118611471-2334-4-40.

30. Freeman MC, Simon TC, Daniels O, Rheingans R.(2013) The impact of school-based hygiene, water quality and sanitation, intervention on soil-transmitted helminth reinfection: A cluster randomized trial: American Journal of Tropical Medicine & Hygiene 213-237.
31. ILembe District Municipality. (2011/2012) Intergrated Development Plan. Annual Review.
32. UThungulu District Municipality (2012/2013) Integrated Development Plan.
33. Kildemoes AO, Kjetland EF, Zulu SG, Taylor M, Vennervald BJ. (2015) Schistosoma haematobium infection and asymptomatic bacteriura in young south african females. Acta Tropica. 144: 14-23. Doi: 10.10.16/j.actatropica.2015.01.008.
34. Senghor B, Diallo A, Doucoure SS, Ndiath MO, et al. (2014) Prevelance and intensity of urinary schistosomiasis among school children in the district of Niakhar, Region of Fatick Senegal. Parasites and Vectors. 7:5 Diol: 10.1186/1756-3305-7-5.
35. WHO/PAHO, (2014) Schistosomiasis Regional meeting: Defining the road map toward verification of elimination of schistosomiasis in Latin America and the Caribbean by 2020.
36. Chipeta MG, Ngwira B, Kazembe L. (2013) Analysis of schistosoma haematobium infection prevalence and intensity in Chikhwawa, Malawi: An application of a two part model. Plos Neglected tropical diseases. 7 (3) 2131-2209.
37. Stothard JR, Sousa-Figueiredo JC, Betson M, Bustinduy A, Reinhard-Rupp J. (2013) Schistosomiasis in African infants and preschool children: let them now be treated! Trends in Parasitology. 29 (4): 197-205.
38. Bustinduy A, King C, Scott J, Appleton S, et al. (2014) HIV and schistosomiasis co-infection in African children. Lancet Infectious Disease:Review. 14: 640-649.
39. Meents EF, Boyles TH. (2010) Schistosoma haematobium prevalence in school children in the rural Eastern Cape Province, South Africa. South African Journal Epidemiology Infection. 25 (4): 28-29.
40. Pitchford RJ. (1981) Temperature and schistosome distribution in South Africa. South African Journal of Science. 77: 252-261.

41. Schutte CHJ, Van Deventer JGM, Lamprecht T. (1981) A cross-sectional study of the prevalence and intensity of infection with Schistosoma haematobium in students of northern KwaZulu-Natal. American Journal of Tropical Medicine and Hygiene. 30: 364-372.
42. Appleton CC, Gouws E. (1996) The distribution of common intestinal nematodes along an altitudinal transect in KwaZulu-Natal, South Africa. Annals of Tropical Medicine and Parasitology. 90(2): 181-188.
43. Stensgaard AS, Utzinger J, Vounatsou P, Hurlimann E, et al. (2013) Large scale determinants of intestinal schistosomiasis and intermediate host snail distribution across Africa: does climate matter? Acta Tropica 128: 378-390. Diol: 10:1016/j.actatropica.2011.11.010.
44. WHO,(2016). Schistosomiasis www.who.int/mediacentre/factshhets/fs115/erv .
45. Joubert PH, Pretorius SJ, De Kock KN, van Eden JA. (1986) Survival of Bulinus africanus (krauss), Bulinus globosus (morelet) and Biomphalaria pfefferi (krauss) at high temperatures. South African Journal of Zoology. 21(1): 85-88. Doi: 10.10801/02541858.1447963.
46. Koukounari A, Gabrielli AF, Toure S, Oliva BE, et al. (2007) Schistosoma haematobium infection and morbidity before and after large-scale administration of praziquantel in Burkina Faso. Journal of Infectious Diseases. 196 (5): 659-69.
47. Census (2011) Municipal Report KwaZulu-Natal. Statistics SA. Report number 03-01-53.
48. De Meillon J, Frank GH, Allanson BR. (1958) Some aspects of snail ecology in South Africa. World Health Organisation. Bulletin: 18: 771-783.
49. Wolmarans CT, De Kock KN, le Roux J, Strauss HD, Killan M. (2001) The high prevalence of schistosomiasis in a rural village in South Africa, despite educational, medical and water reticulation infrastructure. South African Journal of Epidemiology and Infection. 16(1): 15-22.
50. WHO, (2010) Report of an informal working group on urogenital schistosomiasis and HIV transmission. Geneva, Switzerland. 1-35.
51. Moonasar D, Morris N, Kleinschmidt I, Maharaj R, et al. (2013) What will move malaria control to elimination in South Africa? The South African Medical Journal. 103 (10): 801-806.

52. Department of Education KwaZulu-Natal school list, (2012) www.education.gov.za/EMIS/EMISDownloads/tabid/466/Default.aspx. 18.02.2015.
53. WHO, (1995) Health of school children treatment of intestinal helminths and schistosomiasis. WHO/schisto/95.112
54. Chutel L, (2016) South Africa: Drought leads to failed crops and water shortages. Phy.org/news/2016-01-South-Africa-drought-crops-shortages.html. 10.01.2016
55. Edu-Action. GIS and Education consultants. www.eduaction.co.za
56. Geleta S, Alemu A, Getie S, Mekonnen Z, Erko B. (2015) Prevalence of urinary schistosomiasis and associated risk factors among Abobo primary school children in Gambella Regional state, Southwestern Ethopia: a cross sectional study. Parasite and Vectors. 8: 215-224. Doi 10.1186/s13071-015-0822-5
57. Odhiambo GO, Musuva RM, Atuncha VO, Mutete ET, et al. (2014) Low levels of awareness despite high prevalence of schistosomiasis among communities in Nyalendu Informal Settlement, Kisumu City, Western Kenya. Plos Neglected Tropical Disease. 8(4): e2784/Doi 10.1371/journal.pntd.0002784
58. Sokolow SH, Wood CL, Jones D, Swartz SJ, et al. (2016) Global assessment of schistosomiasis control over the past century shows targeting the snail intermediate host works best. Plos Neglected Tropical Disease. 10(7): e0004794. Doi 10.1371/journal.pntd.0004794

APPENDIX A (SPSS TABLES)

SPSS tables

Model Information

Dependent Variable	Prev
Probability Distribution	Normal
Link Function	Identity

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypodissertation Test		
			Lower	Upper	Wald Chi-Square	Df	Sig.
(Intercept)	.567	.0424	.484	.651	179.296	1	.000
[District=Ilembe]	-.156	.0453	-.244	-.067	11.829	1	.001
[District=Uthungulu]	0[a]	.	.	.	.	.	.
[gender=boy]	.043	.0432	-.042	.128	.991	1	.319
[gender=girl]	0[a]	.	.	.	.	.	.
(Scale)	.101[b]	.0097	.084	.122			

Dependent Variable: Prev

Model: (Intercept), District, gender

a. Set to zero because this parameter is redundant.

Maximum likelihood estimate.

Estimates

Gender	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
Boy	.532684	.0313050	.471327	.594040
Girl	.489635	.0313050	.428278	.550992

Covariates appearing in the model are fixed at the following values:
NegInfec=14.99; PInfectio=13.06

Estimates

District	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
Ilembe	.433299	.0268569	.380660	.485937
Uthungula	.589020	.0364513	.517576	.660463

Covariates appearing in the model are fixed at the following values:
NegInfec=14.99; PInfectio=13.06

SPSS Tables for Correlation of prevalence and abiotic factors

Table : Representing correlation coefficient for the girls prevalence vs altitude above sea level

Correlations

			Altitude above sea level (m)	G.Prev%
Spearman's rho	Altitude above sea level (m)	Correlation Coefficient	1.000	-.215*
		Sig. (2-tailed)	.	.035
		N	96	96
	G.Prev%	Correlation Coefficient	-.215*	1.000
		Sig. (2-tailed)	.035	.
		N	96	96

*. Correlation is significant at the 0.05 level (2-tailed).

Table 5: Representing correlation coefficient for the boys prevalence vs altitude above sea level

Correlations

Table 6: Representing correlation coefficient for the girls and boys prevalence vs distance to the nearest river

Correlations

			Distance to nearest river (m)	G.Prev%	B.Prev%
Spearman's rho	Distance to nearest river (m)	Correlation Coefficient	1.000	.010	-.007
		Sig. (2-tailed)	.	.922	.947
		N	96	96	96
	G.Prev%	Correlation Coefficient	.010	1.000	.599**
		Sig. (2-tailed)	.922	.	.000
		N	96	96	96
	B.Prev%	Correlation Coefficient	-.007	.599**	1.000
		Sig. (2-tailed)	.947	.000	.
		N	96	96	96

**. Correlation is significant at the 0.01 level (2-tailed).

Table 7: Representing correlation coefficient for the girls and boys prevalence vs average summer maximum and minimum temperature.

Correlations

			G.Prev%	B.Prev%	Average Summer Maximum (°C)	Average Summer Minimum (°C)
Spearman's rho	G.Prev%	Correlation Coefficient	1.000	.599**	.244*	.121
		Sig. (2-tailed)	.	.000	.017	.242
		N	96	96	96	96
	B.Prev%	Correlation Coefficient	.599**	1.000	.150	.146
		Sig. (2-tailed)	.000	.	.145	.156

	N	96	96	96	96
	Correlation Coefficient	.244*	.150	1.000	.772**
Average Summer Maximum (°C)	Sig. (2-tailed)	.017	.145	.	.000
	N	96	96	96	96
	Correlation Coefficient	.121	.146	.772**	1.000
Average Summer Minimum (°C)	Sig. (2-tailed)	.242	.156	.000	.
	N	96	96	96	96

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

APPENDIX B (PERMISSION LETTER FOR USE OF DATA)

Dear Nathi,

I have just had a meeting with Myra and Khantsho. I understand you would like to try to do the Masters part time. We have decided that we will offer you this opportunity under the preconditions below. Please sign and return to Myra.

Regards, Eyrun

BEFORE anything can start you will need to submit an updated ethics course certificate – report to Khantsho and admin RA (Sneh)

(1) You will spend pre-planned hours at UKZN under Myra Taylor's supervision

(2) The hard copies (photocopies) must be neatly filed in Myra's offices at UKZN

(3) The following time plan and reporting (to different people) must be followed:

   a. Last Tuesday* in January the photocopying has been done by you (on weekdays in Ugu but NOT on January 20th) – report to Khantsho and Sneh who will keep the files until they can be safely transported
   b. Transport of the photocopies will be done by VIBE vehicles – liaise with Sneh about timing (not separate journey)
   c. Last Tuesday in February the first 40 schools of dipstick data have been entered by you - send the data set to Khantsho (CC Myra and Eyrun)
   d. Last Tuesday in March the rest of the schools dipstick results have been entered - send the data set to Khantsho and Roy (CC Myra and Eyrun). At this time you may request the cleaned urine microscopy results for the area.
   e. Last Tuesday in April you have finished school database (as opposed to the individual database of March) - send the data set to Khantsho and Roy (CC Myra and Eyrun)
   f. Last Tuesday in May – you have finished the first draft of the results' chapter – send draft to Myra and Sipho, CC Eyrun

(4) This will be your assignment: To explore the dipstick results as an indicator of *S. haematobium* prevalence and intensity in teenagers taking gender and age into consideration.

(5) You will keep to your agreements with Myra Taylor.

(6) We will provide taxi travel money for 2 journeys to the Ugu clinic, free photocopying there (by you), and files for the photocopies. We will transport files to UKZN. You will get urine microscopy results. No other items / funding will be expected.

(7) Enclosed is data use form which must also be signed.

I declare that I will follow this contract _____Date ___/____/ 2014

APPENDIX C (ETHICS APPROVAL LETTER)

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

19 August 2015

Mr N Banhela (207511537)
School of Nursing and Public Health
Health Sciences
nathi5sifeukzn@gmail.com

Protocol: Investigating the prevalence and intensity of urogenital schistosomiasis among school going pupils in rural Ilembe and Uthungulu Districts, KwaZulu-Natal.
Degree: MMedSc
BREC reference number: BE165/15

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 19 May 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 10 July 2015 to queries raised on 08 July 2015 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have been met and the study is given full ethics approval.

This approval is valid for one year from **19 August 2015.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

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

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

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

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **08 September 2015**

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

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc: supervisor: taylor@ukzn.ac.za
cc: Postgrad: ramlakm@ukzn.ac.za

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000

APPENDIX D (PROTOCOL APPROVAL LETTER)

INYUVESI YAKWAZULU-NATALI

12 March 2015

Mr N Banhela
Student No 207511537
Discipline of Public Health Medicine
School of Nursing & Public Health

Dear Mr Banhela

***Masters in Medical Science*: "Investigating the prevalence and intensity of Urogenital schistomiasis among school going pupils in rural iLembe and uThungulu Districts, KwaZulu-Natal" N Banhela Student No 207511537**

Your protocol has been given final approval of the abovementioned study, on the 9th March 2015. This will be noted at the next Postgraduate and Research & Higher Degrees Committee Meeting.

Please note:

- The Postgraduate Committee must review any changes made to this study
- Please note that the study may not begin without the approval of the Biomedical Research Ethics Committee

May I take this opportunity to wish you every success with the study.

Yours sincerely

Mrs Devi Arumugam
School of Nursing & Public Health

CC. Discipline of Public Health Medicine

Postgraduate Administration
School of Nursing and Public Health
University of KwaZulu-Natal
Postal Address: University of KZN, Durban, 4041, South Africa
Telephone: +27 (0) 31 260 2499

APPENDIX E (TEMPERATURE DATA)

LEGEND

Maximum temperature of the day (in °C)

---- indicates that data is not yet available or was not requested

*** indicates that data is missing or not yet available in the current month

= indicates that the average for the month is unreliable due to missing daily values

Daily Maximum Temperature (C) Data for station [0271699 2] - MANDINI -29.1580 31.4020 113 m 2012 08:00 (Extracted 2015/06/17 15:42)

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	31.3	34.1	32.9	23.2	25.4	25.2	24.7	19.9	23.4	32	21.1	26.9
2	29.9	30.6	31.1	25.6	30.8	25.6	24.5	20.5	21.4	27.5	21.9	27.4
3	29	30.2	28.5	26.8	38.3	24.4	25	23.7	22	32.3	26.8	31.7
4	35.6	36.2	***	31.2	27.5	25.6	32.1	28.9	16.8	24.3	33.3	28.5
5	24	29.2	***	26.6	25.2	31.4	22.5	28.5	14.1	20.9	25.6	26.1
6	29.7	28.1	25	30.3	25.3	25.6	30	20.1	17.3	28.7	25	27.2
7	30.6	29.9	27.7	30.8	26.7	25.7	31.8	15.5	19.4	23.4	23.1	32.7
8	30.3	35.5	27.5	35.6	28.7	26.7	21.8	20.7	28.2	20.4	24.9	29.5
9	32.4	31.1	30.6	25.8	30.1	23.2	24	26.6	33	28.7	26	29.1
10	32.8	29.2	29.8	24.4	31.4	22.1	24.7	27.8	20.4	24.2	28.4	29.4
11	35.5	22.8	***	25.6	25.1	25.1	25.4	34.1	23.6	21	35.2	23.5
12	37.2	28	29.1	23.6	27.6	27	19.7	23.7	29.1	21.5	26.9	26.8
13	35.3	29.4	28.7	25.1	30.8	19.9	22.8	23.5	34.1	28.9	25.5	30.9
14	30.2	35.7	29	25.1	25.1	21.4	27.5	32.5	17.7	26.3	22.1	30.3
15	29.5	28.1	28.1	30.3	21.2	27.3	22.1	36.6	15.5	31.2	29.5	32.2
16	25.9	29.9	30.3	26.4	25.5	27.1	20.8	22.8	21.7	27.3	32.1	27
17	29.1	27.1	29.6	29.6	28.5	28.5	22.2	18.2	24.6	25.9	23.2	27.7
18	29.6	24.2	***	24.9	31	25.4	20.9	28.9	28.4	28.9	22	28.3

17	29.1	27.1	29.6	29.6	28.5	28.5	22.2	18.2	24.6	25.9	23.2	27.7
18	29.6	24.2	***	24.9	31	25.4	20.9	28.9	28.4	28.9	22	28.3
19	29.5	29.5	***	22.9	20.4	25.8	23.5	34.3	23.2	26.2	28.5	31.1
20	30.3	33.8	***	26.4	18.6	23.8	25.5	28.5	27.4	20.2	31.5	29.7
21	30.1	31.8	***	29.6	22.2	20	24.1	22.3	36.5	27	23	31.1
22	29	32.9	***	26.2	26.6	22.1	23	25.2	22.4	24.9	24.8	34.7
23	30	27.7	***	15.8	30.2	20.1	19	20.6	22.3	22	31.1	35.2
24	29.2	32.4	***	21.3	28.1	23	23.4	26	24.8	22.2	32.3	33
25	27.5	33	***	24.2	27.9	24.6	24.5	41.5	24.5	24.8	19.7	34.7
26	27.7	31.3	***	28.2	24.2	24.5	27.4	24.7	32.4	29.5	29.7	28.7
27	28.8	31.4	***	28	25.2	27.7	20.1	25.9	26.5	20.8	20.7	***
28	28.5	37.3	***	25.9	26.4	25.7	21.4	27.9	21.9	20.9	27.8	***
29	28.5	31.4	***	27.6	31.1	31.7	21.1	38.3	22.4	26.7	27.2	***
30	34.3	***	32	30	24.9	26.4	21.6	27	24.8	19.2	26.3	***
31	31.5	***	20.3	***	23.4	***	25.4	39.4	***	22.1	***	***
Avg	30.4	30.8	28.8=	26.6	26.9	25.1	24	26.9	24	25.2	26.5	29.7=

Daily Maximum Temperature (C) Data for station [0304357 6] - MTUNZINI -28.9470 31.7070 41 m 2012 08:00 (Extracted 2015/06/17 15:42

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	32.2	35	32.2	24.5	25.6	25.4	25.8	20	30	32.3	21.1	26.2
2	30.3	30.7	30.2	25.4	30.9	25.4	24.8	20.6	22.4	26.6	21.4	28.9
3	32.1	30.1	28.9	27.5	33.6	24.7	26.5	24.6	23	37.6	26.5	31.7
4	37.1	37.8	25.1	31.3	27	25.9	34.5	30.6	18.2	25	33.8	27.8
5	27	29.2	26.1	27.1	24.8	32.1	22.8	28.6	16.2	22.7	26	25.5
6	29.1	29.5	25.1	31.3	25.8	26.1	29.6	21.6	18.9	30.7	24.6	26.8
7	30.8	30.4	27.4	31.2	27	25.7	31.6	16	19.4	24.4	24.5	32.5
8	30.6	38.5	28.1	34.7	28.6	28.5	21.9	20.6	27.2	22.3	21.9	28.8
9	35.1	31.8	30.4	27	29.4	22.4	26.7	27.7	32.4	29.3	26.6	28.5
10	34.1	30.6	29.8	24.3	32	22.1	24.1	25.7	22.7	25.5	28.1	28.5
11	36.4	24.1	29.1	25.6	25.3	24.3	25.3	35.4	24.3	21.3	34.3	23.7
12	36.8	27.5	27.7	23.6	27.9	24.9	20.6	23.7	29.4	21.2	28.6	25.7
13	38.4	29.7	28.4	24.6	31.9	21.5	22.2	24	33.1	28.7	26.5	30.5
14	30.9	35.9	28.1	24.9	25.5	19.3	28.4	32.7	19.8	28.1	23.5	30.4
15	30.6	28.5	28.1	29.8	22.7	26.1	21.5	35.3	16.3	30.9	28.8	32.3
16	26.7	29.6	29.7	26.6	25.9	26.5	20.6	23.7	20.4	26.4	33.7	26.7
17	29.8	27.7	29.3	29.6	28.8	29.1	22.3	19.9	25.2	26.5	24.4	26.9
18	30.3	24.7	25.7	26	29.2	25.3	20.9	28.8	29.8	31.4	23.1	27.5
19	29.7	29	32.4	22.3	20.7	25.3	24.4	33.1	24.1	25.3	27.7	32.2
20	30.9	33.4	28.8	26.6	19.8	24.1	29.2	27.2	28.4	20.9	31.2	28.8
21	30.9	31.6	33.5	29.9	22.2	20.3	24	22.8	38.5	27.7	25.7	29.9
22	29.7	35.5	30.4	26.1	27.3	24.8	23.8	24.9	23.5	24.7	23.7	34.3
23	30.2	28.2	33	17.1	28.6	21.4	19.9	22	22.9	21.5	30.2	36
24	29.7	31.6	29.1	21.6	28.5	23.2	27.2	25.9	24.5	21.3	31.8	35.4
25	27.6	33.7	32.5	24.4	29	24.5	23.3	42.5	23.7	23.5	21.1	34.8
26	27.4	31.6	33.3	28.2	25.8	26	28.9	24	32.4	29.7	29.9	28.2
27	28.6	31.4	37	31.9	25.6	26.3	22.1	28.3	26.6	20.4	21.4	33.9
28	28.7	36.4	28.8	27.3	26.9	26.9	20.9	28.2	24.8	21	27.9	30.3
29	28.9	31	31.7	27.1	29.9	30	21.1	34.4	22.7	26	26.9	36.7
30	35.2	***	36.1	30.7	25.4	26.7	21.3	26.7	25.9	21.1	27.3	35.6
31	31	***	22.4	***	23.9	***	22.8	38.6	***	21.9	***	38.4
Avg	31.2	31.2	29.6	26.9	27	25.2	24.5	27	24.9	25.7	26.7	30.4

Daily Maximum Temperature (C) Data for station [0305134 6] - RICHARDS BAY AIRPORT -28.7370 32.0930 36 m 2012 08:00 (Extracted 2015/06/17 15:42)

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC

1	29.5	32.1	30.5	22.3	25.7	25.5	23.2	19.3	24.8	29.5	21.8	24.2
2	29.3	29.5	29.4	24.3	29.5	23.8	23.6	19.6	21	31.8	20.7	27.7
3	33.3	29.1	27.4	25.6	32.2	23	23.4	22.4	21.5	33.3	24.8	30.9
4	33.3	34.5	25.7	28.8	26.5	24.2	27	25.6	17.1	24.7	31.3	26.3
5	28.5	28.3	25.6	26.8	25.1	26.3	21.1	25.2	16.8	23.7	25.7	24.5
6	29	29.5	25.1	29.2	25	24.9	24.9	22	19.7	32.5	24.2	25.5
7	29.6	29.6	27.2	29.5	25.8	23.4	28	16	20.6	25.4	24.8	30
8	30.2	34.6	26.8	32.4	27.3	27.3	19.8	18.6	25	22.4	23.3	29.6
9	32.2	34.7	28.9	26.2	27.2	18.9	24.1	25.2	29.8	26.9	24.6	29.2
10	33.2	31.1	28.7	24	28.7	21	23.5	24.6	23.3	26.6	27.2	27.5
11	33.6	24.4	27.7	24.8	25.2	21.8	23	33.9	23.8	20.9	32.5	23.1
12	33.6	27.3	26.5	22.4	27.4	24.2	20.3	23.6	26.1	19.7	28.6	24.9
13	35.1	29.1	27.2	23.5	28.8	20.6	21	22	28.9	25.7	26.5	29.5
14	30.6	32.1	27.4	24	24.7	17.6	26.7	29.6	20.2	26.5	23.7	30.1
15	29.7	27.5	26.9	28.4	21.8	24	20.7	29.8	18.1	29.5	27.1	31
16	25.7	28.4	28.3	25.9	24.2	24.7	19.9	22.8	22.2	25.7	33.6	26.2
17	28.5	29.6	29.8	27.5	26.5	25.8	21	20.5	23.5	24.2	24.4	25.9
18	29.5	24.9	24	24.6	25.6	23.4	20.4	25.8	27.9	32.5	23.4	26.7
19	28	26.9	32.5	21.2	21.6	23.3	21.9	29.3	22.5	23.9	26.1	29.4
20	30	30.9	27.5	25.1	20.1	22.7	25.3	26.6	25.2	22.7	29.4	27.7
21	29.9	31.7	32.1	27.9	21.4	18.3	23.2	21.9	37.1	25.8	24	28.6
22	28.9	33.5	28.6	25.7	24.7	22.6	22.6	22.8	25.2	24.4	23	32.3
23	29.8	27.3	31.4	17.9	27.5	20.7	19.8	21.5	20.8	21	28.1	33.8
24	30.1	29	28.9	18.7	25.7	22	24.3	28	24.3	20.5	28.8	33.6
25	26.3	33.1	31.2	24	27	22	22	37.4	22.5	20.6	21.2	34.3
26	24.7	32.4	32.4	26.7	26.9	23.7	25.3	24.1	29.8	27.9	28.6	25.9
27	27.3	30.2	35.2	29.8	23.5	24.2	22.3	24.7	25.7	22.4	21.7	31.2
28	27.4	32.4	27.7	24.8	24.5	23.9	19.9	25.1	27.7	20	25.3	28.1
29	27.8	31.4	31.1	25.4	26.9	25.8	19.6	31.8	21.6	23.8	26.5	33.8
30	32	***	35.7	29.3	23.9	25	19.7	24.5	23.9	20.9	25.2	34.8

Avg	29.9	30.2	28.7	25.6	25.6	23.2	22.7	25.1	23.9	25	25.9	29

Daily Maximum Temperature (C) Data for station [0337382 5] - BABANANGO -28.3640 31.2060 770 m 2012 08:00 (Extracted 2015/06/17 15:42)

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	29.4	32.6	28.3	23.2	35.7	27.7	22.6	17.3	25.9	28.2	16.6	19.7
2	28.7	29.9	29.2	24	29.6	24.7	30.9	18.7	16.8	33.2	18.7	20.8
3	32.6	32.1	26.4	23.9	36.4	23	23.5	22.2	25.4	35.9	22.7	27
4	31.6	34.9	23.9	30.4	36.5	24.4	29	28.7	14.6	27.1	31.7	25.4
5	27.1	26.2	24.5	25.4	23.6	28.6	19	24.7	11.1	16.5	24.1	21.6
6	28.3	26.7	24.4	27.4	25.3	28.4	28.7	21.5	11.3	29.6	20.6	23.4
7	28.4	29.1	24.4	27.9	27.1	26	30.1	12.1	14.3	26.3	21.7	27.6
8	29.2	32.4	24.7	32.9	25.9	28.6	17	15.4	22.4	18.2	17	27.8
9	30.7	31.7	28.3	27.3	28.7	21.4	23.5	22.9	28	26.6	20.2	28.2
10	31.8	27.1	28.3	25	29.7	19.2	27.5	25.6	20.4	28.4	26.1	25.1
11	31.3	24.6	27.3	24	24.4	22	26.3	29.7	18.9	14.9	30.6	22.8
12	33	24.1	26.7	23.9	27.6	27.9	20	18.2	24.2	13.8	32.4	23.5
13	32.7	27	26.8	22.6	30.4	20.9	23	20.8	26.9	23.9	26.3	25.7
14	29.2	30.3	26.7	24.5	28.6	20.8	26.2	29.6	13.8	24.3	19	26.2
15	29.3	27.8	25.6	31.3	19.8	24.5	23	33.6	11.5	29.2	25.9	26.9
16	23.1	31.4	27.3	27.2	24.6	25	19.6	20.5	15.9	25.5	35	24.7
17	26.8	28.8	31	29.5	27.3	25.7	20.5	15	19.3	21.1	23.6	22.9
18	27.2	22.6	22.2	25.4	28.9	26.6	18.7	25.8	28.5	30.9	18.4	24.8
19	26.3	25.2	31.3	22.9	23.3	23.7	19.7	31.8	20.9	21.3	23.2	29.9
20	27.4	31.7	26.6	27.1	15.2	25	27.1	33.7	23.8	18.7	26.4	29.1
21	29.5	29.7	32.2	28.6	20.7	18.8	27.6	21.1	31.7	22.7	23	27.7
22	27.8	30.7	29	27.9	24	23.3	28.1	24	29	25.3	19.7	30
23	28.2	22.3	31	14.4	30.3	22.6	12.7	20.8	16.2	19.4	27.6	31.5
24	28.3	26.1	28.8	19.8	31.1	22.2	24.6	35.3	25	14.5	30.1	31.8

24	28.3	20.1	28.8	15.8	31.1	22.2	24.0	33.3	23	14.3	30.1	31.8
25	24.5	33.1	30.6	22.5	27	22.2	21	36.6	21.3	19.5	18.2	29.6
26	27.1	31.8	31.5	27.9	30	22.4	26.3	19.4	31.1	27	24.1	22.9
27	26.2	29.8	34.5	32.2	23	28.3	24.4	23.2	33.9	16	20.3	28.6
28	27.1	31.4	27.6	23.6	24	24.1	18.8	23.1	24.5	15.6	23.1	26.7
29	27.1	30.3	28.2	28.3	28.5	30.2	17.1	33.7	20.4	23.6	31.4	31.7
30	31.9	***	35.1	36.2	24.9	30.7	18.7	25	21.9	16.4	20.2	33.1
31	30.1	***	18.8	***	22.8	***	25.3	34.7	***	18.4	***	33.7
Avg	28.8	29	27.8	26.2	26.9	24.6	23.2	24.7	21.6	23	23.9	26.8

LEGEND

Minimum temperature of the day (in °C)

---- indicates that data is not yet available or was not requested

*** indicates that data is missing or not yet available in the current month

= indicates that the average for the month is unreliable due to missing daily values

Daily Minimum Temperature (C) Data for station [0271699 2] - MANDINI -29.1580 31.4020 113 m 2012 08:00 (Extracted 2015/06/17 15:42)

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	18.8	19.7	20	15.6	15.7	11.4	8.4	9.2	14.1	12.6	13.4	17.8
2	20.2	19	19.5	14.2	15.8	12.6	10.4	6.3	13.5	14.9	13.1	18.3
3	21.6	18.4	19.2	13.5	16.6	12	14	6.6	9.8	16.2	15.1	20.3
4	21.7	21	***	13	15.4	9.4	13.1	7.8	12.1	16.9	15	17.8
5	21	21.9	***	17.9	18.3	9.2	11.3	15.5	12.3	16.1	18.5	18.1
6	19.8	21.1	19.5	16.6	17.2	14.4	9.7	12.4	13.2	18.8	17.9	17.9
7	22.1	19.1	18.8	16.1	16.9	13.3	13.1	10.2	14.7	16.4	17.7	19.9
8	21.6	21.2	17.6	16	17.7	11.4	8.9	8.3	13	15.9	15.2	21.7
9	22.3	22.1	17.2	14.3	14.3	11	8.3	6.9	13.8	18.2	12.7	21.4
10	22.2	20.9	20	11.8	13.6	10.5	10.8	9.3	16	15.4	15.3	18
11	22.4	19.6	***	14.2	15.2	8.2	11.3	7.9	15.3	15	15.4	16.6
12	22.9	19.1	19.5	16.3	13.9	4.6	13.6	10.6	11.4	15.9	18.5	16.1
13	22.8	21.1	17.8	12.8	13.9	10.7	10	8	15.9	17	18.3	17.3
14	22	19.6	19.5	11.6	13	12.5	11.5	6	13.3	17.4	17.9	19.6
15	20.8	22.1	19.7	11.1	14.9	9.1	10.2	11.4	13	17.6	18.5	20.1
16	20.4	19.3	17.8	16.6	12.3	8.5	10.2	16.6	13.6	18.4	18	19.2
17	21.6	22.7	19.5	14.4	13.1	9.2	10.7	13.5	13.2	17.8	18.4	19
18	21.2	20.5	***	15.9	10.4	12.6	9.9	12.5	14.4	17.1	17	18.9
19	19.1	20.3	***	15.8	15.9	13.7	7.6	14	16.3	16.2	17.7	18.2
20	19	22	***	13.7	12.6	11.9	6.4	14.8	14.4	15.6	20.7	20
21	19.8	21.7	***	13.8	9.9	13.5	7.5	16.2	18.6	19.2	18	19.4
22	21.4	20.5	***	14.5	11.6	12.4	12	15	14	18	17	19.8
23	20.8	19.9	***	11.5	7.9	11.9	13.5	15.5	14.3	14.9	17.2	19.4
24	21.6	19.3	***	12.9	14.3	12	11.7	15.3	12.4	14.5	19.2	20
25	20.5	16.4	***	14.2	14.3	10.4	10	14.2	14.9	14.4	14.8	21.6
26	18.2	18.5	***	12.4	11.9	8.4	7.8	17	12.8	17.3	15.5	21.3
27	18.9	21.3	***	13.8	17	9.3	8.7	13.9	17.3	15	16.8	***
28	17.9	19.6	***	15.1	15.3	10	13.9	12	14.1	15	16.2	***
29	16.2	20.2	***	15.8	12.1	7.3	11.1	15.8	11.6	17.4	15.5	***
30	18.8	***	18	15.7	14.8	9.6	8.5	16.4	10	15.3	18	***
31	20.2	***	16.4	***	14.5	***	5.7	16.8	***	14.5	***	***

Avg	20.6	20.3	18.8=	14.4	14.2	10.7	10.3	12.1	13.8	16.3	16.7	19.1=

Daily Minimum Temperature (C) Data for station [0304357 6] - MTUNZINI -28.9470 31.7070 41 m 2012 08:00 (Extracted 2015/06/17 15:42)

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	20.9	21.1	20.8	15.8	18.6	14.3	9.3	7.9	15	17.1	14.6	19.2
2	22.3	18.9	20.2	15.2	16.2	10.2	8.1	9	14.2	17.9	14	19.7
3	23.4	19.5	21.2	13.9	16	11.8	14.1	7.9	12.6	21.3	16.2	20.9
4	23	22.7	21.9	17	16.3	8.2	14.5	8.5	13.6	19.9	17.3	19.4
5	22.6	23.4	20.1	17.1	19.5	9.6	10.5	16	14.2	18.9	19.4	20.1
6	21.8	21.8	19.8	16.1	18.7	12	13.5	13.6	15.1	20.5	19.2	19.7
7	23.3	21.1	19.7	19.6	16	12.6	11.2	9.5	16.4	18.5	18.7	22.6
8	22.8	22.7	18.4	19.6	16.6	12.7	10.7	7.3	14.9	17.1	16.9	22.9
9	23.8	23.6	17.7	15	19.1	10.5	7.6	12.1	14.4	19.3	14.1	23.3
10	24.6	22.9	19.8	11	17.2	6.7	9.8	6.5	17.7	17.4	17	19.8
11	23.4	21	20.9	12.8	16.4	7.6	6.6	8.9	16.9	16.4	17.3	18.2
12	24.7	20.3	20.5	17.1	13.3	5.6	14.2	11.5	16	16.7	19.8	17.4
13	24.5	21.6	18.9	12.9	13.2	9.6	12	7.9	17.7	18.6	20.1	18.4
14	23.6	20.5	20.5	12.4	15.4	13.6	13.6	11	14.8	19.5	19.6	20.5
15	21.1	23.5	19.8	14.3	15.1	11.3	10.1	15.1	14.5	19.6	19.4	21.4
16	21.3	20.9	18.3	17.9	12.5	8.4	8.9	15.8	14.2	18.9	20.2	20.6
17	23.7	24.2	18.2	15.8	12.1	6.9	8	15.5	13.6	18.8	20.6	20
18	22.9	21.8	17.7	16.4	10	8.2	10.6	15	17	18.9	18.8	19.6
19	21.2	21.8	19.2	17.2	16.4	14.6	7.2	17.2	16.6	18.4	19.5	18.5
20	20.8	23.5	18.8	15.1	13.8	12.2	8.8	18.6	14.6	17.1	22.2	21.5
21	21.3	23.6	21.4	14.7	8.6	***	6.6	17.1	20.2	20.4	20.2	20.6
22	23.6	22.6	21.9	16.4	11.9	17	10.7	15.2	15	19.5	19	21
23	23.2	21.6	21	13.2	10.9	14.9	14.6	17.1	14.9	16.7	19.9	21.7
24	22.9	21.2	20	14	7.1	12.7	13.9	17.3	13.9	16	20.8	21.1

14	22.3	21.7	21.1	15.4	16.4	14.8	13.2	13	16.2	19	19.2	21
15	21.1	23.3	19.6	16.3	16.5	12.6	9.1	16.8	15	19.9	18.9	21.2
16	22.4	22	18.4	19.7	16.6	13.1	12.2	17.3	14.8	19.6	21	20.8
17	24.5	24.6	19.2	16.9	14.5	13.3	10.6	15.1	14.8	18.9	21.1	18.5
18	22.5	21.9	18	16.7	14.2	12.2	11.4	14.9	16.2	20.2	19.3	18.4
19	21.5	21.9	18.5	17.1	14.4	16.8	9.3	17.1	16.4	19.7	19.3	17.4
20	20.4	23.2	20.5	15.9	13.8	14	11.7	18.7	14	17.3	22.2	21.8
21	22	24.4	21.6	17	12.5	14.2	13.5	14.9	20.4	21.8	20	21.6
22	22.3	23.4	22.2	17.8	13	14.6	13.9	3.9	16.3	20.4	19.1	22.2
23	22.8	23.1	21.7	13.4	15.3	16.7	15.1	17.3	14.6	16.6	19.5	22.3
24	22.4	20.6	21	14.4	10.5	13.6	13.3	18	14.3	16	21.2	21.7
25	21	21.1	19.9	15.8	16.4	11.8	12.6	17.1	16.6	16.3	16.3	22.4
26	19.9	23.7	22.5	16.2	18.3	9.6	9.6	15.8	17.6	18.2	15.8	22.3
27	20.2	22	20.9	19	17.5	13.5	15.6	14.2	20.6	17.8	18.2	20.7
28	19.8	21.3	21.9	17.9	16.4	13.3	14.8	13.8	16.7	16.6	17.6	24.1
29	18.9	21.8	23.9	18.5	16.6	15.5	12.1	17.8	14	18.9	19.4	24.5
30	18.6	***	22.3	19.1	15.1	12.6	10.6	18.9	10.8	17	20.5	24.2
31	21.1	***	16.7	***	14.8	***	9.3	19.2	***	16.2	***	21.6
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g	22.1	22.2	20.6	16.7	16.7	13.2	12.8	13.9	16	18.5	18.4	21.1

ily Minimum Temperature (C) Data for station [0337382 5] - BABANANGO -28.3640 31.2060 770 m 2012 08:00 (Extracted 2015/06/17 15:42)

y	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	15.4	15.2	15.9	12.1	15.5	8.1	4	6	10.8	8.6	9.7	14.1
2	19	15.6	15.6	11.2	13	8.6	8	2.9	8.8	12.5	9.6	14.1
3	18.2	14.4	16.1	8.9	17.9	7	9.8	0.5	9	15.7	10.2	15.6
4	19	16	17.7	9.5	18.3	4.5	12.2	5.5	9	13.6	14.9	13.6
5	18.1	18.3	16.6	12.5	16.1	7.7	6.7	8.6	9.1	13	14.7	15.3
6	18.1	18	15.4	11.6	15.6	13.4	7.1	8.4	8.7	14.8	14.8	15
25	20.8	18.5	20	15.9	14.7	10.6	9.6	14.8	16.7	16.7	16	22.5
26	18.6	23.5	20.9	15.6	17.7	8.8	8.1	19	17.5	18.9	16.5	22.4
27	19.5	22.1	21.5	18.3	17.2	7.2	9.5	14.6	20.5	16.9	17.6	21.8
28	18.1	21.9	21	16	16.1	10.8	14.5	13	15.8	16.6	18.1	24.3
29	17.1	22.2	21.2	18.2	14.2	9.1	11.4	16.4	12.7	18.8	18.7	24.4
30	18.9	***	18.6	15.4	12.4	10.2	8.9	20.4	11.2	16.4	19.7	22.9
31	20.6	***	17.1	***	14.5	***	7	21.1	***	16.2	***	22.6

	21.9	21.9	19.9	15.7	14.8	10.6=	10.5	13.6	15.4	18.2	18.4	20.9

y Minimum Temperature (C) Data for station [0305134 6] - RICHARDS BAY AIRPORT -28.7370 32.0930 36 m 2012 08:00 (Extracted 2015/06/17 15:42)

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	21.1	21.6	21.5	16.3	18.4	15.4	11.3	12.8	17.4	16.2	14.8	19.6
2	22.4	19.1	21.6	15.6	19.3	13.4	15.3	10.9	14.3	18.7	14.1	19.7
3	22.9	20.1	22	14.9	21.8	13.6	15.5	8	15.1	20.6	15.6	21.6
4	22.6	22.7	21.9	15.6	18.7	10.7	17.2	13.6	13.6	20	18	20.6
5	23	23	20.4	16.8	20.1	15.1	14.9	16.3	14.1	19.3	19.6	20.5
6	22.6	22.6	20.1	17.6	19.1	12.4	15.4	15.4	15	20.8	19.9	20.5
7	22.5	21.9	19.7	19.2	17.6	13.7	16.5	8.2	16.8	20	18.5	22.9
8	21.6	20.8	18.4	20.8	18.9	13.6	12	4.7	16.6	18.2	15.6	23
9	24.1	24.2	18.7	15.9	19.7	11.1	10	11	16.7	19	14.1	23
10	24.8	23.5	21.4	15	17.7	9.7	12.5	9	19.2	18	17.8	21.6
11	24.1	21.3	21.8	14	19	10.9	10.2	12.6	16.9	17.1	17.4	19
12	24.6	20.6	20.7	17.3	15.8	12.1	14.7	13.4	16.8	16.9	20.1	18.1
13	24.2	22.3	19.6	15.3	17.5	11.1	13.2	10.2	17.8	18.1	19.1	17.5
14	22.3	21.7	21.1	15.4	16.4	14.8	13.2	13	16.2	19	19.2	21

14	22.3	21.7	21.1	15.4	16.4	14.8	13.2	13	16.2	19	19.2	21
15	21.1	23.3	19.6	16.3	16.5	12.6	9.1	16.8	15	19.9	18.9	21.2
16	22.4	22	18.4	19.7	16.6	13.1	12.2	17.3	14.8	19.6	21	20.8
17	24.5	24.6	19.2	16.9	14.5	13.3	10.6	15.1	14.8	18.9	21.1	18.5
18	22.5	21.9	18	16.7	14.2	12.2	11.4	14.9	16.2	20.2	19.3	18.4
19	21.5	21.9	18.5	17.1	14.4	16.8	9.3	17.1	16.4	19.7	19.3	17.4
20	20.4	23.2	20.5	15.9	13.8	14	11.7	18.7	14	17.3	22.2	21.8
21	22	24.4	21.6	17	12.5	14.2	13.5	14.9	20.4	21.8	20	21.6
22	22.3	23.4	22.2	17.8	13	14.6	13.9	3.9	16.3	20.4	19.1	22.2
23	22.8	23.1	21.7	13.4	15.3	16.7	15.1	17.3	14.6	16.6	19.5	22.3
24	22.4	20.6	21	14.4	10.5	13.6	13.3	18	14.3	16	21.2	21.7
25	21	21.1	19.9	15.8	16.4	11.8	12.6	17.1	16.6	16.3	16.3	22.4
26	19.9	23.7	22.5	16.2	18.3	9.6	9.6	15.8	17.6	18.2	15.8	22.3
27	20.2	22	20.9	19	17.5	13.5	15.6	14.2	20.6	17.8	18.2	20.7
28	19.8	21.3	21.9	17.9	16.4	13.3	14.8	13.8	16.7	16.6	17.6	24.1
29	18.9	21.8	23.9	18.5	16.6	15.5	12.1	17.8	14	18.9	19.4	24.5
30	18.6	***	22.3	19.1	15.1	12.6	10.6	18.9	10.8	17	20.5	24.2
31	21.1	***	16.7	***	14.8	***	9.3	19.2	***	16.2	***	21.6
g	22.1	22.2	20.6	16.7	16.7	13.2	12.8	13.9	16	18.5	18.4	21.1

ily Minimum Temperature (C) Data for station [0337382 5] - BABANANGO -28.3640 31.2060 770 m 2012 08:00 (Extracted 2015/06/17 15:42)

y	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	15.4	15.2	15.9	12.1	15.5	8.1	4	6	10.8	8.6	9.7	14.1
2	19	15.6	15.6	11.2	13	8.6	8	2.9	8.8	12.5	9.6	14.1
3	18.2	14.4	16.1	8.9	17.9	7	9.8	0.5	9	15.7	10.2	15.6
4	19	16	17.7	9.5	18.3	4.5	12.2	5.5	9	13.6	14.9	13.6
5	18.1	18.3	16.6	12.5	16.1	7.7	6.7	8.6	9.1	13	14.7	15.3
6	18.1	18	15.4	11.6	15.6	13.4	7.1	8.4	8.7	14.8	14.8	15
6	18.1	18	15.4	11.6	15.6	13.4	7.1	8.4	8.7	14.8	14.8	15
7	18.2	16.2	14.6	9.7	15.8	10.1	10.5	5.4	10	14.6	13.6	17.6
8	16.6	18.4	15.1	13.6	12.2	8.8	6.3	5.1	10.9	13.1	11.2	16.7
9	18.2	17.6	17.2	12.6	10.5	6.1	6	3.9	11	13.3	9	15.7
10	18.2	18.2	16.9	7.8	10.6	3.9	6.2	6.2	11.8	12.6	11.9	14.3
11	18.1	17.9	15.6	7.9	13.2	4.3	6.8	7.4	10.1	10.4	13.7	13.7
12	16.9	16.7	13.1	14.3	10.6	6.3	8.2	4.3	8.8	10.9	16	11.7
13	16.6	16.7	12.9	9.3	11.2	6.4	7.7	1.8	12	13.2	15.6	14.1
14	18.7	17.2	16.2	6.9	10.8	6.9	8.7	4.5	9.8	15.8	15.1	16.6
15	18.2	16.8	16.8	8.7	10.7	6.3	4.9	15.4	9.3	14.4	14.7	16
16	17	16.3	17	15.5	7.1	7.1	3.3	10.5	9.5	15.4	15.3	15.8
17	17.6	19.4	15.4	12.5	7.3	6.6	3	10.3	10	14.6	15.8	15
18	17.2	17.3	14.7	12.4	6.6	12.5	5.3	9.9	9.2	14.6	14	14.3
19	16.4	17.5	14.7	11.8	10.4	8.4	1.4	13.6	12.7	13.1	14.1	15.5
20	14.2	18.4	13.7	12.9	10.3	9.1	3.2	13.6	12.5	13.3	16.4	17.5
21	15.7	17.5	16.5	11.6	5.8	9.7	7.9	10.4	17.4	15.2	15.5	14.3
22	18	19.9	17.2	11.9	3.5	9	7.7	9.4	12.1	14.5	13.5	17
23	17.6	17.3	17.1	9.1	7	12.6	10.2	12.6	10.6	12.1	15.1	16.8
24	18.2	15	15.2	11.2	8.2	7.7	9.9	12	10.3	10.3	17.4	16.6
25	16.6	15.4	16.3	10.3	7.3	7.3	5.9	15.4	11.1	10.3	11.4	18.5
26	13.8	18.3	15.2	9.5	8.3	4.1	4.7	12.6	10.3	13.5	11.5	16.6
27	14.5	16.7	14.7	11.5	12.5	9.7	6.4	10.1	16	10.5	12.1	16.8
28	14	14.2	18.6	13.3	9.8	6.7	4.8	8.1	13.5	10.6	11.5	18
29	12.5	14	16.2	14.4	8.9	7.5	3.8	13	8.5	14	13.2	18.8
30	14.6	***	18.3	13.7	9.4	5.7	2.8	12.4	6.7	10.2	14.4	18
31	15.9	***	12.9	***	10.7	***	2.7	15.6	***	10.5	***	14.4
vg	16.8	16.9	15.8	11.3	10.8	7.7	6.3	8.9	10.7	12.9	13.5	15.7

APPENDIX F (DISTANCE TO THE NEAREST RIVER, ALTITUDE AND TEMPERATURE MAX AND MIN)

EMIS Number	School Name	District	Latitude	Longitude	Altitude above sea level (m)	Distance to nearest river (m)	Average Summer Maximum (°C)	Average Summer Minimum (°C)	Prevalence %	Boys Pre %
101195	AM Moola S	iLembe	-29.539575	30.850187	558.23	566.18	30.05	19.69	45%	38
102416	Amaphuphesizwe S	iLembe	-29.137947	31.048646	722.46	538.17	30.08	19.72	71%	64
335294	Chief Ngonyama S	iLembe	-29.275059	30.924411	935.15	776.78	30.03	19.65	43%	48
120028	Darnall S	iLembe	-29.263977	31.361573	60.80	418.24	30.32	20.11	38%	55
334961	Dumane S Comm H	iLembe	-29.45763	30.90268	647.19	1459.42	30.07	19.72	63%	0
141303	Esiqhoqhweni Js	iLembe	-29.20947	30.966683	863.20	566.17	30.04	19.65	12%	24
143745	Ezithabeni S	iLembe	-29.142271	31.162062	746.28	1272.27	30.2	19.91	42%	45
156251	Hlangabeza H	iLembe	-28.8971	31.023721	220.15	293.82	29.65	19.01	36%	30
157435	Hloniphani S	iLembe	-29.539282	31.054531	140.34	124.93	30.16	19.87	65%	70
157472	Hlonono S	iLembe	-29.235113	31.110661	581.55	1490.94	30.19	19.88	30%	55
320605	Imbuyiselo S	iLembe	-29.219611	31.320519	243.01	557.77	30.32	20.1	56%	45
163318	Ingobamakhosi S	iLembe	-29.077728	31.589526	80.25	360.65	30.55	20.81	64%	47
164206	Inhlokozi H	iLembe	-29.087915	31.009381	947.77	1791.40	29.99	19.57	6%	0
167425	Isibanisezwe S	iLembe	-29.19724	31.165624	641.81	643.11	30.22	19.93	0%	10
167758	Isifisosethu Ss	iLembe	-29.482546	30.912497	498.67	905.14	30.08	19.73	29%	17
169164	Isinembe S	iLembe	-29.464845	31.077111	310.84	3059.74	30.17	19.89	40%	55
168387	Isinyabusi H	iLembe	-29.071286	31.554986	20.10	359.16	30.48	20.61	15%	13
171606	Jonase H	iLembe	-29.113695	31.104211	684.37	768.60	30.13	19.79	51%	55
173604	Khanyisa S	iLembe	-29.452431	30.823881	798.35	976.64	30.01	19.63	17%	29
174233	Khethimfundo S	iLembe	-29.384116	30.997825	618.06	326.96	30.12	19.8	38%	50
320568	Lethithemba S	iLembe	-29.311615	31.194841	173.92	1124.83	30.25	19.98	57%	62
188589	Lukhasa S	iLembe	-29.379595	30.937753	618.00	133.00	30.08	19.73	0%	4
189884	Mabayana S	iLembe	-29.544728	30.911562	621.77	1405.51	30.08	19.75	48%	61
190476	Macaphuna S	iLembe	-29.180787	31.017246	582.24	1762.84	30.07	19.71	91%	86
192659	Magudwini Js	iLembe	-29.489611	30.844645	642.50	497.05	30.04	19.67	36%	40
193584	Mahlube S	iLembe	-29.403682	30.953058	642.92	641.28	30.09	19.75	11%	33
335257	Manaba S	iLembe	-29.428183	30.92755	638.46	47.32	30.08	19.73	8%	50
324009	Mangcengeza Js	iLembe	-29.24333	31.043634	466.10	1283.40	30.13	19.8	89%	86
200503	Mashiyamahle S	iLembe	-29.499401	31.019433	361.69	1400.45	30.14	19.84	31%	26
201872	Mathubesizwe H	iLembe	-29.118598	31.518759	101.25	1437.89	30.37	20.26	36%	46
204351	Mbhekaphansi H	iLembe	-28.979659	31.027145	259.98	89.03	29.85	19.34	21%	15
209087	Mgandeni H	iLembe	-29.098689	31.35926	238.93	197.12	30.3	20.02	53%	50
218411	Mshiyane H	iLembe	-29.577687	30.8621	402.36	473.04	30.06	19.71	26%	20
219077	Mthengeni H	iLembe	-29.060084	31.457918	203.03	1449.81	30.35	20.21	100%	43
222222	Mzingezwi S	iLembe	-29.454951	30.969319	669.93	183.04	30.11	19.78	24%	0
326599	Ndukende S	iLembe	-29.087566	30.93283	1061.20	472.97	29.9	19.42	47%	21
227661	Ngcongangconga H	iLembe	-29.333052	30.965043	783.79	1677.17	30.09	19.74	49%	63
228845	Ngqokwane H	iLembe	-29.195164	31.121369	705.89	466.38	30.19	19.88	No Data	No
228993	Ngungwini H	iLembe	-29.54225	30.886061	335.59	808.54	30.07	19.72	47%	50
231250	Njubanjuba S	iLembe	-29.33476	31.056783	505.29	1166.16	30.16	19.85	37%	83
233359	Nkwenkwezi S	iLembe	-29.041275	31.432977	422.59	876.30	30.35	20.22	32%	34
234839	Nombika S	iLembe	-29.51004	30.943814	643.21	655.12	30.1	19.77	26%	15
237244	Nqakathela S	iLembe	-29.55469	30.964464	401.95	1137.23	30.11	19.8	60%	50
239094	Ntabinamafutha S	iLembe	-29.370649	30.977491	545.32	504.77	30.1	19.77	33%	36
241388	Nyakana C	iLembe	-29.170515	31.290734	75.69	274.57	30.29	20.01	56%	48
335442	Phakathwayo Js	iLembe	-29.313393	31.064864	237.55	584.79	30.16	19.85	No Data	No
252081	Qalakahle H	iLembe	-29.287602	30.912475	925.80	403.58	30.03	19.64	26%	30
253450	Qinisani H	iLembe	-29.388111	30.986638	645.05	103.83	30.11	19.79	26%	47
253931	Qoqulwazi S	iLembe	-29.472645	31.101788	240.33	908.58	30.19	19.91	29%	43
254375	Qwabe S	iLembe	-29.326352	31.015352	405.20	1369.77	30.12	19.8	54%	64
100011	Sabuyaze H	iLembe	-29.16328	31.068889	585.03	936.57	30.12	19.78	16%	20
257594	Sakhisizwe H	iLembe	-29.290016	31.070897	212.99	501.55	30.16	19.85	33%	33
261701	Shekembula H	iLembe	-29.191542	31.292428	287.15	1523.16	30.29	20.01	25%	71
339919	Siboginhlanhla S	iLembe	-29.504861	30.976903	330.86	933.98	30.12	19.8	83%	57
302475	Sikhonjwa Ss	iLembe	-29.156007	31.011482	867.24	1052.26	30.05	19.67	48%	36
265216	Sikhuthele H	iLembe	-29.185438	31.511939	80.62	951.86	30.34	20.17	15%	26
266178	Simunye S	iLembe	-29.359231	30.914816	822.34	541.43	30.05	19.69	53%	53
267547	Siphinhlanhla H	iLembe	-29.199164	31.082338	564.18	641.88	30.15	19.83	71%	55
267584	Siphiwe S	iLembe	-29.415305	31.056334	407.49	2282.52	30.16	19.86	0%	0
268213	Sisebenzile S	iLembe	-29.530881	31.019288	343.13	929.36	30.14	19.84	23%	28
270174	Siyaphumula S	iLembe	-29.329895	30.908383	884.69	134.59	30.04	19.67	14%	40
273356	Somshoko S	iLembe	-29.12025	31.574199	81.47	2126.71	30.46	20.57	27%	55
274059	Sotobe S	iLembe	-29.435637	30.877799	906.48	382.17	30.05	19.68	15%	0
285344	Tshutshutshu S	iLembe	-29.126089	31.004634	986.22	357.18	30.02	19.62	62%	44
335479	Ubuhlebembiza S	iLembe	-29.263557	31.032459	219.39	329.54	30.12	19.79	60%	10
285899	Ubuhlebesizwe Js (EC)	iLembe	-29.570336	30.888725	252.61	239.14	30.07	19.73	73%	81
302734	Ukukhanyakwezwe Js	iLembe	-29.185014	31.151598	623.95	1062.91	30.21	19.91	0%	5
289821	Umzwangedwa H	iLembe	-29.006919	31.106884	164.25	547.26	30.02	19.61	67%	57
291301	Velangezwi H	iLembe	-29.23175	31.03107	240.44	169.74	30.11	19.77	68%	78
293188	Vukile H	iLembe	-29.144707	31.071931	645.52	113.81	30.11	19.77	48%	57
299589	Zephania Js	iLembe	-29.167272	31.051897	558.52	728.12	30.1	19.75	14%	30

104747	Bagibile H	uThungulu	-28.933157	31.396855	301.77	54.16	30.29	20.11	90%	5
108521	Bhekeshowe H	uThungulu	-28.796274	31.604585	352.65	821.05	30.62	21.31	23%	2
109557	Bhilibana Js	uThungulu	-28.755916	31.17848	1004.53	1229.81	29.42	18.62	56%	3
143005	Ezakheleni H	uThungulu	-28.812705	31.67021	122.52	205.03	30.68	21.42	69%	8
147223	Gawozi S	uThungulu	-28.83499	31.514958	402.86	727.16	30.56	21.07	36%	4
151367	Gqokinsimbi Js	uThungulu	-28.959256	31.28059	619.35	1558.76	30.19	19.9	51%	4
320420	Hhashi S	uThungulu	-28.809495	31.261652	501.36	546.61	29.82	19.32	59%	8
175898	King Cetshwayo Ss	uThungulu	-28.762391	31.076695	408.64	653.33	29.26	18.34	62%	7
306952	Majiya S	uThungulu	-28.94242	31.564115	120.36	281.02	30.63	21.09	46%	7
200207	Mashanandane S	uThungulu	-28.891021	31.731282	80.90	880.02	30.86	21.54	68%	7
311688	Matheku S	uThungulu	-28.796423	31.563942	348.37	1564.83	30.58	21.22	64%	5
202575	Mavumengwane H	uThungulu	-28.927712	31.22758	560.85	2052.20	30.06	19.71	59%	4
209531	Mgitshwa H	uThungulu	-28.766018	31.699751	191.66	1282.29	30.59	21.44	57%	5
220224	Mthunzini H	uThungulu	-28.798167	31.726853	166.70	607.58	30.65	21.48	69%	3
224923	Ndesheni H	uThungulu	-28.847381	31.984199	12.87	1454.10	29.92	21.72	19%	1
225441	Ndlongolwane H	uThungulu	-28.871691	31.238677	666.82	493.03	29.96	19.54	62%	3
225515	Ndluyesilo H	uThungulu	-28.99212	31.296342	655.52	221.39	30.24	19.97	55%	5
229400	Ngwenya S	uThungulu	-28.988913	31.550856	79.23	594.59	30.57	20.9	68%	7
231139	Njingili S	uThungulu	-28.86392	31.579186	362.38	1065.52	30.65	21.23	67%	9
234062	Nokhalela H	uThungulu	-29.007354	31.231072	742.81	735.37	30.19	19.88	75%	4
248936	Phindulimi H	uThungulu	-28.955004	31.471991	223.60	422.80	30.47	20.65	52%	6
267843	Siphoso S	uThungulu	-28.898795	31.57943	304.66	808.73	30.66	21.21	83%	9
280682	Thanduyise H	uThungulu	-28.78211	31.856156	82.34	1020.98	30.3	21.59	30%	5
297887	Yamela S	uThungulu	-28.806863	31.373804	510.20	918.03	30.06	19.78	65%	5
300514	Zinqobele S	uThungulu	-28.889574	31.654927	230.62	1684.25	30.78	21.44	87%	8

APPENDIX G (Manuscript sent for publication to the South African Journal of Infectious Disease)

Environmental factors governing the distribution and prevalence of *Schistosoma haematobium* in school attenders of ILembe and uThungulu Health Districts, KwaZulu-Natal Province, South Africa

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Abstract

Schistosoma haematobium infection is reported to facilitate the development of urogenital diseases. Its symptoms include haematuria, dysuria, and tiredness and it may cause cognitive decline in children. The prevalence of *Schistosoma haematobium* infection needs to be known in endemic areas and a mass treatment programme against the disease implemented.

The aim of this study was to investigate the prevalence and intensity of *S. haematobium* infection in ILembe and uThungulu Health Districts, using the major symptom haematuria as an indicator. A total of 6265 urine samples, from 96 rural schools, was collected for analysis using dipsticks. The prevalence of haematuria for ILembe Health District for boys was 37% (95% CI, 35-39) and girls 39% (95% CI, 37-41) and in uThungulu Health District was 56% (95% CI, 53-59) and 53%

(95% CI, 50-56) for girls and boys, respectively. Light intensity infection was the most common infection level in both health districts. A negative relationship was observed between prevalence and altitude ($r = -0.262$, $p = 0.009$), whereas we found a slight, though significant, positive association with mid-summer temperatures ($r = 0.234$, $p = 0.021$). Associations between prevalence and distance of school to the nearest river were non-significant.

Introduction

The neglected tropical disease of urogenital schistosomiasis is a public health challenge affecting both sexes in many developing countries. South Africa is one of the countries endemic for urogenital schistosomiasis [1]. An estimated 750 million people worldwide and 261 million people in sub-Saharan Africa are infected by this disease [2]. Urogenital schistosomiasis is a waterborne disease caused by the parasite *Schistosoma haematobium*, which is carried by a specific intermediate host snail [3]. People exposed to fresh water containing the infected snails are at risk of attacks by cercariae, which enter the human host, and develop into adult flukes that produce eggs, causing inflammation that affects the urogenital tract. The disease has deleterious effects and symptoms, which include anaemia, tiredness and learning/work problems and target organ dysfunction [3-4]. Morbidity from urogenital schistosomiasis is manifested by blood in urine (haematuria), pain when urinating (dysuria) and frequent urination, in addition to genital symptoms like discharge and dyspareunia [4]. This disease may be a predisposing factor for acquisition of human immunodeficiency virus (HIV) [16, 32].

Schistosomiasis can be diagnosed by microscopy (eggs in urine), or serology, or molecular methods (such as polymerase chain reaction, PCR), but blood in urine is a common symptom of urogenital schistosomiasis, and detection of haematuria may be an indication of *S. haematobium* infection, although this is controversial in females of reproductive age [2, 4, 35]. The urine reagent strip detects haematuria as a proxy diagnostic method for S. *haematobium* infection in endemic areas [7]. The

World Health Organization (WHO) recommends mass treatment of schistosomiasis in regions of high prevalence and intensity, with a particular focus on children, who usually have the highest prevalence and intensity of infection [2, 8, 9]. Although studies were undertaken in this area of KwaZulu-Natal Province in the 1980s and 1990s [10-11], little information is available for the last 20 years. *S. haematobium* is more common in areas of low socioeconomic status [2-4], that is, in disadvantaged areas where water, sanitation and health facilities are inadequate [9]. This poses a challenge for the rapid diagnosis, treatment and control of *S. haematobium* infection. The urine reagent strips (dipsticks) are easy to use, convenient and time-efficient [9, 12]. People in disadvantaged areas are at risk of infection because they often lack adequate safe water sources and are thus compelled to be in contact with river or dam water contaminated by *S. haematobium* [2, 13]. Children playing, women doing laundry and farm workers involved in agricultural and fishery industries are at a heightened risk because of frequent contact with fresh water [6, 13-14]. Furthermore, even if the patients go to the clinic, the laboratory services may not be available and patients may never return for their results due to financial, social or logistic constraints.

Urogenital schistosomiasis is a latent and chronic disease, meaning that the damage it exerts develops over time due to adult worms that produce eggs which disrupt various tissues and organs [4, 15]. The parasite eggs trapped in the body can induce adverse immunomodulatory effects that have been hypothesised to favour the transmission and progression of other diseases [16-17]. Parasite eggs are small, can travel by blood, and have the potential to breach membrane tissues that provide barrier protection against parasite, bacterial and viral entry [4, 16-19]. Research indicates that several diseases such as cancer, HIV and chronic kidney disease may be associated with *S. haematobium* infection [15-16, 20, 32]. The prevalence and intensity of urogenital schistosomiasis is determined by factors such as age, geographical location, contact with contaminated water bodies, season (transmission usually occurs during the hot and humid summer) and the distribution of the intermediate host snail [13, 21]. Environmental factors can serve as indicators to suggest areas that could be at risk for *S. haematobium* infection [30] and include temperature and altitude which can have an influence on the survival and reproduction of the intermediate host snail [22]. In order for the host snail to survive, breed and

complete it life cycle, these factors should occur within tolerable ranges [6, 22, 33]. The aim of this study was to investigate the prevalence and intensity of *S. haematobium* in the ILembe and uThungulu Health Districts and association with abiotic factors such as summer temperature, altitude above sea level and distance from the school to the nearest river.

Methods

Study Setting

Neighbouring ILembe and uThungulu Health Districts are situated on the east coast of KwaZulu-Natal Province, north of the metropolitan city of Durban. All rural high schools with more than 300 pupils were stratified by size and invited to the study starting from the coastline and moving gradually inland. Urine samples were collected from 96 high schools (71 schools from ILembe and 25 schools from uThungulu Health Districts); all learners in grade 8 were invited to participate in the study. Grade 8 learners are usually between 13 and 16 years of age [6]. All girls participating in the study were discreetly asked by a female research assistant whether they were menstruating, and the urine samples of those who were menstruating were not included in this study.

Study Design

In a cross-sectional design, urine samples were collected between years 2012 and 2013.

Urine analyses

Single urine samples collected in the middle of the day were tested for haematuria using urine reagent strips (Neotest 4, Johannesburg as an indicator of urogenital schistosomiasis [7]. Haematuria, if present, was categorised as low/light, medium/(moderate, or high/heavy, corresponding to 1+, 2+, and 3+ readings, respectively, of reagent strips [6].

Temperature data

The temperature data were obtained from the records of the South African Weather Services (SAWS). Temperature data was extrapolated from meteorological stations at Mandini [02716992], Mtunzini [03043576], Babanango [03051346] and Richards Bay Airport [03373825].

Geographical Information

The altitude and the distance of school to the nearest river were calculated using a geographical information system (GIS) programme (ArcMap 9.2), using the geographical co-ordinates for each school provided by the Department of Education website [34]. The altitude data was categorised into low (0-300 m), medium (300-800 m), high (800-1100 m) and very high (1100+ m) [6].

Method of extracting altitude, distance values and temperature for each school

a A 20 m digital elevation model (DEM) of KwaZulu-Natal was used to calculate the altitude of each school in the study. The elevation value represents the altitude above sea level in metres. In order to calculate the distance from each school to its closest perennial river, a spatial join was performed in ArcMap between the Rivers (1:50 000 perennial rivers dataset) and Schools shapefiles. This calculates and adds a distance field to the attribute table. This indicates the distance from the school to the closest river contact point in the GIS programme [36]. The average maximum and minimum summer temperature for each weather station was calculated for the summer months (December, January and February). These values were then used to interpolate average maximum and minimum summer raster coverages for the study area, using the inverse distance weighted (IDW) spatial analyst technique in ArcGIS [36].

Statistics

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) 21 (Armonk, NY, USA). Descriptive analyses were undertaken for all the variables in the study. The mean prevalence of urines testing positive for haematuria were compared for boys and girls, using school, class per grade, health district, altitude and distance from the nearest river/s. The Kolomogorov-Smirnov test was used as a goodness-of-fit test. For data that were normally distributed the student t-test was used and for non-normally distributed data, the Mann-U-Whitey test was used to compare averages. To establish presence and the strength of any relationship between these variables, the Spearman's correlation coefficient (r) was calculated. A p value of $p<0.05$ was used as the level of significance and the 95 % confidence intervals were calculated.

Ethical considerations

Permission for the study was obtained from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal, clearance number BE165/15. Written informed consent was obtained from the parents of participating learners and learners provided assent. Treatment was offered to the Ugu, ILembe and uThungulu Health Districts by the Department of Health in a mass-treatment campaign.

Results

Prevalence

The total sample size was 6265, and the prevalence of haematuria in uThungulu Health District schools was 55% (1289/2360), significantly higher than the 39% (1531/3905) in ILembe Health District schools ($p<0.001$). The prevalence of haematuria indicating urogenital schistosomiasis was 37 % (95% CI, 38-42) for boys in ILembe Health District and for girls, 39 % (95% CI, 37-41) ($p>0.05$). The prevalence of haematuria in uThungulu Health District was 53% (95% CI, 50-56) for boys and 56% (95% CI, 50-56) for girls ($p>0.05$) (Fig. 1).

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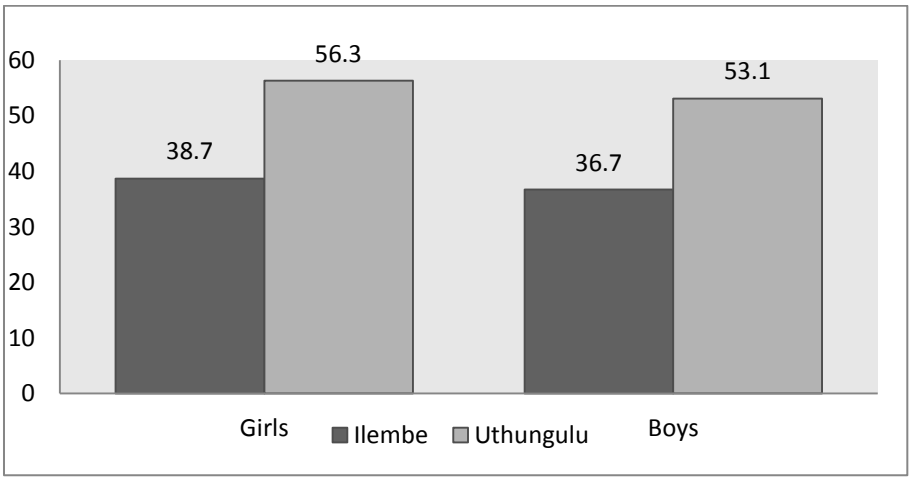

Figure 9. Prevalence of urogenital schistosomiasis by sex in grade 8 learners in ILembe and uThungulu Health Districts, KwaZulu-Natal. n(girls) = 3000; n(boys) = 3265

In ILembe Health District a total of 71 rural public schools were visited and a total of 1888 girls and 2017 boys tested for presence of blood in urine using reagent strips, and 801 boys (37%) and 730 girls (39%) were found to have haematuria. In uThungulu Health District a total of 25 schools was visited and 1112 girls and 1248 boys consented to participate in the study, and 626 girls (56%) and 663 boys (53%) were found to have haematuria.

Intensity

There were no significant differences between the sexes in low, moderate or high levels of haematuria ($p = 0.13$) (Fig. 2). The intensities of haematuria were not significantly different between the health districts ($p > 0.05$). Although the intensity of haematuria followed the same trend for girls in uThungulu (1+ = 40%, 2+ = 30%, 3+ = 30%) and ILembe (1+ = 41%, 2+ = 28%, 3+ = 30%), the uThungulu boys (1+ = 35%, 2+ =28%, 3+ = 36%) had a slightly higher percentage of heavy intensity haematuria compared to boys in ILembe (1+ = 41%, 2+ = 28%, 3+ = 31%) although this difference was not statistically significant ($p = 0.17$).

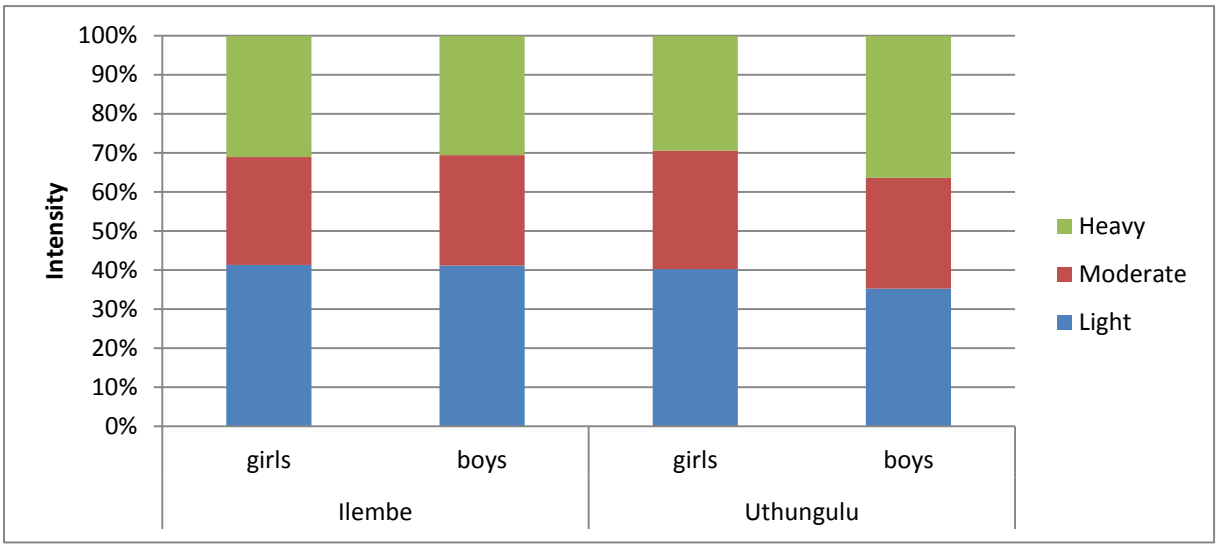

Figure 10. Mean intensity of haematuria obtained from reagent strips at rural schools (n = 96) of ILembe (71 schools) and uThungulu (25 schools) Health Districts (total n = 2842 infected learners: n(girls) = 1370; n(boys) = 1472).

Association of prevalence of haematuria with environmental factors

The map (Fig. 3) shows the schools in the ILembe Health District (74% of sample), where 39% of the schools are in the 0-300 m altitude range. In this altitude range the majority of the schools had a haematuria prevalence greater than 20%. This health district has 59% of schools in the 300-800 m altitude range, which showed a haematuria prevalence ranging from 11-90%.

In uThungulu Health District, 8% of the schools have less than 20 % prevalence and are in the 0-300 m altitude range, but this altitude range also includes 52% of schools that have a haematuria prevalence greater than 50%. The 300-800 m altitude range has 36% of schools with a haematuria

prevalence greater than 50%. In the 800-1100 m altitude range in this Health District there were 4% schools with a prevalence of haematuria greater than 50%.

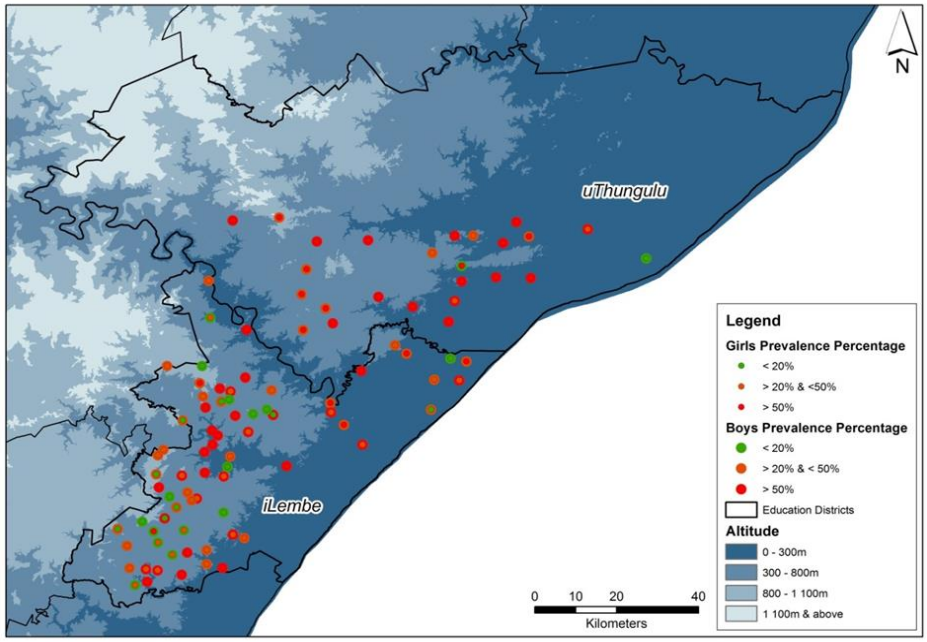

Figure 11. Map of uThungulu and ILembe Health Districts, categorizing the prevalence (<20%, >20-50% and >50%) in boys and girls in selected schools, and the associated altitude (m). [36]

The altitude above sea level for the schools was significantly related to the prevalence of haematuria for both girls and boys and the negative correlation coefficient indicates that increasing altitude was associated with a decrease in the prevalence of infection. For girls there was a statistically significant association between the prevalence of schistosomiasis and the maximum mean summer temperature ($r = 0.244$, $p = 0.017$) but this was not found for boys ($r = 0.150$, $p = 0.145$). The distance from the school to the nearest river contact point did not influence the prevalence of urogenital schistosomiasis in either sex.

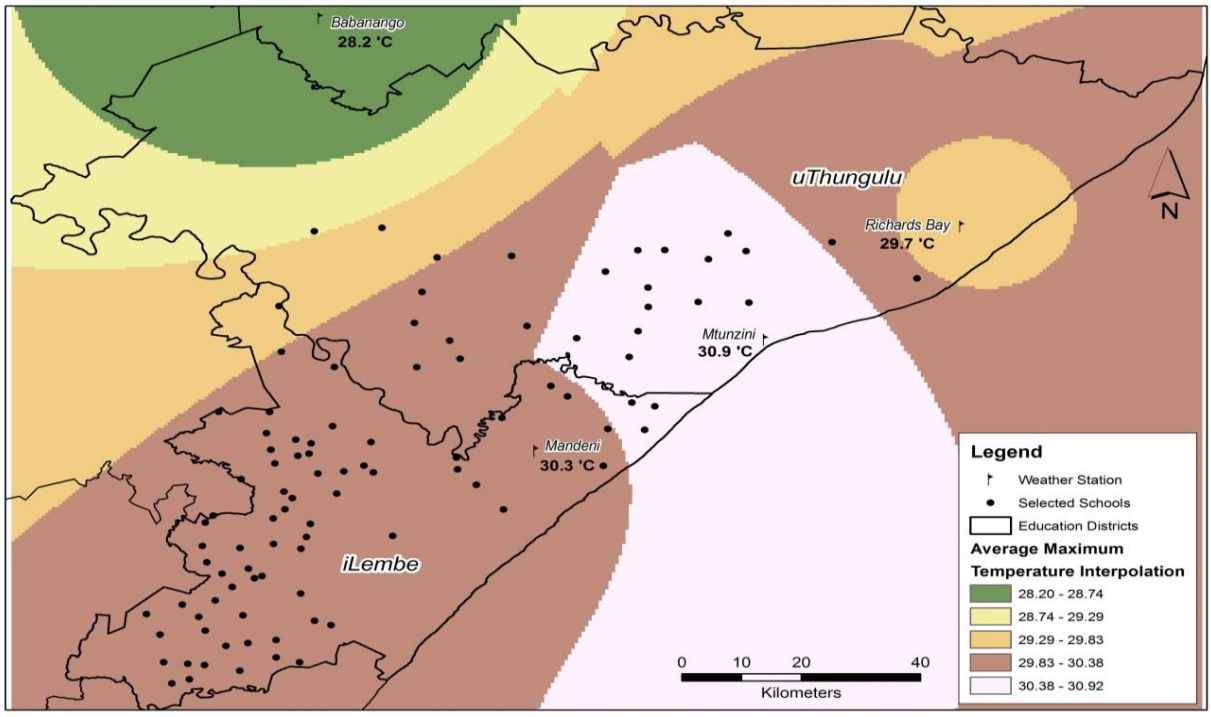

Figure 12. Average maximum summer temperature (°C) of the areas surrounding the schools that were selected in the study [36].

There were 81% of schools in the 29.83-30.38 °C temperature range. When the prevalence of haematuria was combined for boys and girls, there was a relatively weak positive correlation (r = 0.234) with the average summer maximum (see Figure 4) that was significant (p = 0.021) (Table 1).

Table 7. Correlation coefficients (r) between haematuria prevalence and associated abiotic factors (altitude above sea level, distance to nearest river contact point and the maximum and minimum average summer temperature) (n = 6265).

Prevalence	Factor	Correlation coefficient (r)	P value
Girls	Altitude above sea level	-0.215	0.035*
Boys		-0.283	0.005*
Both		-0.262	0.009*
Girls	Distance to nearest river	0.010	0.922
Boys		-0.007	0.947
Both		0.008	0.936
Girls	Average summer	0.244	0.017*
Boys	maximum (°C)	0.150	0.145
Both		0.234	0.021*
Girls	Average summer	0.121	0.242
Boys	minimum (°C)	0.146	0.156
Both		0.174	0.088

*Correlation is significant at $p<0.05$

Discussion

Prevalence and intensity of urogenital schistosomiasis in ILembe and uThungulu Health Districts

The results of this study report that the prevalence of infection with *S. haematobium* is high among school attenders. It was shown that *S. haematobium* infections are significantly higher ($p < 0.001$) in the uThungulu district learners compared to ILembe Health District learners, but in both health districts sexes are infected equally.

Learners in grade eight in the KZN province are usually between the ages of 13-16 years old, and are likely to be swimming or playing in rivers due to the hot weather and lack of alternative recreational facilities [13]. Morgas *et al.* (2010) noted the possibility of higher prevalence among girls in this age group, since they are responsible for most housekeeping chores, which could include washing of dishes or clothes in the river and fetching water from the river for other uses [13]. On the contrary, boys of this age group are at risk because they are still at an explorative stage and are eager to play or swim in river water [13].

The WHO guidelines recommend that all school learners, in the prevalence category in which ILembe Health District falls, receive praziquantel (PZQ) anti-schistosomiasis treatment and that this mass treatment must be done annually for at least one to two years [8]. For uThungulu Health District, which falls in the WHO category 1, the recommendation would be that everyone in the community receives PZQ treatment irrespective of status, age or sex and mass treatment should be provided once a year for a period of three years [8, 24].

Treatment of *S. haematobium* can have profound health improvements as destruction of adult worms by the drug can reduce the number of circulating parasite eggs. Mass treatment thus has the potential to reduce the burden of disease and its complications [31]. From the association reported between female genital schistosomiasis and HIV infection, treatment of urogenital schistosomiasis may reduce the likelihood of transmission of HIV [16, 20, 32]. The reduction of circulating parasite eggs in the body can reduce the damage caused by the development of sandy patches in the cervix and may reduce the development of cancer in the genitals and kidneys [13, 32, 29].

In comparison, Gear *et al* .(1980) reported the prevalence of urogenital schistosomiasis to be 70% for schools in the ILembe Health District [37], whilst for schools in uThungulu Health District a prevalence of 26-50% was reported [37]. For the past 30 years the prevalence of *S. haematobium* has decreased by 32% in ILembe district, whilst for uThungulu district it has increased [37]. It is not fully understood what factors could be responsible for the change in prevalence, but changes could be due to population dynamics, geographical distributions, socioeconomic status and change in climate conditions. However both health districts reported to have experienced delays in the provision of piped water and sanitation [23-24] and this could possibly provide an opportunity for re-infection. Wolmarans *et al*. (2001) found the prevalence of urogenital schistosomiasis in sites in Limpopo Province to be 70% in pupils younger than 14 years of age, but the sample size was smaller (n = 420) [22, 27]. In Mpumalanga Province in 2004 an epidemiological study revealed a prevalence of 35% among primary school pupils in 30 schools [22].

The prevalence and distribution of urogenital schistosomiasis is influenced by environmental factors that determine the survival and replication of the intermediate host snail *Bulinus africanus* [30]. Factors favouring or limiting *B. africanus* survival consequently impact *S. haematobium* transmission and infection [30].

ILembe Health District has most of the schools (21) at a 300-800 m altitude, with a low schistosomiasis prevalence (<20%), whilst schools that are located in the altitude range of 0-300 m tend to have a urogenital schistosomiasis infection percentage higher than 20% and up to 50% (see Figure 3). The majority of schools in uThungulu Health District (0-800 m) showed infection rates greater than 20% and in some schools, greater than 50%.

The intensity of haematuria (light, moderate or heavy) was not affected by gender differences, but intensity of infection varied by health district, with schistosomiasis infection higher in schools located in uThungulu Health District compared to schools in ILembe Health District ($p < 0.005$). In uThungulu Health District boys had a higher tendency towards heavy infection, whilst girls in this health district had a higher distribution in the light infection category (Fig. 2). The majority of learners fell in the

light and/or heavy intensity category in both health districts. A 1+ reading on a reagent strip indicates a light intensity infection, and this is equivalent to a release of 5-10 erythrocytes per µl of urine [6]. This means that the 41% (see Figure 2) of boys and girls in ILembe Health District are losing 5-10 erythrocytes per µl of urine, which can be a significant number of lost red blood cells due to haematuria. In uThungulu Health District 36% of boys lose red blood cells in urine (around 250 erythrocytes per µl of urine) through their heavy intensity of infection. Thirty percent of girls had heavy intensity haematuria in uThungulu Health District. Loss of blood in urine can have serious health implications, such as anaemia and tiredness [3-4].

Campaigns that provide mass treatment to school-age girls and boys are strongly recommended, especially in areas where *S. haematobium* is endemic and prevalent [25]. Treatment of school pupils vulnerable to *S. haematobium* infection is required because genital lesions and membrane damage caused by trapped parasite eggs can be minimized if treatment is offered timeously. Early treatment of urogenital schistosomiasis may reduce the transmission and potentiation of transmission of HIV [20, 32]. Treatment is required equally for both sexes as both boys and girls appear to be similarly infected. The WHO has advised the joint drug delivery for schistosomiasis and soil transmitted helminth infections, after it was observed that these two infections threaten similar communities and are most likely to be found where there is inadequate water and sanitation [26]. With schoolgoing learners being the most at risk, regular treatment is advised because this can be beneficial for potentially anaemic students, who are always tired due to infection with *S. haematobium* and lack the ability to focus at school, and treatment may improve absenteeism among infected learners [26]. Since this is a neglected disease local health facilities in rural areas might lack equipment for diagnosis and sometimes PZQ might not be available at primary healthcare clinics. For these reasons mass treatment with PZQ at schools endemic for *S. haematobium* infection is encouraged [2, 5, 26]. The use of urine reagent strips can assist the diagnosis process, if needed [9].

In this study the prevalence and intensity of urogenital schistosomiasis was measured using urine reagent strips. Urine reagent strips are estimated to have a sensitivity and specificity of 82% and 97%,

respectively, for an active *S. haematobium* infection [12]. This suggests that the results presented here can be trusted, as there is no significant difference between infection levels detected with microscopy or urine reagent strip [6]. Meents & Boyles (2010) reported such congruence local [27]. However, false positives can arise from bacterial or viral infection(s) that cause haematuria, and if menstruating girls were tested [7, 14]. The prevalence presented in this research represents data from the schoolgoing population and may not be a good representation of the entire health districts studied here.

Relationship between prevalence and environmental factors

The prevalence of urogenital schistosomiasis showed a significant negative relationship with altitude above sea level in boys and girls ($p = 0.035$ and 0.005, respectively) (see Table 1). The Spearman's rank correlation showed a weak negative correlation coefficient for the relationship between altitude and prevalence of infection for girls and boys ($r = -0.215$ and -0.283, respectively). This finding is congruent with what other researchers have found, in that locations of low altitude (around 250 m) are conducive for the survival and reproduction of the intermediate snail host, and hence such areas have a high prevalence of *S. haematobium* [28]. However the negative relationship observed between altitude and prevalence of urogenital schistosomiasis is not absolute, as there are schools at the 0-300 m altitude with low prevalence and there are also schools at 300-800 m with high prevalence.

The relationship between prevalence of urogenital schistosomiasis and distance to the nearest river was not significant for both girls and boys ($p = 0.922$ and 0.947). Although in this study there was no statistically significant association between school location and the distance to the nearest river with urogenital schistosomiasis prevalence, one study reported that learners at schools that were close to rivers and/or dams tended to have a higher prevalence of *S. haematobium* infection than those at schools that are further away [29]. In the present area, the distances between the homes and the rivers might be of more importance.

Associations between average summer maximum (°C) temperature and prevalence of urogenital schistosomiasis showed a weak positive relationship for both girls and boys ($r = 0.244$ and 0.150

respectively). This relationship was significant for girls ($p = 0.017$) but it was non-significant for boys ($p = 0.145$). When the prevalence was combined for girls and boys a positive correlation ($r = 0.234$) was observed that was significant ($p = 0.021$) (Table 1). Annual temperature ranges in ILembe and uThungulu Health Districts are adequate for the survival and breeding of the host snail, since the prevalence of haematuria due to urogenital schistosomiasis was moderately high (37-56%).

Conclusion

Urine reagent strips have proved useful in the identification and quantification of haematuria as a proxy for urogenital schistosomiasis infection. Urogenital schistosomiasis infections are moderately high among the schoolgoing population of ILembe and uThungulu Health Districts. Early treatment of urogenital schistosomiasis can prevent the development of genital lesions and kidney damage that is due to the calcification of parasite eggs and formation of fibrous tissue in genitals and the kidneys. The environmental factors explored in this study (temperature, altitude) can serve as useful indicators in the identification of areas at risk for urogenital schistosomiasis.

References

1. Johnson CC, Appleton CC. (2005) Urban schistosomiasis transmission in Pietermaritzburg, South African. The South African Journal of Epidemiology and Infection 20(3): 103-107.

2. World Health Organization (2012) Technical Report Series. Prevention and Control of Schistosomiasis in EndemicAreas.. Geneva: World Health Organization 87: 81-89.

3. Brooker S. (2007) Spatial epidemiology of human schistosomiasis in Africa: risk models, transmission, dynamics and control. Transactions of the Royal Society of Tropical Medicine and Hygiene 101(1): 1-8. Doi: 10.1016/j.trstmh.2006.08.004.

4. Barsoum RS, Esmat G, El-Baz T. (2013) Human schistosomiasis: clinical perspective. Journal of Advanced Research 4(5): 433-44 Available at: http://dx.doi.org/10.1016/j.jare.2013.01.005 (accessed on 15-08-2013).

5. Amazigo UV, Leak SGA, Zoure HGM, Njepuome N, Dikassa PSL. (2012) Community-driven interventions can revolutionise control of neglected tropical diseases. Journal of Neglected Tropical Diseases 26(6): 231-238.

6. Appleton CC, Kvalsvig JD. (2006) A school-based helminth control program successfully implemented in Kwa-Zulu-Natal. The South African Journal of Epidemiology and Infection 21 (2): 55-67.

7. Pugia MJ. (2000) Technology behind diagnostic reagent strips. Laboratory medicine. CE Update Instrumentation ii. 31(2): 46515-0070.

8. World Health Organization (1998) Technical Report Series. Prevention and Control of Schistosomiasis and Soil Transmitted Helminths. Geneva: World Health Organization; 813-911.

9. Taylor M, Jinabhai CC, Naidoo K, Dlamini SB, Sullivan KR. (2004) The epidemiology of schistosomiasis among Zulu children in a rural district in South Africa: determining appropriate community-based diagnostic tools. The South African Journal of Epidemiology and Infection 19(3, 4): 90-95.

10. Pitchford RJ. (1981) Temperature and schistosome distribution in South Africa. South African Journal of Science 77: 252-261.

11. Schutte CHJ, van Deventer JGM and Lamprecht T. (1981) A cross-sectional study of the prevalence and intensity of infection with *Schistosoma haematobium* in students of northern KwaZulu-Natal. American Journal of Tropical Medicine and Hygiene 30: 364-372.

12. King CH, Bertsch D. (2013) Meta-analysis of urine heme dipstick diagnosis of *Schistosoma haematobium* infections including low prevalence and previously-treated populations. PLoS Neglected Tropical Diseases 7(9): e2431. Doi: 10.1371/journal.pntd.0002431.

13. Morgas DE, Kvalsvig JD, Gunderson SG, Taylor M, Kjetland EF. (2010) Schistosomiasis and water-related practices in schoolgirls in rural KwaZulu-Natal, South Africa. The South African Journal of Epidemiology and Infection 25(4): 31-33.

14. Freeman MC, Clasen T, Brooker SJ, Akoko DO, Rheingans R. (2013) The impact of school-based hygiene, water quality and sanitation, intervention on soil-transmitted helminth reinfection: a cluster randomized trial. American Journal of Tropical Medicine & Hygiene 89(5): 875-83.

15. Kayange NM, Smart LR, Tallman JE, Chu EY, et al. (2015) Kidney disease among children in sub-Saharan Africa. A systematic review. Paediatric Research 77(2): 85-189. Doi: 101038/pr2015.189.

16. Mbabazi PS, Andan O, Fitzgerald DW, Chitsulo L, Engels D, *et al*. (2011) Examining the relationship between urogenital schistosomiasis and HIV Infection. PLoS Neglected Tropical Diseases 5(12): e1396. Doi:10.1371/journal.pntd.0001396

17. Jourdan PM, Roald B, Poggensee G, Gunderson SG, Kjetland EF. (2011) Increased vascularity in cervicovaginal mucosa with *Schistosoma haematobium* infection. PLos Neglected Tropical Diseases 5(6): 1-6.

18. Kildemoes AO, Kjetland EF, Zulu SG, Taylor M, Vennervald BJ. (2015) *Schistosoma haematobium* infection and asymptomatic bacteriuria in young South African females. Acta Tropica 144: 14-23. Doi: 10.10.16/j.actatropica.2015.01.008.

19. Senghor B, Diallo A, Doucoure SS, Ndiath MO, *et al*. (2014) Prevalence and intensity of urinary schistosomiasis among school children in the District of Niakhar, Region of Fatick, Senegal. Parasites and Vectors 7:5. Doi: 10.1186/1756-3305-7-5.

20. Hotez JP, Molyneux DH, Fenwick A, Ottesen E, Sachs SE. (2006) Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. A comprehensive pro-poor health policy and strategy for the developing world. PloS Medicine 3(5); 0576-0584.

21. Saathoff E, Olsen A, Magnessen P, Kvalsvig JD, *et al*. (2004) Patterns of *Schistosoma haematobium* infection, impact of PZQ treatment and re-infection after treatment in a cohort of school children from rural KwaZulu-Natal. BMC Infectious Disease 4; 40. Doi: 10.118611471-2334-4-40.

22. De Kock KN, Wolmarans CT. (2004) Distribution and habitats of the *Bulinus africanus* species group, snail intermediate hosts of *Schistosoma haematobium* and *S. mattheei* in South Africa. Water SA 31(1): 117-127.

23. ILembe District Municipality. (2011/2012) Integrated Development Plan. Annual Review.

24. UThungulu District Municipality. (2012/2013) Integrated Development Plan.

25. Randrianasolo BS, Jourdan PM, Ravoniarimbinina P, Ramarokoto CE. (2015) Gynecological manifestations, histopathological findings and schistosoma-specific PCR results among women with *Schistosoma haematobium* infection: a cross-sectional study in Madagascar. Journal of Infectious Diseases: 212(2):275-84. D0i: 10.1093/infdis/jiv035.

26. World Health Organization (WHO). (1995) Health of school children. Treatment of intestinal helminths and schistosomiasis. WHO/schisto/95.112

27. Meents EF, Boyles TH. (2010) *Schistosoma haematobium* prevalence in school children in the rural Eastern Cape Province, South Africa. South African Journal of Epidemiology and Infection 25(4): 28-29.

28. Liao CW, Sukati H, Nara T, Tsubouchi A, *et al*. (2011) Prevalence of *Schistosoma haematobium* infection among schoolchildren in remote areas devoid of sanitation in north western Swaziland, Southern Africa. Japan Journal of Infection and Disease 64: 322-326.

29. Oniya MO, Ishola MA, Jayeoba OD. (2013) Schistosomiasis in Ipogun: update assessment on endemicity and efficacy of praziquantel in chemotherapy. International Journal of Tropical Disease and Health 3(1): 37-44.

30. Appleton CC. (1978) Review on abiotic factors influencing the distribution and the life cycles of bilharziasis host snail. Malacological Review 11: 1-25.

31. World Health Organization/Pan American Health Organization (WHO/PAHO). (2014) Schistosomiasis Regional Meeting: defining the road map toward verification of elimination of schistosomiasis in Latin America and the Caribbean by 2020. www://www.paho.org/hq/index.php?option=com_topics&view=rdmore&cid=6157&Itemid=40770&lang=en (Date accessed: 11-02-2016)

32. Kjetland EF, Leutscher PDC, Ndhlovu PD. (2012) A review of female genital schistosomiasis. Trends in Parasitology 28(2): 1-4.

33. Joubert PH, Pretorius SJ, De Kock KN, van Eden JA. (1986) Survival of *Bulinus africanus* (Krauss), *Bulinus globosus* (Morelet) and *Biomphalaria pfefferi* (Krauss) at high temperatures. South African Journal of Zoology 21(1): 85-88. Doi: 10.10801/02541858.1447963.

34. Department of Education KwaZulu-Natal school list, (2012).. www.kzneducation.gov.za/portals/EMIS/KZN%20List%202012.xls (Date accessed : 03-07-2015)

35. Gundersen SG, Kjetland EF, Poggensee G, Helling-Giese G, Richter J, Chitsulo L, Koummenda N, and Krantz I, Feldmeier H. (1996) Urine reagent strips for diagnosis of *Schistosoma haematobium* in women of fertile age. Acta Tropica 62: 281-287.

36. Edu-Action. GIS and Education Consultants. www.eduaction.co.za (Date accessed : 03-03-2015

37. Gear JHS, Pitchford RJ, van Eeden JA. (1980) Atlas of Bilharzia in Southern Africa. South African Institute for Medical Research, South African Medical Research Council and Department of Health. Johannesburg: SAIMR.